

# Antimicrobial Resistance in thermotolerant *Campylobacter* from a Global Perspective: Insights from Phenotypic and Genomic Analyses

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Michael Zarske

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First Reviewer: Dr. Kerstin Stingl  
National Reference Laboratory for *Campylobacter* –  
German Federal Institute for Risk Assessment (BfR)  
Diedersdorfer Weg 1, 12277 Berlin

Second Reviewer: Prof. Dr. Jens Rolff  
Evolutionary Biology - Institute of Biology – Zoology  
Freie Universität Berlin  
Königin-Luise-Straße 1-3, 14195 Berlin

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## 2 Declaration of Authorship

I hereby declare that I alone am responsible for the content of my doctoral dissertation and that I have only used the sources or references cited in the dissertation.

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place, date

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Michael Zarske

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### 3 Summary

*Campylobacter jejuni* and *Campylobacter coli* are considered the primary causative agents of campylobacteriosis in humans, which has significant public health implications worldwide. In Europe, they account for by far the most cases of bacterial gastroenteritis. Particularly feared, though underestimated by the general population, are the long-term consequences of an infection that can occur in rare cases, such as Guillain-Barré syndrome, reactive arthritis, and irritable bowel syndrome. The excessive use of antibiotics in human and veterinary medicine has led to increasing resistance of these bacteria to antimicrobial agents, limiting treatment options. Consequently, the Centers for Disease Control and Prevention (CDC) classifies *Campylobacter*, especially due to its fluoroquinolone resistance, as a high-priority resistant bacterium and a serious public health threat.

This study aimed to compare the antibiotic resistances of *Campylobacter* isolates from different regions of the world to identify resistance determinants relevant to human medicine and imminent in Europe, which develop under high selection pressure and pose a challenge to global public health. Based on the results of this comparative study, a novel warning tool should be developed that can be used at the molecular level in routine monitoring programs.

The results of our comparative study showed differences in the extent of various resistances depending on the origin of the *Campylobacter* isolates. For example, the isolates from Georgia and Germany showed particularly high resistance to fluoroquinolones and tetracyclines. In contrast, resistance to aminoglycosides and macrolides was less common. The *Campylobacter* isolates from Vietnam, however, showed nearly complete resistance to fluoroquinolones and tetracyclines, and particularly the Vietnamese *C. coli* isolates exhibited very high resistance to aminoglycosides and macrolides. These high resistance rates in Vietnam are possibly

due to the extensive use of antibiotics in livestock farming.

Through the application of whole genome sequencing, phenotypic resistances could be linked to the presence of resistance determinants in the individual isolates. This showed that distinct *Campylobacter* populations carry different resistance markers and that Vietnamese *Campylobacter* isolates carried more resistance determinants.

We further investigated to what extent a data analysis based on whole genome data can be used to predict the expression of phenotypic resistance. We found that for the most part, the prediction matched the phenotypic resistance. However, discrepancies between the whole genome sequencing data and the phenotypic resistance profiles were also discovered. Problems included missing or inaccurately annotated AMR genes, detection issues due to multiple gene copies or variants, and novel mutations affecting gene functionality. Additionally, unknown resistance mechanisms were identified, such as resistance to ciprofloxacin without concurrent nalidixic acid resistance.

Another resistance mechanism leading to aminoglycoside resistance was deciphered and characterized in the course of this study. By applying natural transformation and analyzing genome data, a point mutation in the 16S rRNA of *Campylobacter* was identified, which was causally related to aminoglycoside resistance. We were able to show that the stability of the resistance depended on how many of the three copies of the 16S rRNA present in *Campylobacter* had undergone this mutation.

Using Nanopore long-read sequencing technology, combined with short-read data, hybrid assemblies were created for individual isolates. This allowed several isolates to be represented with their fully circular chromosome and, where applicable, additional epichromosomal units (e.g. plasmids). This provided insights into the localization and



mode of spread of the identified resistance determinants.

Ultimately, based on the whole genome data, it was possible to design new primers and probes and subsequently develop a pentaplex real-time PCR system. This was adequately tested and validated during this study and will be available in the future as a cost-effective alternative to whole genome sequencing to routinely monitor the most important resistance markers such as fluoroquinolone, macrolide, and tetracycline resistances.

In conclusion, the great genetic diversity and observed resistances in *Campylobacter*, especially in regions with intensive antibiotic use, stresses the necessity for continuous monitoring of circulating resistances in a global context to control and contain the spread of resistant strains. Furthermore, this work serves as an incentive to improve public health in the future and raise awareness about reducing antibiotic consumption.

### 4 Zusammenfassung

*Campylobacter jejuni* und *Campylobacter coli* gelten als die wichtigsten Erreger der Campylobacteriose beim Menschen, die weltweit große Auswirkungen auf die öffentliche Gesundheit hat. In Europa stellen sie mit Abstand die meisten Fälle von bakteriell verursachter Gastroenteritis dar. Besonders gefürchtet, aber dennoch von der allgemeinen Bevölkerung unterschätzt, sind die Langzeitfolgen einer Erkrankung, die in seltenen Fällen auftreten können, wie das Guillain-Barré-Syndrom, reaktive Arthritis und das Reizdarmsyndrom. Der übermäßige Einsatz von Antibiotika in der Human- und Veterinärmedizin hat zu einer zunehmenden Resistenz dieser Bakterien gegen antimikrobielle Mittel geführt, was die Behandlungsmöglichkeiten einschränkt. Entsprechend wird *Campylobacter* von der Centers for Disease Control and Prevention (CDC) insbesondere wegen seiner Fluorchinolon-Resistenz als resistentes Bakterium mit hoher Priorität und als ernsthafte Bedrohung der öffentlichen Gesundheit eingestuft.

Diese Studie hatte zum Ziel, die Antibiotikaresistenzen von *Campylobacter*-Isolaten aus unterschiedlichen Regionen der Welt zu vergleichen, um für die Humanmedizin relevante und für Europa bevorstehende Resistenzdeterminanten zu identifizieren, die sich unter hohem Selektionsdruck entwickeln und eine Herausforderung für die öffentliche Gesundheit darstellen. Aufgrund der Ergebnisse dieser vergleichenden Studie sollte ein neuartiges Warninstrument entwickelt werden, welches auf molekularer Ebene für den Einsatz in Routineüberwachungsprogrammen verwendet werden kann.

Die Ergebnisse unserer vergleichenden Studie zeigten Unterschiede in der Ausprägung verschiedener Resistenzen je nach Herkunftsort der *Campylobacter*-Isolate. So wiesen die Isolate in Georgien und Deutschland im besonderen hohe Resistenzen gegenüber Fluorchinolonen und Tetracyclinen auf. Hingegen waren

Resistenzen gegenüber Aminoglykosiden und Makroliden eher seltener zu beobachten. Die *Campylobacter*-Isolate aus Vietnam zeigten indes eine nahezu vollständige Resistenz gegen Fluorochinolone und Tetracykline und besonders die vietnamesischen *C. coli* Isolate wiesen sehr hohe Resistenz gegenüber Aminoglykosiden und Makroliden auf. Diese hohen Resistenzraten in Vietnam sind womöglich auf den umfangreichen Einsatz von Antibiotika in der Tierhaltung zurückzuführen.

Durch die Anwendung der Ganzgenomsequenzierung konnten die phänotypischen Resistenzen mit dem Vorhandensein von Resistenzdeterminanten in den einzelnen Isolaten verknüpft werden. Dies zeigte, dass distinkte *Campylobacter* Populationen unterschiedliche Resistenzmarker tragen und dass vietnamesische *Campylobacter* Isolate mehr Resistenzdeterminanten trugen.

Wir fragten uns ferner inwieweit eine Datenanalyse basierend auf Ganzgenomdaten zur Vorhersage einer Ausprägung einer phänotypischen Resistenz herangezogen werden kann. Wir stellten fest, dass zwar für einen Großteil die Vorhersage mit der phänotypischen Resistenz übereinstimmte. Jedoch konnten auch Diskrepanzen zwischen den Daten der Ganzgenomsequenzierung und den phänotypischen Resistenzprofilen aufgedeckt werden. Zu den Problemen gehörten fehlende oder ungenau annotierte AMR-Gene, Nachweisprobleme aufgrund von Mehrfachgenkopien oder -varianten sowie neuartige Mutationen, die die Genfunktionalität beeinflussten. Außerdem wurden auch unbekannte Resistenzmechanismen identifiziert, wie die Resistenz gegen Ciprofloxacin ohne gleichzeitige Nalidixinsäure-Resistenz.

Ein weiterer Resistenzmechanismus, der zu einer Aminoglykosidresistenz führt, konnte im Rahmen der Studie entschlüsselt und charakterisiert werden. Durch Anwendung der natürlichen Transformation und Analyse von Genomdaten, konnte eine Punktmutation in der 16S rRNA von *Campylobacter* ausfindig gemacht werden,

die im kausalen Zusammenhang mit der Aminoglykosidresistenz stand. Wir konnten zeigen, dass die Stabilität der Resistenz davon abhängt, wie viele der drei Kopien der in *Campylobacter* vorkommenden 16S rRNA dieser Mutation unterlaufen waren.

Durch Anwendung der Nanopore Long-Read Sequenzieruntechnik, konnten zusammen mit den Short-Read Daten für vereinzelte Isolate sogenannte Hybrid Assemblies erstellt werden. Somit konnten mehrere Isolate mit ihrem vollständig zirkulärem Chromosom und ggf. weiteren epichromosomalen Einheiten (Plasmide) dargestellt werden. Dies gab Aufschluss über die Lokalisierung und Art der Verbreitung der identifizierten Resistenzdeterminanten.

Anhand der Ganzgenomdaten war es letztendlich möglich neue Primer- und Sonden zu designen und daraufhin ein Pentaplex-Real-Time PCR System zu entwickeln. Dieses wurde während der Zeit der Studie hinreichend getestet und validiert und steht in Zukunft als kostengünstige Alternative zur Ganzgenomsequenzierung bereit um routinemäßig die wichtigsten Resistenzmarker wie Fluorochinolon-, Makrolid- und Tetrazyklin Resistenzen zu beobachten.

Schlussendlich lässt sich sagen, dass die große genetische Vielfalt und die beobachteten Resistenzen bei *Campylobacter*, insbesondere in Regionen mit starkem Antibiotikaeinsatz, die Notwendigkeit einer kontinuierlichen Überwachung zirkulierender Resistenzen im globalen Kontext unterstreichen, um die Ausbreitung resistenter Stämme zu kontrollieren und einzudämmen. Ferner stellt diese Arbeit einen Anreiz dar, zukünftig die öffentliche Gesundheit zu verbessern und das Bewusstsein im Sinne der Antibiotikaverbrauchsmengenreduzierung zu schärfen.

## 5 Introduction

### 5.1 The Genus *Campylobacter* spp.

The bacteria of the genus *Campylobacter* were probably first identified in 1886. At this time the German-Austrian pediatrician Theodor Escherich observed spiral-shaped bacteria under the microscope, but was unable to cultivate them (1). In 1913 a *Vibrio*-like organism was isolated from aborted fetuses and subsequently named “*Vibrio fetus*” (2). Fifty years later, in 1963, Sebald and Véron introduced the name “*Campylobacter*” to the genus, attributing it to the bacteria's distinctive shape and specific growth preferences, while also highlighting biological differences from *Vibrio* species (3). The name *Campylobacter* originates from the Greek words “campylo,” meaning “curved,” and “bacter,” which translates to “rod.” Yet, it wasn't until the 1970s that they were successfully isolated from stool samples of humans with acute enteritis, a significant achievement given the challenges associated with cultivating these bacteria under known conditions due to their specific growth requirements (4-6). *Campylobacter* species commonly exhibit a helical morphology, are classified as Gram-negative, and have a microaerobic metabolism (7). To date, the Genus *Campylobacter* comprises 48 species and 13 subspecies (8), of which *Campylobacter jejuni* and *Campylobacter coli* play the most important role for public health as they are the two primary agents in *Campylobacter* associated gastroenteritis (9). Nonetheless, several other species, such as *C. upsaliensis*, *C. hyointestinalis*, and *C. lari*, can also cause infections in humans (10, 11). They are highly motile due to their flagella, which are situated at the polar ends of the bacterium (12). Unlike other pathogenic bacteria they are very susceptible to various environmental conditions such as desiccation (13), osmotic stress (14), oxidative stress (15), and low pH (16).

### **5.2 Campylobacteriosis as zoonotic disease**

#### **5.2.1 Prevalence**

Thermotolerant *Campylobacter* have been recognized as the leading cause of gastroenteritis worldwide and rank among the most prevalent human enteric pathogens in both developed and developing countries (9, 17-20). In 2022, Campylobacteriosis surpassed 137,000 reported cases EU-wide, which is more than twice the reported number of *Salmonella* infections (65,208) (21). Thus, *Campylobacter* spp. remains the leading cause of bacterial gastroenteritis in the European Union. However, the true incidence is likely even higher. For instance, Havelaar and colleagues suggest it could be as much as 47 times greater than what is reported by EU member states (22). However, they also note that asymptomatic cases may not always be consistently classified, and there are differences between countries in reporting practices, which can affect the comparability of the data.

#### **5.2.2 Clinical presentation, health burden, and economic impact**

Campylobacteriosis is characterized by symptoms such as watery and/or bloody diarrhea, abdominal pain, fever, and nausea but can also only show mild symptoms (23, 24). While the illness is typically self-limiting, it can have severe outcomes. A study suggested, that individuals infected with ciprofloxacin-resistant *Campylobacter* experienced prolonged diarrhea compared to those with ciprofloxacin-susceptible *Campylobacter* infection, while the reason for this is still unclear (25). Particularly concerning are the potential long-term autoimmune sequelae, such as Guillain-Barré syndrome, reactive arthritis, and irritable bowel syndrome (26, 27). These autoimmune diseases contribute to a considerable public health burden, often underestimated by the general population. Hence, the Foodborne Disease Burden Epidemiology

Reference Group (FERG) of the WHO published an estimation of the global *Campylobacter* disease burden. In 2010, a total of 600 million cases of foodborne illnesses were attributed to thirty-one distinct hazards, with *Campylobacter* estimated to account for 96 million of these cases. Among these *Campylobacter* cases, approximately 21,000 deaths and over 2.1 million Disability Adjusted Life Years (DALYs) were recorded, primarily associated with Guillain-Barré Syndrome, as data on other long-term sequelae was limited. DALYs are a measure of overall disease burden, expressed as the total number of years lost due to illness, disability, or premature death. Thus, globally, *Campylobacter* ranked sixth among the thirty-one hazards associated with foodborne illnesses with respect to the amount of DALYs per 100,000 population (28).

In the United States, the estimated annual cost of illness is approximately 1.9 billion US dollars, with over half of this (56%; 1.1 billion US dollars) attributed to Guillain-Barré syndrome (29), although only 0.07% of acute cases develop this long-term sequelae (26). Based on data from 2017, Schorling et al. estimated the total cost of *Campylobacter* enteritis in Germany to be 95.2 million Euros. Sequelae also contributed notably to these costs, comprising approximately 30%. Here, chronic inflammatory bowel disease substantially contributed to the calculated costs (30). In addition to healthcare expenses, illness and mortality result in additional non-healthcare costs, such as productivity losses (31, 32).

### **5.2.3 Transmission routes and risk factors**

In high-income countries, the majority of *Campylobacter* spp. infections are caused by consuming undercooked contaminated animal derived food or by cross-contamination of ready-to-eat food by contaminated meat. Fresh meat from poultry, notably chicken, is considered the primary source of human infections associated with

the pathogen (33, 34). Animals colonized asymptomatically serve as carriers, shedding bacteria into the environment and becoming a potential source of infection for both uncolonized animals and susceptible humans (35-37). Transmission from living animals can occur through direct contact or indirectly via environments contaminated with feces (38). Apart from these transmission routes, *Campylobacter* infections are also commonly associated with the consumption of raw milk (21, 34, 39). According to a meta-analysis, the primary risk factor for campylobacteriosis is international travel, followed by eating undercooked chicken, environmental exposure, and direct contact with farm animals (40).

### 5.2.4 Diagnosis and treatment

The diagnosis of *Campylobacter* in stool samples can be conducted through conventional culture methods (27, 41-43) or assessed culture-independently, e.g. using enzymatic immunoassays (EIA) (44) or polymerase chain reaction (PCR) (45). Recently, culture-independent methods are replacing traditional culture techniques (46). Nevertheless, cultural evidence is important in order to enable antimicrobial resistance testing and whole-genome sequencing if necessary.

The World Health Organization (WHO) recommends oral rehydration as the first-line treatment to replace water, electrolytes, and nutrient deficiencies caused by diarrhea (47). To further support the reduction of dehydration, ancillary drugs such as antiemetics (48) or the antiperistalsis drug loperamide (49) may be utilized as adjuncts in diarrhea treatment. Since 2017, healthcare professionals in the US have been advised to use azithromycin, a macrolide, as the preferred antimicrobial for treating campylobacteriosis, and ciprofloxacin, a fluoroquinolone, as an alternative option (27). In the US, the prevalence of oral antibiotic prescriptions shows that macrolide and fluoroquinolone treatments range from 35% to 37% in patients with and without



post infection irritable bowel syndrome, respectively, with combinations of both prescribed 12% and 13% of the time (50). In a case-control study performed from 2011 to 2014 in Germany, 31% of the patients reported that they were treated with antibiotics, mostly with ciprofloxacin and erythromycin (51). Meanwhile, Schorling et al., based on data gathered in Germany over one year from a large health insurance, found that 8.7% of patients diagnosed with severe campylobacteriosis and 13.1% of those with moderate cases received antibiotics (30). A study from 2011 conducted in Georgia (Caucasus) found that 45% of Georgian health care practitioners' use antimicrobials in the case of diarrheal disease, of which 65% used antibiotics only in case of presence of blood in stool (52). In China, children that suffer from severe or prolonged campylobacteriosis receive antibiotic treatment with azithromycin or erythromycin as preferred antimicrobials (53).

### **5.3 Antimicrobial resistance in *Campylobacter* spp.**

The discovery of antibiotics in the early 20<sup>th</sup> century revolutionized the treatment of bacterial infections, allowing for the effective control and cure of various diseases that were once life-threatening, thus extending the average human lifespan by approximately 23 years since their introduction (54). Initially, antibiotic resistance was rare, but over time, the prevalence of resistant strains increased rapidly. Organisms, particularly those that developed resistance to multiple, unrelated antibiotics, had a considerable impact on human health, as antibiotic treatment was no longer effective for the first time (55). Today, antibiotic resistant bacteria are a major threat to human health globally (56). Notably, *Campylobacter* has been identified as a serious threat by the Centers for Disease Control and Prevention (CDC) (57) and is classified as a high-priority antibiotic-resistant pathogen by the WHO (58).

### 5.3.1 Antimicrobial resistance mechanisms in *Campylobacter* spp.

Phenotypic antimicrobial resistance in *Campylobacter* is driven by various molecular mechanisms such as target site modification by point mutations or modifying enzymes, enzymatic inactivation of the antimicrobial, and efflux pumps. Antimicrobial resistance may complicate the treatment of infections, particularly with commonly used antibiotics like quinolones. Understanding these resistance mechanisms is crucial for developing effective strategies to combat *Campylobacter* infections and resistance spread.

#### 5.3.1.1 Macrolide resistance

Macrolide antibiotics are a class of antibiotics that include erythromycin, azithromycin, and clarithromycin. These antibiotics bind to the large subunit of the ribosome, specifically the 50S subunit, at the peptidyl transferase center (59).

*Campylobacter* spp. can develop resistance to macrolide antibiotics by point mutations in their 23S rRNA genes. Mutations at positions 2074 or 2075, such as A2075G, A2074G, A2074C, and A2074T, are described, which can lead to high-level resistance to erythromycin when present in all three copies of the gene (60-62). There have also been isolates identified with fewer than three copies of the altered 23S rRNA gene, yet they exhibit similar minimum inhibitory concentration (MIC) values to those harboring mutations in all three copies (63). One *C. jejuni* isolate with even two different mutations in all three copies was detected by Real-time PCR using a melting curve analysis (64). The A2075G mutation is the predominant alteration found in isolates from food animals (65-67). 23S rRNA mutations associated with macrolide resistance have been shown to be transferable via natural transformation and were, apart from one exception of seven strains, stable upon subculturing in absence of selection pressure (63). The point mutation A2075G resulted in bacterial fitness loss for colonization of *C. jejuni* in the chicken host, as evidenced by pairwise *in vivo*

competition tests with mutated resistant and isogenic susceptible strains (68). Meanwhile, Zeitouni et al. found that when mono-inoculated or co-inoculated into chickens, macrolide susceptible *C. jejuni* outcompeted the macrolide resistant population. However, a spontaneous mutant that evolved in vivo showed a colonization capacity similar to the susceptible strain. In contrast, macrolide susceptible and resistant *C. coli* displayed similar levels of colonization in chickens, both in separated inoculations and during competitive assays (69).

Another target site modification mechanism is the methylation of binding sites of the macrolide antibiotic by rRNA methyltransferases. The genes encoding these enzymes were described in 1995 in *Campylobacter rectus* from patients with periodontitis (70). These adenine-specific N-methyltransferases are encoded by the *erm* gene class (erythromycin ribosome methylation). They utilize S-adenosylmethionine (SAM) to methylate a single adenine residue in the 23S rRNA gene, specifically targeting A2058 in *E. coli* numbering. This methylation results in the formation of either N6-mono- or dimethyladenine (71). *Campylobacter* spp. mainly harbors the *erm(B)* gene, which has already been identified in isolates from different continents such as Africa (72), Australia (73), Asia (67, 74, 75), Europe (76, 77) and North America (78). It was described first in a *C. coli* strain isolated from swine in China (79) and probably derived from gram-positive bacteria (80).

### 5.3.1.2 Quinolone resistance

Quinolones have been utilized since the 1960s, starting with the clinical application of nalidixic acid as the initial agent. The incorporation of a fluorine molecule at position 6 marked a major advancement in inhibiting bacterial growth, exhibiting minimum inhibitory concentrations 100 times greater compared to those observed with nalidixic acid (81). Today fluoroquinolones are frequently used in human (82, 83) and veterinary medicine (84). Fluoroquinolones (e.g. ciprofloxacin, enrofloxacin) target bacterial DNA

synthesis through inhibition of two enzymes crucial for DNA replication: DNA gyrase, formed by GyrA and GyrB, which introduces negative supercoils into DNA, and topoisomerase IV, consisting of ParC and ParE, responsible for decatenating DNA molecules and resolving DNA entanglements (85). In *Campylobacter*, however, the ParC/E appear to be absent, leaving DNA gyrase as the sole target for fluoroquinolones (86). Thus, resistance to fluoroquinolones mainly arises from amino acid substitutions within the quinolone resistance-determining region (QRDR), specifically due to mutations in the *gyrA* gene (87). The most frequent mutation associated with fluoroquinolone resistance is the T86I mutation (66, 67, 88-90). Additionally, other mutations have been identified, such as T86V (67), T86K (91), D90N (91), D90Y (92), and T86A (92), although the latter only confers low-level resistance to ciprofloxacin (2 mg/L). *In vivo* studies on the fitness cost of the T86I mutation highlighted an even increased bacterial fitness for some *C. jejuni* strains, as they were able to outcompete their susceptible counterparts (93). Similarly, observations of the growth kinetics of susceptible and resistant isogenic *C. jejuni* in competitive *in vitro* experiments indicated that fluoroquinolone-resistant strains might exhibited a small but significant growth advantage over the fluoroquinolone-susceptible strains (94). Zeitouni and Kempf found that fluoroquinolone resistance in *Campylobacter* strains incurs fitness costs *in vitro*, *in vivo*, and on food matrices (95). *In vitro* experiments revealed general fitness costs associated with fluoroquinolone resistance, while *in vivo* studies using chicken models showed that fluoroquinolone-resistant strains were outcompeted by susceptible strains during competitive colonization. Additionally, on food matrices such as chicken skin, the acquisition of fluoroquinolone resistance led to the rapid disappearance of resistant strains, indicating reduced survival in competitive scenarios.

### 5.3.1.3 Aminoglycoside resistance

Aminoglycosides are derived from the bacteria *Actinomycetes* and function by inhibiting protein synthesis (96). An ATP-dependent transport mechanism is used by aerobic bacteria to import aminoglycosides into the cell. Once inside, these agents specifically attach to the A-site decoding area of the 16S rRNA (97). Streptomycin, introduced in 1944, was the first aminoglycoside utilized as an antibiotic. Throughout the years, several additional antibiotics belonging to this class have either been isolated from bacteria (such as gentamicin, kanamycin, and tobramycin) or created through semi-synthetic methods (like amikacin) (96).

The mechanism of action of aminoglycosides relies on their chemical structure. While gentamicin, kanamycin, and tobramycin have a 2-deoxystreptamine (2-DOS) core structure in common, streptomycin exhibits a streptidine moiety, leading to different binding patterns. The 2-DOS aminoglycosides directly interact with the ribosomal RNA, specifically targeting the aminoacyl-(A) tRNA decoding site situated in the helix 44, resulting in translational misreading. In contrast, streptomycin additionally binds to helices from all four different domains and also interacts with the ribosomal protein S12, resulting in a different mode of action (98).

In *Campylobacter* spp., resistance to aminoglycosides is primarily correlated with the presence of genes encoding enzymes that alter the chemical structure of the aminoglycosides (99). These enzymes are thought to convey either acetylation of an amino group (*N*-Acetyltransferases, AAC), adenylation of a hydroxyl group (*O*-Adenyltransferases, ANT), or phosphorylation of a hydroxyl group (*O*-Phosphotransferases, APH) of the target aminoglycoside (100). Gentamicin, kanamycin, and tobramycin resistance is correlated with the presence of aminoglycoside 2"-phosphotransferase genes (*aph(2'')*) (100), of which several distinct variants have been identified in *Campylobacter* to date (101-104). Furthermore,

bifunctional enzyme encoding genes like *aac(6')-Ie/aph(2'')-Ia* and *aac(6')-Ie/aph(2'')-If* may also play a pivotal role in resistance to these substances (67, 104). Kanamycin resistance was suggested to additionally be conferred by 3'-phosphotransferase genes (*aph(3')*) such as *aph(3')-IIIa* (105) and *aph(3')-VIIa* (106). Meanwhile, streptomycin resistance in *Campylobacter* is associated with the occurrence of 6-Adenyltransferase genes (*ant(6)*), of which *ant(6')-Ia* (also named *aadE*) is the most frequently encountered gene (88, 90, 107). Additionally, the streptomycin resistance genes *ant(6')-Ib* (88) and a *C. coli* specific version of *aadE*, designated *aadE-Cc* (65, 90), have also been identified in *Campylobacter*. Besides the inactivation of streptomycin by enzymes, mutations in the ribosomal protein S12 (encoded by the *rpsL* gene) can confer resistance, with the point mutations K43R and K88R being identified in *Campylobacter* (67, 107, 108).

### 5.3.1.4 Tetracycline resistance

Discovered in the 1940s, tetracyclines, including tetracycline, doxycycline, and minocycline, hinder protein synthesis by binding to the 16S rRNA of the 30S ribosomal subunit, thereby obstructing the binding of aminoacyl-tRNA to the ribosomal acceptor site (109). Unlike what is observed with macrolides and aminoglycosides, resistance to tetracyclines is rarely mediated by mutations in ribosomal RNA or proteins, nor by methylation of specific RNA residues. Instead, it is conferred by other proteins. These proteins either i) export tetracycline across the cell membrane using an energy-dependent efflux mechanism (110, 111), ii) chemically alter the drug to deactivate it (112), or iii) mimic elongation factors to displace the bound antibiotic from the ribosome, thereby protecting the ribosome, and thus are designated ribosomal protection proteins (RPPs) (113, 114). Tetracycline resistance in *Campylobacter* is mainly conferred by the RPPs of which the later designated Tet(O) was probably the first to be described (113, 115). The RPP encoding gene *tet(O)* is the predominantly

found resistance gene conferring tetracycline resistance in *Campylobacter* spp. (66, 104, 116). Other types of RPP encoding genes have also been identified in *Campylobacter*, such as *tet(32)* (117) and *tet(W)* (67). In addition, mosaic like RPP encoding genes have been observed in *Campylobacter* spp. like *tet(O/M/O)* (118) and *tet(O/32/O)* (119). Additionally, the presence of the efflux pump coding gene *tet(L)* has recently been discovered in China (120).

### **5.3.2 Prevalence of antimicrobial resistance in *Campylobacter* spp.**

The EU summary report on antimicrobial resistance in 2021/22 showed a prevalence of 69.1% and 70.6% ciprofloxacin-resistant *C. jejuni* and *C. coli* human isolates from 22 member states, respectively. Meanwhile, resistance to erythromycin was less pronounced with 0.9% of *C. jejuni* and 7.8% of *C. coli* resistant human isolates. Similar to the higher proportion of macrolide-resistant *C. coli*, they were also more resistant to tetracyclines (71.2%) compared to their *C. jejuni* counterparts (46.6%). Gentamicin resistance was also low, with 0.5% in *C. jejuni* and 3.0% in *C. coli*. Similar prevalences in the occurrence of resistance were observed in cecal samples from broilers from 27 EU member states and the United Kingdom. The highest resistances were observed in *C. coli* from cattle under the age of 1 year, with 90.5% resistance to tetracycline, 35.7% to erythromycin, 79.7% to ciprofloxacin, and 12.4% to gentamicin (121).

Antibiotic resistances among *Campylobacter* isolates in Southeast Asia vary considerably by country. For instance, Vietnam exhibited very high resistance rates to ciprofloxacin (63 - 100%), nalidixic acid (88 - 100%), and tetracyclines (75 - 100%), along with moderate to high resistance rates to gentamicin (25 - 56%), streptomycin (63 - 100%), and erythromycin (25 %) in *C. spp* from chicken and pork (122-124). In a study conducted by Lim et al. (2017), poultry meat products from Manila (Philippines) displayed very high resistance rates to erythromycin (98.6%) and clindamycin (98.6%)

(125). Additionally, frequent resistance to ciprofloxacin and tetracyclines was observed in Thailand (81.2% and 40.6%, respectively), while here the isolates were moderately resistant to erythromycin (9.4%) (126).

During a study spanning from 2017 to 2018 in Beijing, China, elevated rates of resistance to ciprofloxacin (94.5% and 94.4%) and tetracycline (93.5% and 94.4%) were observed in human isolates of *C. jejuni* and *C. coli*, respectively. Erythromycin resistance was more prevalent among *C. coli* isolates (44.4%) compared to *C. jejuni* (9.0%), showing more than four times higher prevalence. Likewise, gentamicin resistance was present in 50.0% of *C. coli* isolates and in 13.0% of *C. jejuni* isolates. Difference in streptomycin resistance was even more pronounced in *C. coli* (72.2%) than in *C. jejuni* (9.5%) (127). Notably, *Campylobacter coli* often exhibits higher resistance levels than *C. jejuni*, as evidenced by the different studies mentioned. Additionally, *Campylobacter* spp. isolates from Asia tend to be more resistant than their European counterparts.

### **5.3.3 Surveillance of antimicrobial resistance**

Since the primary driver of the rise in antimicrobial resistance is the use of antimicrobial agents (57), several countries and regions have introduced antimicrobial resistance surveillance systems along with mitigation strategies to minimize antimicrobial use and resistance in bacteria.

#### *5.3.3.1 AMR Surveillance in the United States*

In 1996, the National Antimicrobial Resistance Monitoring System (NARMS) was established to prospectively monitor changes in antimicrobial susceptibilities of selected zoonotic enteric pathogens like *Salmonella* spp. and *Campylobacter* spp. (128, 129). As NARMS initiated monitoring of antimicrobial resistance in bacterial



isolates from humans, it wasn't long until data from this program (130) and another source (131) revealed an increase in fluoroquinolone resistance among *Campylobacter* from humans subsequent to the approvals of the fluoroquinolones sarafloxacin and enrofloxacin in poultry (129). This prompted the withdrawal of fluoroquinolones, particularly enrofloxacin, from poultry use in the United States (132). This withdrawal was the first case of an animal drug being taken off the market due to the associated emergence of resistance in humans. This led the FDA to develop an evidence-based approach for approving animal antimicrobial drugs of clinical importance to humans for use in primary production (133). Nowadays NARMS is being crucial in monitoring antimicrobial resistance in enteric bacteria in the US, aiding in identifying emerging threats across humans, animals, and food. Its data inform policies, regulatory actions, and educational efforts to reduce resistance and protect public health, continuously evolving to address changing bacterial environments and technologies (129).

### 5.3.3.2 AMR Surveillance in the European Union

Over the past decades, several EU Member States had established own surveillance programs to monitor antimicrobial resistance in bacterial isolates from animals raised for food production (134-137). However, with the introduction of Directive 2003/99/EC, a harmonized approach was established, mandating all European Member States to monitor antimicrobial resistance in zoonotic and public health-threatening agents (138). This led to the adoption of Implementing Decision 2013/652/EU (139) in 2013, which was later updated to Implementing Decision (EU) 2020/1729 (140). Within the EU-Decision, it is stated that Member States shall monitor thermotolerant *Campylobacter* spp. from different food-producing animals and the fresh meat thereof. For *Campylobacter*, this involves collecting samples, such as cecal content, from specified food-producing animals at the time of slaughter. This sampling is conducted

on a rotational basis, with the focus on poultry one year, and on bovine animals and pigs the following year. The antimicrobial susceptibility testing is performed by using the broth micro dilution method specified by the Clinical and Laboratory Standards Institute (CLSI) (141, 142). The interpretation of antimicrobial susceptibility data relies on epidemiological cut-off values (ECOFFs) regularly updated and published by the European Committee on Antimicrobial Susceptibility Testing (EUCAST), which was first formed in 1997 to also harmonize prior existing national committees (143). The findings are subsequently submitted on an annual basis to the European Commission, which in turn tasks the European Food Safety Authority (EFSA) with writing and publishing a summary report on antimicrobial resistance for the European Union.

In 2008, Germany started its own strategy to fight antimicrobial resistance named DART (Deutsche Antibiotika-Resistenzstrategie) (144). Following this, the 16th amendment of the Medicinal Products Act (16th AMG Amendment), which came into force in 2014, established regulations for implementing an antibiotic reduction strategy for livestock (145). The Working Group on Antibiotic Resistance of the Federal Office of Consumer Protection and Food Safety (BVL) and the Federal Institute for Risk Assessment (BfR) evaluated the antibiotic reduction strategy and found that the amount of veterinary antimicrobials dispensed has seen a notable decrease between 2011 and 2020, with a reduction of 58.9% (from 1,705.7 to 700.7 t) (146). Since the reduction strategy was considered successful (147), follow up programs were established such as DART 2020 (148) and the still ongoing DART 2030 (149).

### *5.3.3.3 AMR Surveillance in Asia*

Southeast Asia is considered a region that contributes to emergence of drug resistance (150). Additionally, the WHO stated in a report that Southeast Asian countries lack systematic data collection regarding antimicrobial resistance (151). Hence, in 2015, the WHO adopted a global action plan, encouraging member states to create their own

tailored national action plans (NAPs) to combat antimicrobial resistance based on the One Health approach (152). Although governments showed their willingness for accelerated development of NAPs in Southeast Asia, progress on implementation should be strengthened, with e.g. regard to accountability, equity, sustainability and transparency (153). Antimicrobial usage in food animals in Myanmar, Indonesia, and Vietnam, is projected to increase by 205%, 202%, and 157%, respectively, between 2010 and 2030 (154), due to higher demand for animal products and an increase in larger production types with greater use of antimicrobial drugs.

In 2005, China established its first two nationwide surveillance systems to monitor antimicrobial usage in clinical settings, followed by another surveillance system in 2009 that aimed to track antimicrobial usage in agriculture. China then became one of the first countries to implement its own NAP, leading to further measures to reduce antimicrobial use in both clinical and agricultural settings (155). Thus, in 2016, several fluoroquinolones, such as lomefloxacin, ofloxacin, and norfloxacin, were banned in food animals but are suspected to still be used illegally (156). In 2017, colistin was also banned as a growth promoter (157). These mitigation strategies showed effectiveness, as antibiotic prescriptions decreased from 19.4% in 2010 to 7.7% in 2017 for outpatients, and from 67.3% to 36.8% for inpatients (155). Still, China is the world's largest producer and consumer of antibiotics, with per capita antibiotic use approximately ten times higher than in the United States (158). Additionally, it accounts for the largest share of antimicrobial consumption in food animal production, representing 23% of the overall global consumption in 2010 (66).

### **5.4 Aim of the study**

Our study aimed to investigate the antibiotic resistance profiles of *Campylobacter* isolates collected from poultry samples from Germany and Vietnam and from human

and chicken samples from Georgia. Our primary objective was to gain insights into the underlying genetic mechanisms driving antimicrobial resistance in *Campylobacter* populations. This was done by correlating phenotypic resistance with the presence of genomic determinants. We aimed to identify the limitations in current predictive tools for antimicrobial resistance. For this purpose, knowledge gaps were addressed through in-depth analysis of whole-genome sequencing data combined with comprehensive phenotypic assessment. Additionally, our goal was to enhance routine resistance monitoring through the development of novel Real-time PCR assays, in order to facilitate more effective surveillance of emerging resistances within *Campylobacter* populations.

## 6 Publications

### 6.1 List of publications and own contribution

**Publication 1: Comparison of Antimicrobial Susceptibility Profiles of Thermotolerant *Campylobacter* spp. Isolated from Human and Poultry Samples in Georgia (Caucasus)**

M. Metreveli, S. Bulia, L. Tevzadze, S. Tsanova, M. Zarske, J. C. Goenaga, S. Preuß, G. Lomidze, S. Koulouris, P. Imnadze, K. Stingl

*Antibiotics (Basel)*. 2022;11(10) <https://doi.org/10.3390/antibiotics11101419>

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I contributed to NGS methodology and data analysis. I additionally reviewed and edited the manuscript.

**Publication 2: Multiplex Real-Time PCR for the Detection of Tetracycline, Ciprofloxacin, and Erythromycin Resistance Determinants from Human and Foodborne *Campylobacter jejuni* and *Campylobacter coli***

V. Zeller-Péronnet, N. Bretschneider, J. Lausch, N. Hanifi, M. Pavlovic, M. Zarske, H. Q. Luu, U. Busch, K. Stingl, I. Huber

*Microorganisms*. 2023;11(12). <https://doi.org/10.3390/microorganisms11122927>

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I took part in the gene target definition based on NGS analysis of Publication 3. Additionally, I contributed to the manuscript review and editing.

**Publication 3: Identification of knowledge gaps in whole-genome sequence analysis of multi-resistant thermotolerant *Campylobacter* spp.**

M. Zarske, H. Q. Luu, C. Deneke, M.-T. Knüver, M. Thieck, H. T. T. Hoang, N. Bretschneider, N. T. Pham, I. Huber, K. Stingl

*BMC Genomics*. 2024;25(1):156. <https://doi.org/10.1186/s12864-024-10014-w>

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I performed standardized microdilution analysis and designed the custom plate formats for microdilution, sequenced Vietnamese isolates and some of the German isolates by Illumina short-read sequencing technology and contributed all Oxford Nanopore sequences. I processed the data through the Aquamis, Bakcharak and MiLongA pipelines and, if appropriate, did further analysis via webtools or Geneious Prime software. Furthermore, I contributed most of the result interpretation. I wrote the initial draft.

**Publication 4: The point mutation A1387G in the 16S rRNA gene confers aminoglycoside resistance in *C. jejuni* and *C. coli***

M. Zarske, C. Werckenthin, J.C. Golz, K. Stingl

*Antimicrob Agents Chemother* 68:e00833-24. <https://doi.org/10.1128/aac.00833-24>

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I took part in the conceptualization of the study. Except for isolation of the original strain, I performed all the experiments and contributed the most to data analysis and result interpretation. Additionally, I wrote the initial draft.

**6.2 Publication 1: Comparison of Antimicrobial Susceptibility Profiles of Thermotolerant *Campylobacter* spp. Isolated from Human and Poultry Samples in Georgia (Caucasus)**



## Article

# Comparison of Antimicrobial Susceptibility Profiles of Thermotolerant *Campylobacter* spp. Isolated from Human and Poultry Samples in Georgia (Caucasus)

Maia Metreveli <sup>1,\*</sup>, Salome Bulia <sup>2</sup>, Liana Tevzadze <sup>3</sup>, Shota Tsanova <sup>3</sup>, Michael Zarske <sup>4</sup> , Juan Cruz Goenaga <sup>4</sup>, Sandra Preuß <sup>4</sup>, Giorgi Lomidze <sup>5</sup>, Stylianos Koulouris <sup>6</sup>, Paata Imnadze <sup>1,3</sup>  and Kerstin Stingl <sup>4,\*</sup> 

<sup>1</sup> Faculty of Medicine, Ivane Javakhishvili Tbilisi State University, 0179 Tbilisi, Georgia

<sup>2</sup> Department of Gastroenteric Infection Diseases, Tbilisi Children Infectious Diseases Clinical Hospital, 0171 Tbilisi, Georgia

<sup>3</sup> National Center for Disease Control and Public Health, 0198 Tbilisi, Georgia

<sup>4</sup> National Reference Laboratory for *Campylobacter*, Department of Biological Safety, German Federal Institute for Risk Assessment, 12277 Berlin, Germany

<sup>5</sup> Faculty of Medicine, European University, 0189 Tbilisi, Georgia

<sup>6</sup> European Commission, Directorate General for Health and Food Safety (DG-SANTE), 1049 Brussels, Belgium

\* Correspondence: maia.metreveli349@med.tsu.edu.ge (M.M.); kerstin.stingl@bfr.bund.de (K.S.);

Tel.: +995-599115591 (M.M.); +49-30-1841224206 (K.S.)



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**Abstract:** Antimicrobial resistance remains a public health concern globally. This study presents antimicrobial resistance by microdilution and genetic diversity by the whole-genome sequencing of *Campylobacter* spp. from human and poultry samples isolated in Georgia in 2020/2021. The major species in poultry samples was *C. coli*, while *C. jejuni* was preferentially isolated from human samples. Resistance against tetracycline was highest (100%) in *C. coli* from industrial chicken and lowest in *C. jejuni* from clinical isolates (36%), while resistance against ciprofloxacin varied from 80% in *C. jejuni* from backyard chicken to 100% in *C. jejuni* and *C. coli* from industrial chicken. The point mutations in *gyrA* (T86I) and *tet* (O) genes were detected as resistance determinants for (fluoro-)quinolone or tetracycline resistance, respectively. Ertapenem resistance is still enigmatic. All isolates displayed sensitivity towards erythromycin, gentamicin and chloramphenicol. Multi-resistance was more frequently observed in *C. coli* than in *C. jejuni*, irrespective of the isolation matrix, and in chicken isolates compared to human isolates, independent of the *Campylobacter* species. The Georgian strains showed high variability of multi-locus sequence types (ST), including novel STs. This study provides the first antibiotic resistance data from *Campylobacter* spp. in Georgia and addresses the need for follow-up monitoring programs.

**Keywords:** EUCAMP3; microdilution; cgMLST; backyard chicken; whole-genome sequencing; resistance determinant; campylobacteriosis; gastroenteritis; WGS

## 1. Introduction

The emergence and spread of multi-resistant bacteria continues to be a global public health concern. In the European Economic Area (EEA), it was estimated that more than 670,000 diseases were caused by antimicrobial-resistant (AMR) bacteria yearly, with about 33,000 associated deaths [1].

Campylobacteriosis is a disease caused by thermotolerant *Campylobacter* spp. It is one of the four major causes of diarrhea worldwide, and is considered to be the most common cause of bacterial food-borne human gastroenteritis [2]. *Campylobacter* species are motile, curved, microaerobic, Gram-negative rods that commonly reside in the intestinal tract of many wild and domestic warm-blooded animals.

Although campylobacteriosis is mostly self-limiting, recent reports showed that a substantial proportion (31%) of reported *Campylobacter* infections have been treated with

antibiotics [3], probably those infections with severe outcome. Concordantly, a considerable number of 21% of the reported campylobacteriosis cases resulted in hospitalization in the EU in 2020, while for comparison, salmonellosis led to 29.9% and infections by shiga-toxin-producing *E. coli* to 40.9% of hospitalization [4].

Based on the joint report of European Food Safety Authority (EFSA), European Centre for Disease Prevention and Control (ECDC), European Medicines Agency (EMA) and Organization for Economic Co-operation and Development (OECD) the overall consumption of antibiotics in humans decreased by 23% and in food-producing animals by 43% between 2011 and 2020 in the EEA [5]. Harmonized AMR key indicator bacteria, such as fully susceptible *Escherichia coli* for food-producing animals and Methicillin-resistant *Staphylococcus aureus* (MRSA) for humans varied depending on the country and the years. In the majority of the countries, the proportion of fully susceptible *E. coli* increased and MRSA decreased between 2014 and 2018, being in-line with reduced use of antibiotics [6]. However, the percentage of *E. coli* from human samples resistant against third-generation cephalosporins increased in half of the countries and decreased in the other half. Of particular concern is the increase in carbapenem resistance with, e.g., almost a quarter of EU/EEA countries reporting at least 10% carbapenem-resistant *K. pneumoniae* [1]. Carbapenems are not authorized for use in veterinary medicine in the EU [7] and in Georgia [8]. Combined resistance to both ciprofloxacin and erythromycin, which is considered critically important for treatment of campylobacteriosis, was marginal with 0.5% in *C. jejuni* and still low with 8.9% in *C. coli* in 2020. However, relatively high levels of combined resistance were reported by Finland and Portugal for *C. coli* (36.8–40.6%) [6]. In a global world, emerging resistant strains identified at one location can be spread around the world, thus, the issue requires a global systematic approach and international action [9].

AMR surveillance data from Georgia are scarce in the public health system and absent at the food production and veterinary sectors. The Central Asian and European Surveillance of Antimicrobial Resistance (CAESAR) 2019 report [10] described resistance data gathered in twelve countries of the WHO European Region including Georgia. Data from Georgia were assessed reliable with limitations of small number of samples, focus on samples from the capital and lack of harmonized AST guidelines [10]. Data on *Campylobacter* spp. were lacking.

Antimicrobial resistance monitoring in *Campylobacter* from poultry samples in Europe is performed based on the regulation 2003/99/EC, laying down the monitoring of zoonoses and zoonotic agents isolated from distinct food and animal matrices and their characterization using harmonized panels of antimicrobial substances [11]. In several countries, an increase in resistance in *C. jejuni* from broilers against tetracycline and ciprofloxacin was detected. In addition, *C. jejuni* isolates from human samples also showed increasing resistance to these antimicrobials [6].

On the way of EU integration, the regulation for monitoring of zoonoses and zoonotic agents based on the 2003/99/EC went into force in Georgia in 2020. According to this regulation monitoring of antimicrobial resistance has to be carried out at primary production level and/or at other stages of the food chain. The regulation covers zoonoses including *Campylobacter* spp.; however, implementation of the regulation is not in action yet.

Our study presents first data on genetic diversity of *Campylobacter* spp. strains from human stool and poultry samples isolated in Georgia based on whole genome sequencing analysis and identifies antimicrobial resistance patterns of *C. jejuni* and *C. coli* including their genetic determinants. The study encourages future monitoring programs for in-depth analysis of thermotolerant *Campylobacter* spp. in Georgia in order to improve food safety.

## 2. Materials and Methods

### 2.1. Sampling and Transport

In total, 160 *Campylobacter* isolates were obtained from chicken cecal samples from February 2020 until September 2021 in Georgia. The 110 so-called “backyard” chicken samples were gathered at the Digomi live animal market in Tbilisi, where poultry is sold

reared at small farms and households from all over the country and directly processed on the market slaughterhouse. Another 50 *Campylobacter* strains were isolated from samples collected at a medium-sized 'intensive-rear' poultry farm slaughterhouse, located at the eastern part of Georgia. In addition, 382 human stool samples had been previously collected from July 2020 to July 2021 at the Tbilisi Children Infectious Diseases Clinical Hospital from hospitalized children with diarrhea, from which 60 were positive for *Campylobacter* spp. [12]. Human stool samples were transported on Cary-Blair medium (Biolife Italiana srl, Milan, Italy) at cooling temperatures without microaerobic conditions and analyzed within 24 h. Chicken cecal samples were transported in plastic bags on ice and analyzed within 3–6 h after sampling.

## 2.2. Detection and Phenotypic Identification of *Campylobacter* spp.

*Campylobacter* detection was performed according to ISO 10272-1:2017 part C on modified charcoal cefoperazone deoxycholate agar (mCCDA) (Thermo Fisher Specialty Diagnostics Ltd., Hampshire, UK). For the clinical samples, *Campylobacter* Chromogenic agar *Campylobacter* (CHROMagar, France) was applied as an additional second selective medium to increase sensitivity [12]. Less than 20% of the clinical samples were also enriched with Preston broth (Biolife Italiana S.r.l., Milan, Italy) [13], but the results showed no enhanced detection [12].

Ceca were aseptically cut and the content mixed. One 1 µL loop of the cecal material was directly streaked on the mCCDA agar plate and distributed over the surface by using a fresh loop. The human stool samples were treated similarly but in addition to mCCDA a second selective plate was used in parallel. Incubation was performed at 42 °C in a microaerobic gas mixture consisting of 85% nitrogen, 10% carbon dioxide and 5% oxygen (LTD Argoni, Tbilisi, Georgia).

Suspicious colonies were sub-cultured on Columbia Blood Agar (ColbA; AES Laboratories, Bruz Cedex, France). Confirmation of colonies was initially performed applying the Biomerieux system ApiCampy (Biomerieux Inc, Marcy-l'Etoile, Lyon, France), consisting of 20 microtubes containing dehydrated substances. One half contained enzymatic tests and the other half substrates for assimilation or inhibition. In the latter, growth of bacteria is monitored. The specific pattern of growth and presence of enzymatic activity is used as read-outs for identification of bacteria. In addition, colonies were observed by microscopy after Gram-staining. All isolates were stored at –80 °C for further characterization.

## 2.3. Confirmation of *Campylobacter* Species and Differentiation by Real-Time PCR Analysis

At the National Reference Laboratory for *Campylobacter* at BfR the 220 strains, from which 160 were derived from chicken and 60 from human sources, were re-cultured on ColbA for 48 h under microaerobic atmosphere. In case no growth or some contamination was obtained, a parallel enrichment in Bolton broth (Oxoid, Thermo Fisher Scientific Inc., Waltham, MA, USA) with 5% lysed defibrillated horse blood (Oxoid, Thermo Fisher Scientific Inc., Waltham, MA, USA) was streaked on mCCDA and incubated for another 48 h. Single suspected colonies were sub-cultured on ColbA and incubated 24 h under similar conditions.

Isolates of *Campylobacter* spp. were species-differentiated by real-time PCR [14]. For this purpose, cell material of isolates was resuspended in 5% Chelex 100 resin (Bio-Rad Laboratories GmbH, Feldkirchen, Germany) and heated for 15 min at 95 °C for thermal lysis. Cell debris was centrifuged for 5 min at 14,000× g, and the supernatant containing bacterial DNA was used for PCR analysis at a volume of 2.5 µL after 1:100 dilution. Oligos and dark-quenched (DQ) probes in HPLC-grade were as follows: for *C. jejuni*, *mapA*-F, 5'-CTG GTG GTT TTG AAG CAA AGA TT-3', *mapA*-R, 5'-CAA TAC CAG TGT CTA AAG TGC GTT TAT-3' and *mapA*-probe, 5'-FAM-TTG AAT TCC AAC ATC GCT AAT GTA TAA AAG CCC TTT-3'-DQ; for *C. coli*, *ceuE*-F, 5'-AAG CTC TTA TTG TTC TAA CCA ATT CTA ACA-3', *ceuE*-R, 5'-TCA TCC ACA GCA TTG ATT CCT AA-3' and *ceuE*-probe, 5'-JOE-TTG GAC CTC AAT CTC GCT TTG GAA TCA TT-DQ; for *C. lari*, *gyrA1*-F1, 5'-GAT AAA GAT

ACG GTT GAT TTT GTA CC-3', *gyrA1*-R1, 5'-CAG CTA TAC CAC TTG ATC CAT TAA G-3', *gyrA1*-F2, 5'-GAT AAA GAT ACA GTT GAT TTT ATA CC-3', *gyrA1*-R2, 5'-TGC AAT ACC ACT TGA ACC ATT A-3' and *gyrA1*-probe, 5'<sup>Cy5</sup>-TTA TGA TGA TTC TAT GAG TGA GCC TGA TG-DQ; for the internal amplification control, IPC-ntb2-F, 5'-ACC ACA ATG CCA GAG TGA CAA C-3', IPC-ntb2-R, 5'-TAC CTG GTC TCC AGC TTT CAG TT-3' and IPC-ntb2-probe, 5'<sup>TAMRA</sup>-CAC GCG CAT GAA GTT AGG GGA CCA-DQ. Note that *gyrA1*-F2 bears one base exchange T3A relative to the original publication due to oligo optimization for the validation study [15]. Oligos at final concentrations of 300 nM (Sigma Aldrich, Steinheim, Germany), 100 nM dark-quenched probes (TIB MOLBIOL, Berlin, Germany) and 1 U of Platinum Taq DNA polymerase (Thermo Fisher Scientific Inc., Waltham, MA, USA) were used. As amplification control, 25 copies of the IPC-ntb2 plasmid [16] was added per PCR reaction.

#### 2.4. Antimicrobial Susceptibility Testing

Isolates were tested for AMR according to the prescriptions given in Commission Implementing Decision (CID) (EU) 2020/1729 (European Commission, 2020) [17]. Broth microdilution susceptibility testing was performed according to M45-A (Clinical and Laboratory Standards Institute [CLSI], 2015) [18] and VET06 (CLSI, 2017) [19] with the in-house validated modification of the use of fetal calf serum (PAN-Biotech GmbH, Aidenbach, Germany) instead of lysed horse blood in the culture medium for improved readability of *Campylobacter* growth. For this purpose, strains were subcultured on Columbia blood agar for 24 ± 2 h at 42 °C under microaerobic atmosphere (5% O<sub>2</sub>, 10% CO<sub>2</sub>, 85% N<sub>2</sub>). Cation-supplemented Mueller–Hinton broth (TREK Diagnostic Systems, United Kingdom) supplemented with 5% fetal calf serum was inoculated with 2–8 × 10<sup>5</sup> colony forming units/mL. Minimum inhibitory concentrations (MICs) were determined using the European standardized microtiter plate format EUCAMP3 (TREK Diagnostic Systems). Antimicrobials tested included chloramphenicol (CHL; 2–64 mg/L), erythromycin (ERY; 1–512 mg/L), gentamicin (GEN; 0.25–16 mg/L), ciprofloxacin (CIP; 0.12–32 mg/L), tetracycline (TET; 0.5–64 mg/L) and ertapenem (ETP; 0.12–4 mg/L). Epidemiological cut-off values (ECOFFs) were taken from the European Committee for Antimicrobial Susceptibility Testing (EUCAST; <https://mic.eucast.org/Eucast2> (accessed on 7 September 2022)) laid down in the CID 2020/1729. For *C. spp.* ECOFFs were as follows: 16 mg/L (CHL), 0.5 mg/L (CIP), 0.5 mg/L (ETP) and 2 mg/L (GEN). For ERY and TET, species-specific cut-off values were used (4 or 8 mg/L (ERY) and 1 or 2 mg/L (TET) for *C. jejuni* or *C. coli*, respectively). Incubation was performed for 44 ± 4 h at 37 °C under microaerobic atmosphere. MICs (mg/L) were semi-automatically analyzed using the Sensititre Vizion system (TREK Diagnostic Systems), which has an integrated camera and a mirror, recording a translucent picture from the microtiter plates. The MIC data were stored and exported using Sensi Vizion Software 2.0 (MCS Diagnostics BV, Swalmen, The Netherlands).

#### 2.5. NGS Methodology

Genomic DNA was extracted from *Campylobacter* strains sub-cultured overnight using the PureLink Genomic DNA Mini Kit (Thermo Fisher Scientific, Waltham MA, USA) according to the manufacturer's instructions. DNA was fluorimetrically quantified by Qubit 3.0 Fluorometer (dsDNA HS Assay Kit 0.2–100 ng; Thermo Fisher Scientific, Waltham, MA, USA). The quality of the DNA was evaluated by spectral analysis (NanoDrop Spectrophotometer, Thermo Fisher Scientific, Waltham, MA, USA). DNA libraries were prepared using the Illumina DNA Prep, (M) Tagmentation Kit according to manufacturer's instructions (Illumina Inc., San Diego, CA, USA) but with using half of the volume of all reagents. Paired-end sequencing was performed on the Illumina MiSeq System (2 × 151 cycles) using the MiSeq Reagent Kit v3 (600 cycles, Illumina Inc., San Diego, CA, USA). Trimming and de novo assembly of raw reads were carried out using the AQUAMIS pipeline v1.3.8 ([https://gitlab.com/bfr\\_bioinformatics/AQUAMIS](https://gitlab.com/bfr_bioinformatics/AQUAMIS) (accessed on 7 September 2022)). The quality of the assembled genome contigs was au-

tomatically evaluated using the teQuilR in-house pipeline. Sequences were published within the BioProject No. PRJNA844526 at the NCBI sequence read archive (SRA). Ridom Seqsphere+ v8.2.0 (Ridom, Muenster, Germany) was used to perform phylogenetic analysis on assembled genome contigs using the cgMLST scheme of 1343 gene targets previously defined [20] with 98% required identity and 98% required percentage of coverage to one of the alleles of the reference sequence NC\_002163.1.gb (*C. jejuni* NCTC 11168). At least 95% “good targets” were found for cgMLST-based analysis using the previously proposed cgMLST scheme. New MLST alleles and MLST-ST types were uploaded to PubMLST ([www.pubmlst.org](http://www.pubmlst.org)). Prediction of antimicrobial resistance determinants and plasmid markers within assembled genome contigs was performed by using the BakCharak pipeline v2.0 ([https://gitlab.com/bfr\\_bioinformatics/bakcharak](https://gitlab.com/bfr_bioinformatics/bakcharak) (accessed on 7 September 2022)). Tools in the pipeline include ABRicate v1.0.1 (<https://github.com/tseemann/abricate> (accessed on 7 September 2022)) and AMRFinderPlus v3.6.15 [21] and its associated database for antimicrobial resistance determinant, as well as Platon v1.1.0 for plasmid prediction (<https://github.com/oschwengers/platon> (accessed on 7 September 2022), [22] and plasmid blaster, a tool that performs a BLAST analysis against the NCBI RefSeq plasmid database. BLAST results were filtered with at least 20% coverage of the contig length.

### 2.6. Statistical Analyses

Isolates were categorized into susceptible and resistant, using the epidemiological cut-off values as mentioned in Section 2.4. The dependent variable was resistant vs. susceptible (reference category) to the antimicrobial in question. In addition to the individual antimicrobial, an outcome variable “2-3-fold resistance” was defined for an isolate resistant against two or three tested antimicrobials. This means that first, isolates were categorized according to their MIC and the epidemiological cut-off value (ECOFF) as sensitive or resistant towards every individual antimicrobial. Second, the number of resistances per isolate was counted and those with 2 or more resistances were defined as displaying “2-3-fold resistance”.

Multiple logistic regression with forward selection was used to establish independent predictors for tetracycline resistance (variables of matrix source (human vs. chicken (reference category)) and bacterial species (*C. coli* vs. *C. jejuni* (reference category)) were included). A Nagelkerke R Square and a non-standardized beta coefficient (B) were calculated. An odds ratio with 95% confidence interval (CI) was calculated as an exponential of the B coefficient (Exp [B]).

For all analyses, *p*-values of less than 0.05 were considered statistically significant. Statistical analyses were performed using SPSS (IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY, USA: IBM Corp).

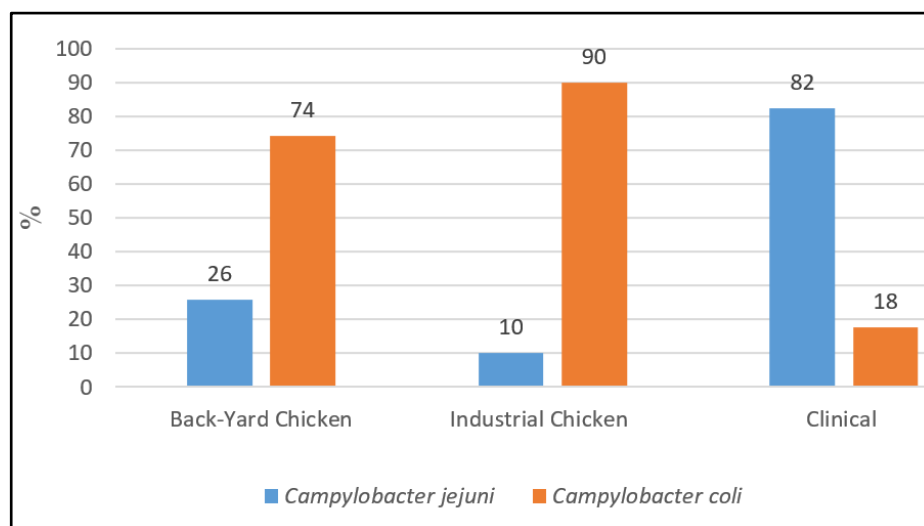
## 3. Results

### 3.1. Collection of *Campylobacter* spp. Strains and Identification of Species

*Campylobacter* spp. isolates from chicken cecal content were obtained from February 2020 until September 2021. “Backyard” chicken samples aged between several days to one year were collected from chicken reared on small farms and in households all over the country and sold at a live market in Tbilisi. In addition, *Campylobacter* strains were isolated from samples collected at a medium-sized industrial poultry slaughterhouse, located at the eastern part of Georgia and supplying Tbilisi with fresh chicken meat. Those chickens were “standardized” with an age between 38 and 42 days. In addition, human stool isolates had been previously collected from hospitalized children with diarrhea from July 2020 to July 2021 [12]. Hence, the samples correlated in time and space. From a total of 220 isolates—160 derived from chicken and 60 from human sources (Supplementary Materials Table S1)—sixteen were non-culturable after transport to BfR. However, from these sixteen non-culturable samples, *Campylobacter* spp. were still detectable by real-time PCR in twelve of the enrichment inoculums, showing either *C. coli*

(4/12) or *C. jejuni* (3/12) in seven cases and mixed cultures of *C. coli* and *C. jejuni* in five cases (41%,  $n = 5/12$ ).

Out of 204 strains re-cultured, 37.7% ( $n = 77$ ) were identified as *C. jejuni* and 62.3% ( $n = 127$ ) as *C. coli* applying real-time PCR [14]. The distribution of isolated species differed between human stool samples and cecal chicken samples (Figure 1).



**Figure 1.** *Campylobacter* species distribution (%) in poultry and human samples.

From the isolates of backyard chicken, 25.8% were identified as *C. jejuni* ( $n = 25/97$ ) and 74.2% ( $n = 72/97$ ) as *C. coli*; in cecal samples from industrial chicken, *C. coli* was even more dominant with 90% ( $n = 45/50$ ). In contrast, out of 57 clinical strains of children stool samples, 82.5% ( $n = 47/57$ ) were identified as *C. jejuni* and 17.5% ( $n = 10/57$ ) as *C. coli* (Figure 1) [12].

### 3.2. Prevalence of Antimicrobial Resistance in *Campylobacter* Isolates

All isolates were tested for their resistance to the six antimicrobials chloramphenicol, ciprofloxacin, ertapenem, erythromycin, gentamicin and tetracycline according to the European standardized EUCAMP3 plate format. Results from resistance testing are shown in Table 1. All tested strains were sensitive towards gentamicin, erythromycin and chloramphenicol. Resistance in both human and poultry isolates and in both bacterial species was highest against ciprofloxacin and tetracycline.

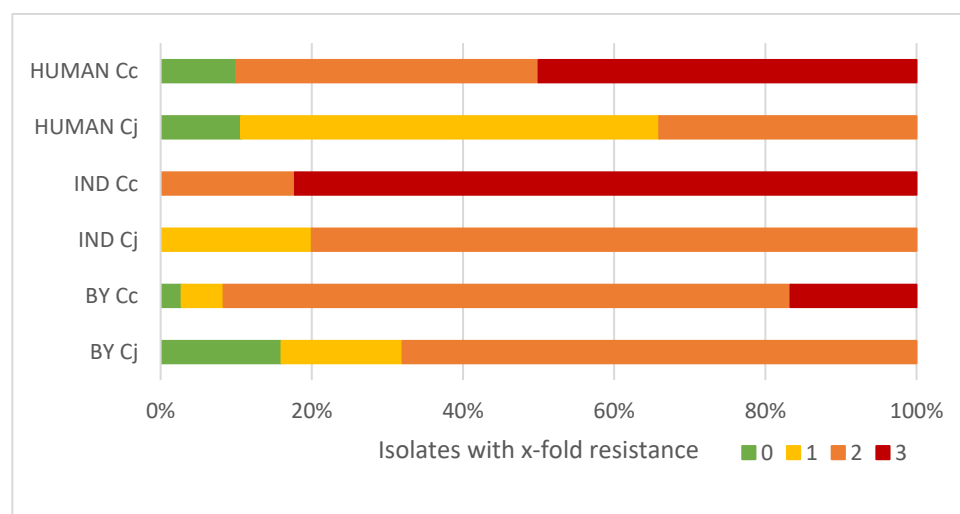
**Table 1.** Antimicrobial susceptibility of *Campylobacter jejuni* and *Campylobacter coli* strains isolated from three different sources.

| Antimicrobial   | ECOFF<br>( $\mu\text{g/mL}$ ) (R>) |     | No. (%) of Resistant Isolates    |                                |                                    |                                |                                  |                                |                                  |                                 |
|-----------------|------------------------------------|-----|----------------------------------|--------------------------------|------------------------------------|--------------------------------|----------------------------------|--------------------------------|----------------------------------|---------------------------------|
|                 |                                    |     | Backyard Chicken<br>( $n = 97$ ) |                                | Industrial Chicken<br>( $n = 50$ ) |                                | Human<br>( $n = 57$ )            |                                | Total<br>( $n = 204$ )           |                                 |
|                 |                                    |     | <i>C. jejuni</i><br>( $n = 25$ ) | <i>C. coli</i><br>( $n = 72$ ) | <i>C. jejuni</i><br>( $n = 5$ )    | <i>C. coli</i><br>( $n = 45$ ) | <i>C. jejuni</i><br>( $n = 47$ ) | <i>C. coli</i><br>( $n = 10$ ) | <i>C. jejuni</i><br>( $n = 77$ ) | <i>C. coli</i><br>( $n = 127$ ) |
| Chloramphenicol | 16                                 | 16  | 0                                | 0                              | 0                                  | 0                              | 0                                | 0                              | 0                                | 0                               |
| Ciprofloxacin   | 0.5                                | 0.5 | 20 (80%)                         | 69 (96%)                       | 5 (100%)                           | 45 (100%)                      | 41 (87%)                         | 9 (90%)                        | 66 (86%)                         | 123 (97%)                       |
| Erythromycin    | 4                                  | 8   | 0                                | 0                              | 0                                  | 0                              | 0                                | 0                              | 0                                | 0                               |
| Ertapenem       | 0.5                                | 0.5 | 0                                | 27 (37%)                       | 0                                  | 37 (82%)                       | 0                                | 6 (60%)                        | 0                                | 70                              |
| Gentamicin      | 1                                  | 1   | 0                                | 0                              | 0                                  | 0                              | 0                                | 0                              | 0                                | 0                               |
| Tetracycline    | 1                                  | 2   | 18 (72%)                         | 52 (72%)                       | 4 (80%)                            | 45 (100%)                      | 17 (36%)                         | 8 (80%)                        | 39 (51%)                         | 105 (83%)                       |

ECOFF, epidemiological cut-off for definition of resistance against antimicrobial substances (EUCAST.org); R>, maximal MIC that represents sensitivity; any MIC exceeding this concentration is defined as resistant. Note that ECOFF for erythromycin and tetracycline differs for *Campylobacter* species. n, number of tested isolates; numbers in table represent numbers of resistant isolates; in brackets, percentage of resistant isolates.

Both human and poultry *C. coli* strains showed resistance against ertapenem—37% of the strains from backyard chicken, 60% of human isolates and 82% of industrial chicken strains, while *C. jejuni* isolates were fully susceptible to this antimicrobial. Among the ertapenem-resistant *C. coli*, 89% ( $n = 62$ ) had a MIC value of 1  $\mu\text{g}/\text{mL}$ , just above the current cut-off value, 10% ( $n = 7$ ) displayed a MIC of 2  $\mu\text{g}/\text{mL}$  and a single strain had a MIC of 4  $\mu\text{g}/\text{mL}$ . From the strains with MIC values  $\geq 2$   $\mu\text{g}/\text{mL}$  ETP, three were derived from human samples, four from backyard chicken and one from industrial chicken.

Overall, isolates of *C. coli* were less frequently fully susceptible (3/127, 2.4%) than isolates of *C. jejuni* (9/77, 11.6%), with each six strains isolated from backyard poultry and human samples and lack of susceptible strains among the industrial isolates (Figure 2).



**Figure 2.** Resistance against antimicrobial classes in *Campylobacter* spp. isolates from different sources. Green, sensitive; yellow, 1-fold-resistant; orange, 2-fold-resistant; red, 3-fold-resistant. *Cj*, *C. jejuni*; *Cc*, *C. coli*; BY, backyard chicken; IND, industrial chicken; HUMAN, human isolates. Resistances against individual antimicrobials detailed in Table 1 were counted per isolate and percentage of isolates with resistances against x-fold antimicrobial classes are depicted here.

*C. coli* were more likely resistant—compared to *C. jejuni*—against ciprofloxacin (OR 5.1, 95% CI 1.6–16.7) and tetracycline (OR 4.6, 95% CI 2.5–8.8). In addition, isolates from clinical samples were less likely resistant to tetracycline compared to chicken isolates (OR 0.18, 95% CI 0.1–0.4). No statistically significant difference was observed for resistance to ciprofloxacin between human and poultry isolates.

Overall, *C. coli* was 18.5 times more likely resistant against two or more antibiotics compared to *C. jejuni* (OR 18.5, 95% CI 7.7–44.8). The same was observed in clinical isolates, where *C. coli* was 17.4 times more likely resistant to two or more antimicrobials than *C. jejuni* (OR 17.4, 95% CI 2.03–150.1); for poultry samples *C. coli* OR showed 7.9 times more probability to have resistance against two or more antibacterial agents compared to *C. jejuni* (OR 7.9, 95% CI 2.6–24.6).

There was a significant association of multi-resistance probability with isolation source in *C. jejuni* strains. In particular, the probability of resistance against two or more antimicrobials for chicken isolates of *C. jejuni* was 4.5 times higher compared to human isolates (OR 4.5, 95% CI 1.7–12.1); however, we did not find a significant association between clinical and chicken isolates for *C. coli* species, probably due to low number of *C. coli* isolates from human stool samples. Additionally, no statistically significant difference was found for the presence of two or more resistances in *C. jejuni* or in *C. coli* isolates from industrial compared to backyard chicken.

Variables of bacterial species and isolates were subjected to logistic regression analysis to test association with resistance to two or more antimicrobials as dependent variables. Both variables were retained in the final model as independent variables. The Nagelkerke

pseudo R squared was 0.435 indicating that more than 43% of the variability of dependent variables is due to the independent variables model.

Multi-variate logistic regression was performed with two variables which showed significant association with tetracycline resistance. Both variables, bacterial species and sample sources were retained into final model as independent predictors. The regression model can explain more than 20% of the variation in the dependent variable (tetracycline resistance), according to the Nagelkerke pseudo R squared of 0.204. (Table 2). In other words, the predictive model, consisting of the variables “bacterial species” and “sample sources”, can explain 20% of the variability of the dependent variable “tetracycline resistance”. Alternatively, this means, that the remaining 80% of the variability of the dependent variable could be explained with variables, that were not measured within the study and/or are not identified as a possible predictor for the outcome variable. Nagelkerkes R squared 43% for the dependent variable “2-3-fold resistance” can be interpreted in the same way.

**Table 2.** Association of full susceptibility and resistance to tetracycline and resistance against  $\geq 2$  antimicrobials of *Campylobacter* spp. with bacterial species and sample sources.

| Anti-Microbial      | Covariate                           | Coefficient of Regression | Standard Error | Wald   | Degrees of Freedom | p-Value | Odds Ratio | 95% Confidence Interval of Odds Ratio |        | Nagelkerke Pseudo R Squared |
|---------------------|-------------------------------------|---------------------------|----------------|--------|--------------------|---------|------------|---------------------------------------|--------|-----------------------------|
|                     |                                     |                           |                |        |                    |         |            | Lower                                 | Upper  |                             |
| TET                 | Chicken vs. human                   | 1.153                     | 0.403          | 8.203  | 1                  | 0.004   | 3.167      | 1.439                                 | 6.971  | 0.204                       |
|                     | <i>C. coli</i> vs. <i>C. jejuni</i> | 0.947                     | 0.391          | 5.858  | 1                  | 0.016   | 2.577      | 1.197                                 | 5.547  |                             |
| 2-3-fold resistance | Chicken vs. human                   | 1.361                     | 0.442          | 9.487  | 1                  | 0.002   | 3.901      | 1.641                                 | 9.276  | 0.435                       |
|                     | <i>C. coli</i> vs. <i>C. jejuni</i> | 2.271                     | 0.496          | 21.000 | 1                  | <0.001  | 9.693      | 3.669                                 | 25.607 |                             |

TET, tetracycline; Coding of variables: *C. coli* (1) vs. *C. jejuni* (0); poultry isolates (1) vs. human isolates (0).

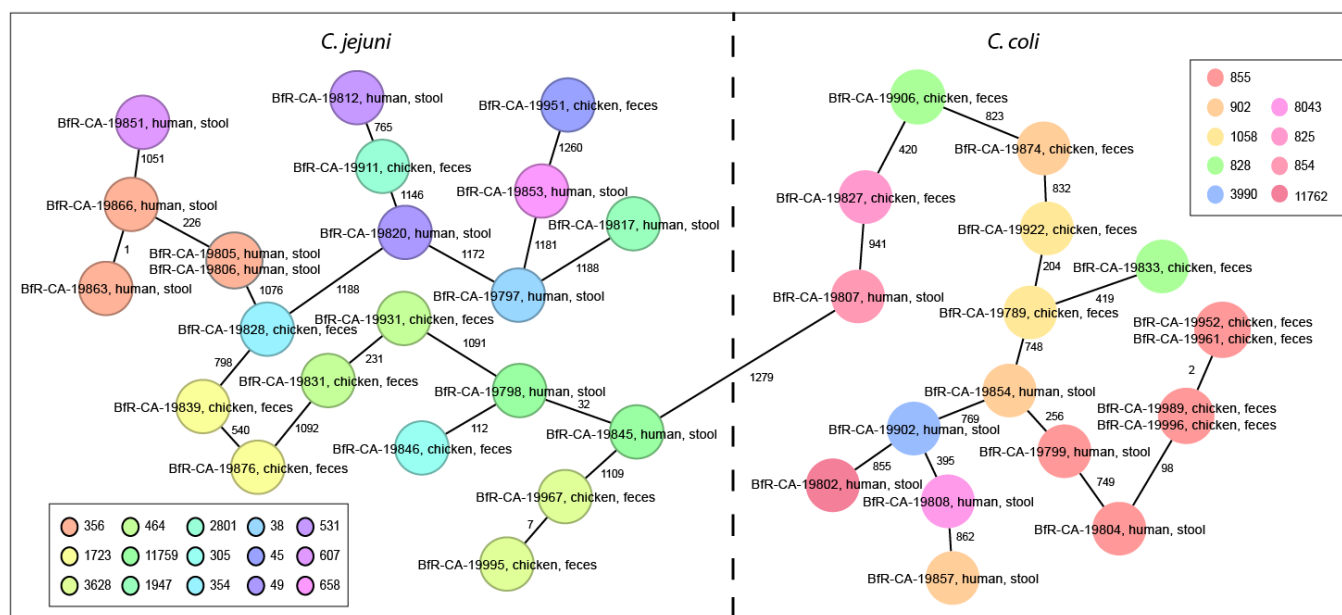
### 3.3. *Campylobacter* spp. Isolates Are Phylogenetically Diverse

We additionally analyzed forty *Campylobacter* strains by whole-genome sequencing, twenty derived from poultry and another twenty from human samples, approximately each ten *C. jejuni* and *C. coli* per matrix. The poultry isolates were both from backyard samples ( $n = 14$ ) and from industrial chicken ( $n = 6$ ). After de novo assembly of the raw reads, multi-locus sequence type analysis (MLST, based on 7 housekeeping genes) and, for more precise resolution, the core-genome MLST (cgMLST) scheme based on the comparison of 1343 gene alleles was used for phylogenetic analysis. Missing cgMLST loci were pairwise ignored.

As expected, we obtained a high variability of multi-locus sequence types (ST,  $n = 24$ ), including three strains with either unknown *uncA* allele and/or unknown ST-type. The *C. jejuni* ( $n = 22$ ) belonged to 15 different ST-types, while the *C. coli* ( $n = 18$ ) displayed 9 different ST-types (Figure 3). The most frequent ST-types were ST-855 ( $n = 6$ ), ST-356 ( $n = 4$ ), and ST-902 ( $n = 3$ ). The *C. coli* ST-types most frequently grouped within the common clonal complex ST-828 (17/18). Supplementary Materials Table S2 highlights new ST-types and their respective allelic combinations not previously reported in the PubMLST database as well as the metadata of the dataset.

Within the limited number of sequenced strains, we even found three sequence clusters. One of this clusters (ST-855) included four highly similar *C. coli* strains from industrial chicken, collected in June/July 2021 during three independent samplings, with maximal two cgMLST allele differences. Two further *C. jejuni* clusters with each two strains identified among the human isolates belonged both to ST-type 356 and were separated from each other by 226 allele difference. One of these clusters included two *C. jejuni* strains isolated from children in September and October 2021, harboring identical pairwise cgMLST. The other cluster included two *C. jejuni* strains isolated from children in July and September 2021.





**Figure 3.** Whole-genome sequences of the isolates from chicken and human samples in Georgia displayed high variability. Minimum spanning tree of cgMLST analysis was based on 1343 core genes defined previously [20]. Missing alleles were pairwise ignored. Each colored circle with (*C. jejuni*) or without frame (*C. coli*) represents an ST-type of the 7 housekeeping genes MLST scheme as depicted in the inset boxes per species. Numbers next to the connecting lines illustrate the number of allele differences analyzed by cgMLST between nearest neighbors. One new *uncA* allele and two new ST-types were found. More details, including all ST-types are shown in Supplementary Materials Table S2.

Eighteen *Campylobacter* isolates (45%) putatively carried plasmids (Supplementary Materials Table S2), since contigs of the whole genome assembly were predicted as epichromosomal elements by Platon and BLAST analysis using the NCBI RefSeq plasmid database. All plasmids had at least 20% coverage of homology to known *Campylobacter* spp. plasmids (Supplementary Materials Table S3), except for BfR-CA-19911, which harbored a small plasmid without any match in the RefSeq database.

### 3.4. Detection of Antimicrobial Resistance Genes

Whole-genome sequencing analysis also revealed several resistance genes, responsible for the observed phenotypes. The presence of the *tet(O)* gene, which mediates resistance to tetracycline, was detected in all tetracycline-resistant strains (70%,  $n = 28/40$ ). The most common mutation in the *gyrA* gene (T86I) was identified in all ciprofloxacin-resistant isolates (90% ( $n = 36/40$ )). The presence of *bla*<sub>OXA-61</sub> family genes (OXA-193, OXA-452, OXA-460, OXA-461, OXA-489, OXA-594), which confer resistance to beta-lactams, was observed in 75% ( $n = 30/37$ ) of strains. In addition, we found the *aadE-Cc* gene in three *C. coli*, putatively conferring streptomycin resistance. Streptomycin and ampicillin are not part of EUCAMP3 plate format, so the phenotype was not confirmed. The AMRFinderPlus database also annotated the mutation 50S\_L22\_A103V of the L22 ribosomal protein as a putative resistance marker for macrolide resistance in 30% ( $n = 12/37$ ) of the strains; however, all isolates were sensitive towards erythromycin. The resistance mechanism against ertapenem is still unknown. According to Platon prediction, all resistance determinants were chromosomally located.

## 4. Discussion

EU countries have made significant strides in developing and implementing national monitoring plans on antimicrobial resistance [6]; however, in Georgia, monitoring programs are still lacking.

Our study results on antibiotic resistance in Georgian *Campylobacter* spp. isolates from chicken show similarities to the AMR data profiles of *Campylobacter* spp. in EU member states. In particular, both *C. jejuni* and *C. coli* from poultry sources in the EU exhibited high resistance against (fluoro-)quinolones and tetracycline, which is in line with our data [6,23,24]. However, notably, the resistance rate to ciprofloxacin and tetracycline was 100% in isolates from industrial poultry samples in Georgia, while in backyard chicken and in human isolates *Campylobacter* strains displayed slightly lower resistance against both antimicrobials. Comparing multi-resistance in *C. jejuni* or *C. coli* in industrial versus backyard chicken, no significant difference could be found. Interestingly, all isolates were sensitive towards gentamicin, chloramphenicol and erythromycin.

Use of (fluoro-)quinolones was shown to be the major risk factor for ciprofloxacin resistance in *Campylobacter* spp. on broiler farms [25]. However, it was shown that the *gyrA* mutation, conferring resistance against (fluoro-)quinolones, can also contribute to a fitness increase in *C. jejuni* in poultry depending on the strain background [26]. The clonal spreading of (fluoro-)quinolone-resistant clones was suggested to occur in Europe [27], although the contribution of whether the resistance was selected through (fluoro-)quinolone use in individual countries and/or transmission between countries is still unclear [28]. Moreover, the differences in resistance rates between the bacterial species from the same source and, therefore, the same antimicrobial exposure indicated that antimicrobial use alone cannot explain differences in resistance profiles of *C. jejuni* and *C. coli* [29]. *C. coli* from the same matrix exhibited higher resistance than *C. jejuni* towards multiple antimicrobials tested [29]. The reason for this phenomenon is still unclear. (Fluoro-)quinolones are among WHO's "Highest Priority Critically Important Antimicrobials" (HPCIA) [30]. Increases in resistance to (fluoro-)quinolones in *Campylobacter* spp. are of concern, as resistance in *Campylobacter* from animals has been shown to be associated with resistance of *Campylobacter* from human infections [6]. When Georgian isolates were compared according to their origin, the chicken *C. coli* or *C. jejuni* isolates were each significantly more resistant towards two and three classes of antimicrobials than the human strains. This might hint to additional infection routes other than cross-contamination from preparing fresh chicken meat and/or direct contact to animals on chicken farms in Georgian children suffering from campylobacteriosis. In addition to the preparation of poultry meat and contact with poultry animals, contact with sand in a sandbox with putative contact to animal feces such as that from dogs and wild animals was also identified in a German study as risk factor positively associated with a *Campylobacter* infection for children under 5 years of age [3].

Furthermore, our study showed a high prevalence of *C. coli* in comparison to *C. jejuni* from poultry samples, which was untypical in a number of countries even in the Caucasus region [6,31–33]. However, there are other studies that identified a higher prevalence of *C. coli* than *C. jejuni* in swab samples from farms and neck skins at slaughter in Italy [34] or some alterations of species distribution depending on the stage of broiler production [35]. A long-term study over seven years showed a gradual decrease in the prevalence of *C. jejuni* and a concomitant increase in *C. coli* in cecal samples from chicken in China [36], while in Malaysia both species were frequently isolated from different broiler parts [37].

One explanation for different species distribution might be age and race of the chicken, which is not likely in our study, since we obtained a similar species distribution from backyard chicken of different age and industrial chicken with standardized rearing period of 38–42 days. Our results may additionally hint at the fact that initially, we might have isolated mixed cultures of both *C. jejuni* and *C. coli* in some cases, since PCR results of inoculums identified the presence of both species, which in turn could not be recultivated together.

All tested isolates from Georgia were sensitive towards erythromycin and gentamicin, which was similar for isolates in the EU. Erythromycin resistance in *Campylobacter* isolates from human cases of campylobacteriosis and from broilers in sixteen EU member states was either absent or detected at very low levels in *C. jejuni*, but was observed at higher levels in *C. coli* isolates. Overall, erythromycin resistance was reported in 10% (2020)

and 12.9% (2019) of human isolates and 4.4% of broiler isolates. Combined resistance to both ciprofloxacin and erythromycin, which is considered critical for the treatment of campylobacteriosis, was reported to be 8.9% (2020) and 10.4% (2019) in isolates from humans and 4.1% in broilers. In 2020, EU countries reported low prevalence of gentamicin resistance [6]. Data from *C. jejuni* and *C. coli* of human and animal origin in 2019–2020 showed very high to extremely high levels of resistance to (fluoro-)quinolones, which are also critically important antimicrobial agents (CIAs) for the treatment of *Campylobacter* infections in humans [30]. WGS of isolates, especially those with multi-drug resistance, high-level resistance to erythromycin or ciprofloxacin, or resistance to gentamicin or ertapenem, is strongly recommended in order to decipher the antimicrobial resistance determinants involved, their genetic location, and the potential for horizontal transmission [38].

## 5. Conclusions

Preventive and control activities in Georgia are still limited concerning the monitoring and antimicrobial susceptibility profiling of thermotolerant *Campylobacter* spp. Our first national study showed similar AMR patterns of thermotolerant *Campylobacter* spp. strains isolated in Georgia to those reported by the European Union. In particular, resistances against (fluoro-)quinolones and tetracycline were high and should be considered in local therapeutic protocols for severe human cases. Antimicrobial resistance and the prevalence of thermotolerant *Campylobacter* spp. in animals, food and humans need further approaches in order to gain a representative picture of concurrent strains in the Caucasian region.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/antibiotics11101419/s1>, Table S1: Complete sample list, including phenotype of antibiotic resistance; Table S2: WGS data overview and antibiotic resistance of tested samples; Table S3: WGS data-plasmid annotation.

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**6.3 Publication 2: Multiplex Real-Time PCR for the Detection of Tetracycline, Ciprofloxacin, and Erythromycin Resistance Determinants from Human and Foodborne *Campylobacter jejuni* and *Campylobacter coli***



## Article

# Multiplex Real-Time PCR for the Detection of Tetracycline, Ciprofloxacin, and Erythromycin Resistance Determinants from Human and Foodborne *Campylobacter jejuni* and *Campylobacter coli*

Véronique Zeller-Péronnet <sup>1</sup>, Nancy Bretschneider <sup>1</sup> , Johanna Lausch <sup>1</sup>, Nadera Hanifi <sup>1</sup>, Melanie Pavlovic <sup>1</sup>, Michael Zarske <sup>2</sup> , Huong Quynh Luu <sup>3</sup> , Ulrich Busch <sup>1</sup> , Kerstin Stingl <sup>2</sup> and Ingrid Huber <sup>1,\*</sup>

<sup>1</sup> Department for Food and Food Hygiene, Bavarian Health and Food Safety Authority (LGL), 85764 Oberschleissheim, Germany; veronique.zeller-peronnet@lgl.bayern.de (V.Z.-P.); nancy.bretschneider@lgl.bayern.de (N.B.); nadera.hanifi@lgl.bayern.de (N.H.); melanie.pavlovic@lgl.bayern.de (M.P.); ulrich.busch@lgl.bayern.de (U.B.)

<sup>2</sup> National Reference Laboratory for Campylobacter, Department of Biological Safety, German Federal Institute for Risk Assessment (BfR), 10589 Berlin, Germany; michael.zarske@bfr.bund.de (M.Z.); kerstin.stingl@bfr.bund.de (K.S.)

<sup>3</sup> National Institute of Veterinary Research (NIVR), Hanoi 100000, Vietnam; lqhuongvet@yahoo.com

\* Correspondence: ingrid.huber@lgl.bayern.de; Tel.: +49-9131-6808-5158



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**Abstract:** *Campylobacter jejuni* and *Campylobacter coli* are the predominant thermophilic species responsible for foodborne gastroenteritis worldwide. Elevated resistance to certain antibiotics was observed due to antimicrobial therapy in farm animals and humans, while reduced antimicrobial usage partially reduced antibiotic resistance. Monitoring the antimicrobial resistance demonstrated a substantial fraction of multi-resistant isolates, indicating the necessity of reliable tools for their detection. In this study, resistance determinants in 129 German and 21 Vietnamese isolates were selected to establish a novel multiplex real-time PCR (qPCR), facilitating the simultaneous detection of four resistance determinants. These comprised *tet*(O) gene variants associated with tetracycline resistance, point mutations GyrA\_T86I and GyrA\_T86V associated with ciprofloxacin resistance, and the *erm*(B) gene together with the point mutation A2075G in the 23S rRNA gene, associated with erythromycin resistance. Moreover, the performance of the qPCR assay was evaluated by comparing the results of qPCR to phenotypic antimicrobial resistance profiles, obtained with standardized EUCAMP3 microdilution panel, which showed 100% similarity (inclusivity and exclusivity). Variation in measurement methods, including qPCR machines and master mixes showed robustness, essential for laboratories. The assay can be used for the rapid detection of resistance determinants, and is beneficial for monitoring the spread of antibiotic resistance in *C. jejuni* and *C. coli*.

**Keywords:** food safety; *Campylobacter* spp.; food and clinical isolates; antimicrobial resistance determinants; susceptibility testing; real-time PCR assay

## 1. Introduction

*Campylobacter* is the most frequently reported foodborne bacterial pathogen in humans in the European Union [1]. Consumption of poultry meat contaminated with thermotolerant *Campylobacter* species can cause severe gastroenteritis. *C. jejuni*, followed by *C. coli*, are the predominant thermotolerant *Campylobacter* species in poultry samples and are mainly responsible for foodborne human infections [2,3]. The use of antibiotics in animal farming and for the treatment of human diseases promotes antimicrobial resistance. The relationship between antibiotic use and increasing occurrence of resistance has been frequently described [4]. In January 2022, a new Veterinary Medicinal Products Regulation (2019/06) was implemented throughout the European Union (EU) [5], which updated the rules on

the authorization and use of veterinary medicines in the EU to preserve the effectiveness of antibiotics for the future. However, due to enhanced and prolonged antimicrobial usage in high selection areas, such as Southeast Asian countries, the prevalence of antibiotic-resistant *Campylobacter* on poultry meat and the risk of multi-drug resistance islands (MDRI) are increasing [6]. It is becoming crucial to identify resistance determinants independently of time- and labor-consuming phenotypic characterization and to develop fast tools for the use in European monitoring surveys of circulating resistance determinants.

Several studies addressing the impact of antibiotic usage on the formation of resistance have already revealed multiple resistance mechanisms, such as duplicated genes, mosaic genes, gene mutations, plasmids carrying resistance determinants, and transposons, all of which contribute to the spread of antibiotic resistance [4,7,8]. For ciprofloxacin resistance, the point mutation T86I in the gyrase A subunit is the most frequent resistance determinant in *Campylobacter* spp. [9–11]. Erythromycin resistance in *Campylobacter* spp. was shown to be mainly conferred by the point mutation 23S rRNA A2075G [12,13]. However, in Asian countries [14], also sporadically in Europe (Spain) [15], and in the United States [16], the *erm(B)* gene, encoding a methyltransferase presents a second, highly transferable resistance determinant in *C. coli*. Tetracycline resistance in *Campylobacter* spp. is based on the presence of a ribosomal protection protein encoded by *tet(O)* [17] and mosaic variant genes [18,19].

Zarske et al. [20] investigated resistance determinants in German and Vietnamese thermotolerant *Campylobacter* spp. populations. Moreover, they demonstrated the presence of different resistance determinants, such as resistance genes, gene variants, and point mutations in distinct genes (*gyrA*, 23S rRNA, *rpsL*). Based on this genomic knowledge, worldwide prevalent resistance determinants were selected to develop a multiplex real-time PCR assay capable of covering resistance to tetracycline, ciprofloxacin, and erythromycin antibiotics.

In the last decade, polymerase chain reaction (PCR)-based detection systems have increasingly been applied to explore determinants of antimicrobial resistance among thermophilic *Campylobacter* spp. isolates. With combination of singleplex PCRs, the presence of two tetracycline resistance genes *tet(O)* and *tet(A)* can further be screened [21]. Laprade et al. [22] developed four conventional multiplex PCR assays that detect tetracycline resistance gene *tet(O)* in combination with virulence and toxin genes. A real-time PCR assay based on the amplification of a fragment of the 23S rRNA gene, surrounding bases 2074 and 2075, was developed to detect macrolide-associated mutations [23]. Additionally, Zhang et al. [13] identified the presence of the mutation in the 23S rRNA gene by mismatch amplification mutation assay (MAMA) PCR and DNA sequencing; for the presence of the *erm(B)* gene, a conventional PCR was applied. Zirnstein et al. [24] published a MAMA PCR assay, and Espinoza et al. [8] published a real-time PCR for the detection of the point mutation T86I in the gyrase A that is associated with resistance to ciprofloxacin.

In a further study, Nguyen et al. [25] characterized Vietnamese *Campylobacter* isolates in antibiotic susceptibility testing EUCAMP2 and identified resistance determinants, using MAMA PCRs for point mutations at positions 2074 and 2075 of the 23S rRNA gene, as well as for the screening of the point mutation T86I in the gyrase A. A specific conventional PCR was applied to detect the presence of the *tet(O)* gene.

In the current study, we developed a multiplex real-time PCR assay to simultaneously detect the presence of four resistance determinants in *C. jejuni* and *C. coli*. In these assays, the widely distributed resistance gene *tet(O)*, encoding the *Tet(O)* ribosomal protection protein [26] and the point mutations T86I and T86V within the gyrase subunit A [8,24], were retained to screen tetracycline and ciprofloxacin resistance, respectively. In order to cover erythromycin resistance, two detection systems, including the resistance gene *erm(B)*, encoding the Erm(B) ribosomal methyltransferase [13,27] as well as the point mutation A2075G in the 23S ribosomal RNA gene [13,28], were selected. The selection of these targets for multiplexing the real-time PCR assay was based on the European Union Summary Report on Antimicrobial Resistance of EFSA and ECDC (2023) [29], which indicated that combined resistance to both ciprofloxacin and erythromycin is considered critically important for the treatment of campylobacteriosis.



A test panel consisting of 129 German isolates obtained from food and human sources, as well as 21 Vietnamese isolates derived from chicken feces and exhibiting thermotolerant characteristics were phenotypically tested for resistance to six antibiotic classes and used for the validation of the novel multiplex real-time PCR.

## 2. Materials and Methods

### 2.1. *Campylobacter* Isolates and Growth Conditions

A total of 68 human isolates of *Campylobacter* (*C.*) spp. were obtained from systematical screenings performed during the 2018–2023 period in stool samples from gastroenteritis patients at LGL, department of human bacteriology, as well as private laboratories in the south of Germany. A total of 61 food isolates of *Campylobacter* spp. were isolated at LGL or provided by the German Federal Institute for Risk Assessment (BfR) during the 2019–2022 period, mostly from chicken neck skins from slaughterhouses and chicken breast from retail shops. Vietnamese isolates were previously isolated from chicken feces [20]. The classical microbiological method to detect *Campylobacter* spp. was carried out according to ISO 10272-2:2017 [30]. Briefly, 1 mL meat rinse was spread onto the surface of three selective mCCD agar (modified Charcoal-Cefoperazone-Deoxycholate Agar, Merck, Darmstadt, Germany) plates and incubated at 42 °C for 44 ± 4 h with a concentration of 10% carbon dioxide (CO<sub>2</sub>). Subsequently, all isolates were identified at the species level by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) using the MALDI Biotyper (MBT) platform (Bruker Daltonics, Bremen, Germany) according to Huber et al. [31] to ensure the identification of each isolate.

In total, 85 *C. jejuni* and 44 *C. coli* isolates from Germany were collected for this study. *C. jejuni* strain DSM 4688 (DSMZ-German Collection of Microorganisms and Cell Cultures GmbH, Braunschweig, Germany) and *C. coli* strain 2012-70-443-2 (Technical University of Denmark, Lyngby, Denmark) were used as a negative control strains for phenotypic resistance testing and the multiplex real-time PCR-assay. All *Campylobacter* isolates and strains were stored at –80 °C using the MAST Cryobank system (Mast Diagnostica GmbH, Reinfeld, Germany) and are listed in Supplementary Material Table S1.

These 129 *Campylobacter* isolates, together with 21 Vietnamese isolates, were characterized phenotypically and genotypically, and formed a test panel for the design, development, and validation of a real-time PCR assay. All isolates were phenotypically tested for resistance to six antibiotics in standardized microtiter plate format EUCAMP3. For genotypical characterization, an NGS-based approach was applied to identify different genetic determinants conferring antimicrobial resistance.

### 2.2. Antibiotic Susceptibility Testing EUCAMP3

The European standardized Sensititre™ EU Surveillance *Campylobacter* EUCAMP3 plate system (Thermo Fisher Scientific Inc., Waltham, MA, USA) was used to identify phenotypic resistance patterns of isolates from Germany and Vietnam against six antimicrobial agents: chloramphenicol, erythromycin, gentamicin, ciprofloxacin, tetracycline, and ertapenem. According to the European Union Summary Report on Antimicrobial Resistance of EFSA and ECDC [29], these antimicrobials have been reported to be mandatory for *C. jejuni* and *C. coli* as representatives of six different antibiotic classes of phenicols, macrolides, aminoglycosides, (fluoro)-quinolones, tetracyclines, and carbapenem, respectively.

Isolates stored at –80 °C were grown on Columbia agar (ColbA), supplemented with 5% sheep blood (Oxoid, Thermo Fisher Scientific Inc.) for 24 h with a concentration of 10% CO<sub>2</sub> at 42 °C and subcultured once for additional 20 ± 2 h before antibiotic susceptibility testing. Isolates were inoculated at a bacterial concentration between 2 × 10<sup>5</sup> and 8 × 10<sup>5</sup> CFU/mL in cation-supplemented Mueller–Hinton broth (Thermo Fisher Scientific Inc.) with 5% fetal bovine serum (PAN-Biotech, Aidenbach, Germany) (CAMHB/FBS). A volume of 100 µL inoculated CAMHB/FBS (5 × 10<sup>5</sup> CFU/mL) was added to each well

of EUCAMP3 format plates, and the plates were incubated at 37 °C for 44 ± 4 h with a concentration of 10% carbon dioxide (CO<sub>2</sub>).

Minimal inhibitory concentrations (MICs; in mg/L) were determined using the semi-automatically Sensititre™ Vizion™ system (Thermo Fisher Scientific Inc.) and the Sensivizion V2.0 software (MCS Diagnostics BV, Swalmen, The Netherlands). Epidemiological cut-off values (ECOFFs, Table 1) for resistance determination were based on the European Committee on Antimicrobial Susceptibility Testing [32–34].

**Table 1.** Epidemiological cut-off values (ECOFFs) for evaluation of antibiotic susceptibility testing results of thermotolerant *Campylobacter* spp. from Germany.

| Antimicrobial   | MIC [mg/L]<br>Resistant > <i>C. jejuni</i> | MIC [mg/L]<br>Resistant > <i>C. coli</i> | Reference                            |
|-----------------|--|--|--------------------------------------|
| ciprofloxacin   | 0.5  | 0.5                                      | ECOFFs for <i>C. spp.</i><br>[32–34] |
| tetracycline    | 1  | 2  |                                      |
| ertapenem       | 0.5  | 0.5                                      |                                      |
| erythromycin    | 4  | 8  |                                      |
| chloramphenicol | 16   | 16                                       |                                      |
| gentamicin      | 2  | 2  |                                      |

MIC, minimum inhibitory concentration.

### 2.3. DNA Extraction and Quantification

*Campylobacter* isolates were subcultured on ColbA or Tryptone Soy Agar with Sheep Blood (Thermo Fisher Scientific Inc., Waltham, MA, USA) (TSASB) for 20 ± 2 h with a concentration of 10% CO<sub>2</sub> at 42 °C. Bacteria were resuspended from agar plates in 200 µL phosphate-buffered saline buffer with pH of 6.7–6.9 (Sigma Aldrich 79383-250ML, Merck, Darmstadt, Germany) (1 × PBS) and harvested by centrifugation at 14,000 × g for 5 min. The cell pellet was either directly used for DNA extraction or stored at −20 °C. For DNA extraction, the PureLink Genomic DNA Mini Kit (Thermo Fisher Scientific Inc.) was used according to the manufacturer’s instructions, using the Gram-negative bacteria genomic DNA purification protocol. Elution buffer EB (Qiagen 19086-250ML, Hilden, Germany) was used for DNA elution.

DNA concentration was quantified using a Qubit Fluorometer and the Qubit dsDNA BR Assay Kit (Thermo Fisher Scientific Inc.) according to the manufacturer’s instructions. DNA concentration was adjusted for real-time PCR analysis to 10 pg/µL with sonicated salmon sperm DNA (10 ng/µL) (Agilent Technologies, Santa Clara, CA, USA) used as background DNA.

### 2.4. Next-Generation Sequencing (NGS) and Assembly

For short-read sequencing, DNA libraries with an average insert size of about 400 bp were generated using the NEB (New England Biolabs GmbH, Frankfurt am Main, Germany) Ultra II DNA Library Prep Kit according to the manufacturer’s instructions and sequenced on the Illumina MiSeq benchtop sequencer using the MiSeq reagent kit v2 (2 × 150 bp, Illumina, Inc., San Diego, CA, USA). Paired-end reads were processed using the AQUAMIS pipeline v1.3.7 [35], which comprised quality control, trimming, and de novo assembly using Shovill. All assemblies fulfilled the quality criteria of Q30 for at least 75% and minimum coverage of 30×. The 21 Vietnamese isolates were sequenced and assembled at BfR as described in [20].

### 2.5. Design of Primers and Probes

Hundred *Campylobacter* isolates available at LGL (HS\_1 to FS\_100) and a worldwide collection of *Campylobacter* isolates from NCBI were used to design the oligonucleotides. Primers and probes were designed with the help of the NCBI Primer Blast Tool. An

additional 29 German isolates from service laboratories in southern Germany and BfR (HS\_101 to FS\_129), as well as 21 Vietnamese isolates (VE\_01 to VE\_21), were applied for validation of the designed oligonucleotides.

Prevalent resistance determinants in *Campylobacter* isolates were retained to develop a pentaplex real-time PCR (multiplex real-time PCR with detection systems in 5 channels), allowing simultaneous detection of resistance genes and point mutations associated with tetracycline, ciprofloxacin, and erythromycin resistance. The IPC-ntb2 gene fragment from *Nicotiana tabacum* was used as internal amplification control (IAC, [36]) and extracted from *E. coli* DSM 116329 (DSMZ-German Collection of Microorganisms and Cell Cultures GmbH, Braunschweig, Germany). It was applied to detect PCR inhibition and to confirm negative results. Simultaneously, a triplex real-time PCR assay (3 fluorescence channels for *tet*(O), *GyrA*\_T86I/V, and IAC) combined with a duplex real-time PCR assay (2 fluorescence channels for *erm*(B) and 23S *rRNA*\_A2075G) were validated in case of limited optical modules available in the real-time PCR instruments in user laboratories.

#### 2.6. In Silico Screening for Primer Binding Sites and Gene Alignments

In silico primer screening [37] for the selection of designed primers and probes was performed to evaluate the specificity of the real-time PCR assay.

Assembly sequences were screened for primer and probe sequences using fastaRegex-Finder [38].

NCBI reference sequences of resistance determinants (*gyrA* *C. coli*: GeneID: 66544015 *gyrA* *C. jejuni*: GeneID: 905319, *tet*(O) *C. jejuni*: GeneID: M18896.2) were blasted against a custom BlastDB based on all assembly sequences to identify and extract corresponding sequences from the assemblies. These were aligned using muscle 5.1 [39] and visualized with Aliview 1.2.6. [40].

#### 2.7. Multiplex Real Time PCR Assay for Detection of Resistance Determinants

The real-time PCR assays were validated with QuantiNova Multiplex PCR master mix (Qiagen, Hilden, Germany) for the pentaplex assay in two different probe dye combinations, either FAM-ROX-Cy5-HEX-ATTO425 on AriaMx instrument (Agilent Technologies) or FAM-ROX-Cy5-HEX-Cy5.5 on Quantstudio5 (Thermo Fisher Scientific) and on CFX96 Touch System (Bio-Rad, Hercules, CA, USA). QuantiNova Multiplex PCR Kit was also appropriate for the combination of the triplex and duplex assays.

A total of 50 copies of the IPC-ntb2 plasmid [36] were added as IAC in the pentaplex and triplex assays. The reaction mix was filled with PCR-grade water to 20 µL. A volume of 5 µL DNA with a concentration of 10 pg/µL was added to the reaction mix. The protocols for all three reaction mix variations are given in Supplementary Material Tables S3–S6. Two Vietnamese isolates *C. coli* BfR-CA-15062 (VE\_01, *tet*(O/M/O), *GyrA*\_T86I, *erm*(B)) and *C. jejuni* BfR-CA-16092 (VE\_14, *tet*(O/M/O) + *tet*(O)<sub>x</sub>, *GyrA*\_T86I, 23S *rRNA*\_A2075G) were used as positive control strains for the real-time PCR-assays.

The primer and the probe concentrations were optimized on the AriaMx instrument to achieve an optimal fluorescence signal for all primer–probe detection systems. The optimal annealing temperature of 60 °C was determined via a gradient PCR experiment on Quantstudio5 in which an annealing temperature gradient between 58 °C and 62 °C was applied. No significant differences were detected in real-time PCR results between 58 °C and 62 °C, but the fluorescence of the amplification curves was optimal for all detection systems at an annealing temperature of 60 °C. Amplification conditions with QuantiNova Multiplex PCR Kit on all three PCR instruments consisted of enzyme activation at 95 °C for 2 min followed by 40 cycles of 95 °C for 10 s, 60 °C for 20 s, and 72 °C for 20 s.

For the detection systems *tet*(O), *erm*(B), and IAC, labeled double-quenched probes were used, as they reduce background signals and crosstalk between the different channels of the real-time PCR instruments in multiplex PCR.

## 2.8. In-House Validation of the Pentaplex Real-Time PCR Assay

### 2.8.1. Selectivity

The applicability of the pentaplex real-time PCR assay for detecting the resistance determinants was checked on all 129 DNAs of German *Campylobacter* isolates (HS\_101 to FS\_129) as well as on all 21 DNAs of Vietnamese isolates (VE\_01 to VE\_21) supplied by BfR.

### 2.8.2. Determination of Efficiency and LOD<sub>95%</sub>

To access the efficiency and the limit of detection (LOD<sub>95%</sub>) of the detection systems for resistance determinants on AriaMx equipment (Agilent Technologies, Santa Clara, CA, USA), serial dilution of two *Campylobacter* isolates were applied to cover both erythromycin resistance determinants (*erm*(B) gene and the point mutation 23S *rRNA*\_A2075G), as well as both GyrA\_T86I detection systems (*C. jejuni* and *C. coli*). *C. coli* isolate VE\_01 with *erm*(B) gene and *C. jejuni* isolate VE\_14 with 23S *rRNA*\_A2075G were selected.

The DNA copy number was adjusted to 5000 copies/ $\mu$ L DNA in ddPCR [41] based on an absolute quantification of DNA copy number. All DNAs were diluted to five dilution levels (5000, 1000, 500, 100, and 50 copies/ $\mu$ L DNA). Each dilution level was measured in three technical replicates to evaluate the efficiency of the pentaplex real-time PCR. The percentage of efficiency and the coefficient of determination  $R^2$  were calculated.

To determine the lowest copy number still detectable with a 95% confidence interval (LOD<sub>95%</sub>) a serial dilution of the target DNAs was prepared at 8 low copy number levels (20, 10, 4, 2, 1, 0.4, 0.2, and 0.02 copies/ $\mu$ L) and each dilution level was measured in 12 independent technical replicates. The probability of detection (POD curve) and LOD<sub>95%</sub> was computed via a web service provided by QuoData (QuoData Web Service [42]) according to BVL guidelines [43,44].

### 2.8.3. Robustness

The robustness of the real-time PCR assay was tested on two different real-time PCR machines from two additional manufacturers (Quantstudio5, Thermo Fisher Scientific, and CFX96 Touch System, Bio-Rad). The HiDi<sup>®</sup> Taq DNA Polymerase and 10 $\times$  buffer (MyPols Biotec, Konstanz, Germany) were used to check the suitability of a single components master mix in the real-time PCR assay. A 25  $\mu$ L PCR reaction mix contained 1  $\times$  HiDi<sup>®</sup> buffer, 2 IU per reaction of HiDi<sup>®</sup> Taq DNA Polymerase, 1.5 mM MgCl<sub>2</sub> (Thermo Fisher), 200  $\mu$ M of each deoxynucleoside triphosphate (Takara Bio Inc., Kusatsu, Japan) and 5  $\mu$ L of the sample DNA. The amplification conditions with HiDi<sup>®</sup> Taq Polymerase were enzyme activation at 95 °C for 2 min followed by 40 cycles of 95 °C for 15 s, 60 °C for 30 s, and 72 °C for 30 s.

The combination of both factors (master mix/PCR equipment) was tested to detect potential effects on the real-time PCR performance. For this purpose, the efficiency was calculated using standard curves, as described in 2.8.2, with two *Campylobacter* isolates at five dilution levels (5000, 1000, 500, 100, and 50 copies/ $\mu$ L DNA).

## 3. Results

For the development of a multiplex real-time PCR assay, a test panel consisting of 129 *Campylobacter* isolates from Germany and 21 isolates from Vietnam was genotypically and phenotypically characterized for antimicrobial resistance. The correct assignment of phenotypic results (see Section 3.1) to genotypic results was verified in silico (see Section 3.3) and validated in the real-time PCR assay (see Sections 3.4 and 3.5).

### 3.1. Antimicrobial Resistance Profiles

All 129 *Campylobacter* isolates from Germany were categorized into sensitive and resistant strains using the epidemiological cut-off values, which were based on the European Committee on Antimicrobial Susceptibility Testing and the European Food Safety Authority ([32–34], Table 1). The results of resistance profiles for all 129 isolates from Germany upon susceptibility testing against the six antimicrobials of the European-wide harmonized

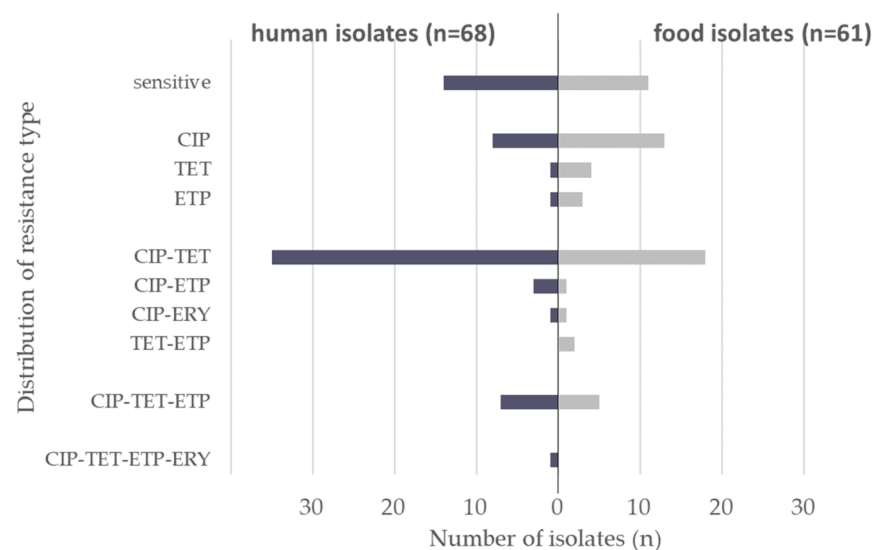
EUCAMP3 plate format are outlined in Supplementary Material Table S1 and summarized in Table 2. The two strains *C. jejuni* strain DSM 4688 and *C. coli* strain 2012-70-443-2 served as complete sensitive controls.

**Table 2.** Prevalence of phenotypic resistance of human and food isolates from Germany in EU-CAMP3 panel.

| Antibiotic      | Percentage of German Isolates Resistant to Antimicrobials Tested (%) |                            |                                      |                              |                            |                                      |
|-----------------|--|----------------------------|--------------------------------------|------------------------------|----------------------------|--------------------------------------|
|                 | Human Isolates (n = 68)  |                            |                                      | Food Isolates (n = 61)       |                            |                                      |
|                 | <i>C. jejuni</i><br>(n = 44)   | <i>C. coli</i><br>(n = 24) | <i>C. jejuni</i> +<br><i>C. coli</i> | <i>C. jejuni</i><br>(n = 41) | <i>C. coli</i><br>(n = 20) | <i>C. jejuni</i> +<br><i>C. coli</i> |
| ciprofloxacin   | 81.8   | 79.2                       | 80.9                                 | 68.3                         | 50.0                       | 62.3                                 |
| tetracycline    | 65.9   | 62.5                       | 64.7                                 | 43.9                         | 55.0                       | 47.5                                 |
| ertapenem       | 6.8  | 37.5                       | 17.6                                 | 7.3                          | 40.0                       | 18.0                                 |
| erythromycin    | 0.0  | 8.3                        | 2.9                                  | 0.0                          | 5.0                        | 1.6                                  |
| chloramphenicol | 0.0  | 0.0                        | 0.0                                  | 0.0                          | 0.0                        | 0.0                                  |
| gentamicin      | 0.0  | 0.0                        | 0.0                                  | 0.0                          | 0.0                        | 0.0                                  |

Resistance to gentamicin and chloramphenicol was not observed in German isolates. Food and human isolates were both predominantly resistant to ciprofloxacin (62.3 to 80.9%), followed by tetracycline (47.5 to 64.7%) and finally to ertapenem (17.6 to 18.0%). Resistance to erythromycin was observed always in combination with resistance to ciprofloxacin at a low level (1.6 to 2.9%) and only in *C. coli* isolates.

The distribution of combined resistance (1-fold to 4-fold) is displayed in Figure 1. In total, 23% of the German isolates showed resistance to a single antibiotic ( $n = 10$  for humans,  $n = 20$  for food). Overall, 47% of the isolates were resistant to two antibiotics in different combinations ( $n = 39$  for humans,  $n = 22$  for food). Finally, 9% of the isolates showed resistance to three antimicrobial agents ( $n = 7$  for humans,  $n = 5$  for food) and one human isolate showed resistance to the four antimicrobial agents ciprofloxacin, tetracycline, ertapenem, and erythromycin. The occurrence of combined resistance to ciprofloxacin and tetracycline is very frequent. Among 129 German isolates, 11 human isolates as well as 14 food isolates displayed no resistance to any of the six antibiotics tested in the EUCAMP3 panel.



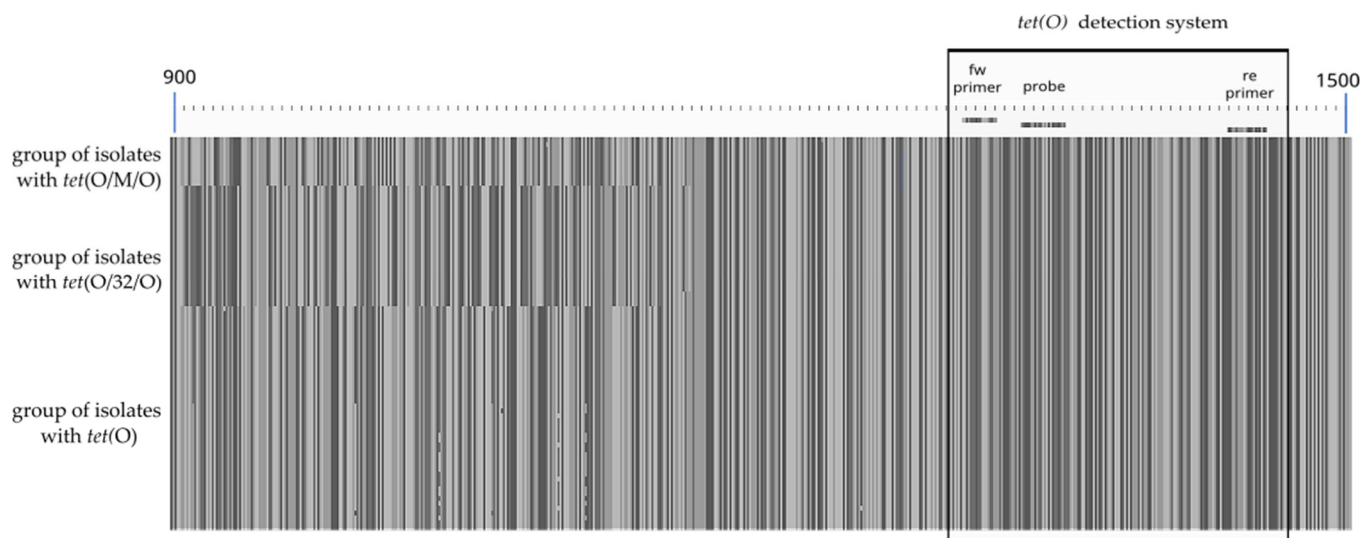
**Figure 1.** Distribution of resistance type (1-fold to 4-fold in EUCAMP3) for human and food isolates from Germany. HS, human isolates; FS, food isolates; CIP, ciprofloxacin; TET, tetracycline; ETP, ertapenem; ERY, erythromycin.

### 3.2. Design of Primer and Probes for Real-Time PCR Assays

The pentaplex real-time PCR assay included four detection systems combined with the IAC. Four resistance determinants were detected simultaneously: suitable fragments of the resistance genes *tet(O)* and *erm(B)* as well as the point mutations GyrA\_T86I/V and A2075G in the 23S rRNA gene.

For the point mutation GyrA\_T86I, ATT in *C. coli* and ATA in *C. jejuni* codes for isoleucine, whereas in wild-type ACT in *C. coli* and ACA in *C. jejuni* codes the threonine. In point mutant A2075G in the 23S rRNA gene, contrary to the wild type, the base A is substituted with G. In all cases, labeled single-quenched probes with 4 LNA (Locked Nucleic Acid) bases [45–47] were used to stabilize hybridization and increase thermal stability. Additionally, unlabeled LNA probes with the wild-type nucleotide sequence were added, in order to improve specificity of the detection of *gyrA* and 23S rRNA gene-resistant mutants and to suppress the unspecific binding of the labeled LNA probes to wild-type sequences. The base sequences in *gyrA* for the point mutation T86I showed considerable differences between *C. coli* and *C. jejuni*; therefore, two different primer–probe sets were needed to screen ciprofloxacin resistance in both species simultaneously. Sequences and final concentrations of primers and probes (IDT, Coralville, IA, USA and metabion, Planegg, Germany) for pentaplex real-time PCR are listed in Table 3.

Tetracycline resistance can be established by the presence of *tet(O)* and/or mosaic variants *tet(O/M/O)*, and *tet(O/32/O)* [18,19]. The designed detection system for *tet(O)* delimited an area, similarly for all gene variants (Figure 2). The alignment of the sequenced tetracycline resistant *Campylobacter* isolates in Figure 2 shows the binding sites to *tet(O)* primers and probe, independently of *tet(O)* variants.



**Figure 2.** Detection system for *tet(O)* and *tet(O)* mosaic variants (Aliview 1.2.6.). From the top, 3 groups of isolates, with mosaic variant *tet(O/M/O)*, with mosaic variant *tet(O/32/O)* and with gene *tet(O)*. Figure 2 shows a segment of the alignment from nucleotides 900 to 1500. The detection system for *tet(O)* covers the nucleotides between 1304 and 1460. The complete sequence alignment extends over 1920 nucleotides.

**Table 3.** Oligonucleotides for pentaplex assay: *tet*(O); GyrA\_T86I; *erm*(B); 23S *rRNA*\_A2075G, IAC.

| Antimicrobial Resistance and Target                            | Primer/Probe Name | Oligonucleotide Sequence 5' → 3'  | Amplicon Size [bp]   | Final Concentration in qPCR [nM] | Reference             |
|--|-------------------|---|--|----------------------------------|-----------------------|
| Tetracycline<br><i>tet</i> (O) <sup>1</sup>                    | tet(O)-fw         | AAGTCCC GCCAAATCT   | 157<br>(Acc. No. NG_048257.1)                              | 150 nM                           | the current study     |
|  | tet(O)-re         | TGCTCGCAGCCATAAAGAA<br><b>6-FAM</b> <sup>6</sup> —  |  | 150 nM                           |                       |
|  | tet(O)-probe      | TCGGGTTGT*CCATAGAGCCG<br>—IABkFQ <sup>12</sup>  |  | 100 nM                           |                       |
| Ciprofloxacin for<br><i>C.jejuni</i><br>GyrA_T86I <sup>2</sup> | gyrA_Cj_fw        | GTATAGTGGGTGCTGTTAT   | 118<br>(Acc. No. wt AB104527.1, pm CP053659.1)             | 400 nM                           | the current study [8] |
|  | gyrA_Cj_re        | CCTTGCTCTGTAATACTTG   |  | 400 nM                           |                       |
|  | gyrA_Cj_wt        | CCACATGGAGAT+A+C+A+GCAGTTTATG<br><b>ROX</b> <sup>7</sup> —  |  | 600 nM                           |                       |
|  | gyrA_Cj_pm        | CCACATGGAGAT+A+T+A+GCAGTTTATG<br>—BHQ2 <sup>13</sup>  |  | 200 nM                           | the current study     |
| Ciprofloxacin for<br><i>C.coli</i><br>GyrA_T86I <sup>2</sup>   | gyrA_Cc_fw        | GTATAGTAGGGGATGTTATCG   | 118<br>(Acc. No. wt CP092026.1, pm CP091310.1, CP082881.1) | 400 nM                           | the current study     |
|  | gyrA_Cc_re        | CCTTGTCATCGATACTTG  |  | 400 nM                           |                       |
|  | gyrA_Cc_wt        | CCACATGGYGAT+A+C+T+GCTGTTTACG <sup>17</sup><br><b>ROX</b> <sup>7</sup> —  |  | 600 nM                           |                       |
|  | gyrA_Cc_pm        | CCACATGGYGAT+A+T+T+GCTGTTTACG<br>—BHQ2 <sup>13,17</sup>   |  | 200 nM                           |                       |
| Erythromycin<br><i>erm</i> (B) <sup>3</sup>                    | erm(B)-fw         | AGGGTTGCTCTTGCACACTC  | 125<br>(Acc. No. MF134831.1)                               | 400 nM                           | the current study     |
|  | erm(B)-re         | GAACATCTGTGGIATGGCGG  |  | 400 nM                           |                       |
|  | erm(B)-probe      | Cy5 <sup>8</sup> —AGCTGCCAG*CGGAATGCTTTCA<br>—IAbRQSp <sup>14</sup>   |  | 200 nM                           |                       |
| Erythromycin<br>23S <i>rRNA</i> _A2075G <sup>4</sup>           | 23S_A2075G_fw     | GTGGAGGTGAAAATTCTC  | 113<br>(Acc. No. wt CP020776, pm GU384931.1)               | 400 nM                           | the current study     |
|  | 23S_A2075G_re     | CAAAGCCTCCACCTATC   |  | 400 nM                           |                       |
|  | 23S_A2075G_wt     | CAAGACGG+A+A+A+GACCCCGTG<br><b>HEX</b> <sup>9</sup> —   |  | 600 nM                           |                       |
|  | 23S_A2075G_pm     | CAAGACGG+A+G+A+GACCCCGTG<br>—BHQ1 <sup>15</sup>   |  | 200 nM                           |                       |
| Internal PCR control<br>(target gene ntb2 <sup>5</sup> )       | IPC-ntb2-fw       | ACCACAATGCCAGAGTGACAAC  | 125  | 300 nM                           | [36]                  |
|  | IPC-ntb2-re       | TACCTGGTCTCCAGCTTTCAGTT<br><b>AriaMx: ATTO425</b> <sup>10</sup> —   |  | 300 nM                           |                       |
|  | IPC-ntb2 probe    | CACGCGCAT*GAAGTTAGGGGACCA<br>—IABkFQ <sup>12</sup><br><b>QuantStudio5 and CFX96: Cy5.5</b> <sup>11</sup> —<br>CACGCGCAT*GAAGTTAGGGGACCA<br>—NFQ-2 <sup>16</sup> |  | 150 nM                           |                       |

<sup>1</sup> Resistance gene *tet*(O); <sup>2</sup> point mutation in GyrA; <sup>3</sup> resistance gene *erm*(B); <sup>4</sup> point mutation in the 23S rRNA gene; <sup>5</sup> methyltransferase gene of *Nicotiana tabacum*; <sup>6</sup> FAM, 6-carboxyfluorescein; <sup>7</sup> ROX, carboxy-X-rhodamine; <sup>8</sup> Cy5, cyanine dye; <sup>9</sup> HEX, hexachlorofluorescein; <sup>10</sup> ATTO425, tetrazine dye; <sup>11</sup> Cy5.5, cyanine dye; <sup>12</sup> IABkFQ, Iowa Black® FQ quencher; <sup>13</sup> BHQ2, Black Hole Quencher; <sup>14</sup> IAbRQSp, Iowa Black® RQ quencher; <sup>15</sup> BHQ1, Black Hole Quencher; <sup>16</sup> NFQ-2, Non-Fluorescent quencher; <sup>17</sup> Y (C/T), degenerated nucleotide; +A, +G, +C, +T, base notation for Locked Nucleic Acid (LNA) bases; \* = ZEN™ or TAO or abNFQ-2 (internal quencher for FAM and ATTO425 or Cy5 or Cy5.5 respectively); point mutation in labeled probes (pm) and wild type in unlabeled probes (wt) are underlined.

### 3.3. In Silico Screening in Comparison to Phenotypic Results

Binding sites were screened in silico for the designed primers and probes for the test panel to assess their ability to detect resistance genes and point mutations. Scanning the generated assemblies revealed the presence of binding sites in 94 isolates to *tet*(O), 10 isolates to *erm*(B), 12 isolates to A2075G point mutation in the 23S rRNA gene and 114 isolates (47 *C. coli*, 67 *C. jejuni*) to the GyrA\_T86I mutation. The results of the binding site screening for the designed primer sets correlated with the results of the phenotypic resistance screening in EUCAMP3 (Table 4). The presence of primer and probe binding sites are summarized in Supplementary Material Table S2. For the two sensitive control strains *C. jejuni* strain DSM 4688 and *C. coli* strain 2012-70-443-2, no primer binding to the four designed resistance detection systems was predicted. For some assemblies based on short-read sequence data, the in silico screening predicted more than one copy of the *tet*(O)

gene. This could be confirmed only with long-read sequencing, as shown in [20]. Since thermotolerant *Campylobacter* spp. harbor three copies of the ribosomal RNA operon, 23S rRNA A2075G was occasionally detected as multiple copies in some of the assemblies [48].

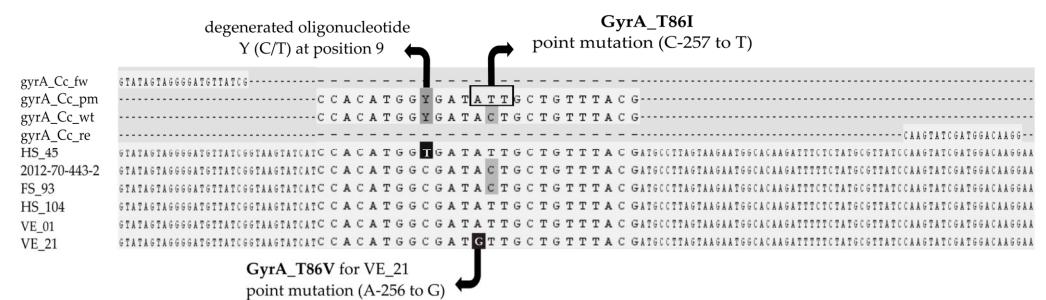
**Table 4.** Correlation between phenotypic resistance results and primer binding sites (theoretical genotypic results).

|            |       | Tetracycline  |    | Ciprofloxacin    |    |                               |        | Erythromycin                  |        |                     |    |               |
|------------|-------|---------------|----|------------------|----|-------------------------------|--------|-------------------------------|--------|---------------------|----|---------------|
|            |       | <i>tet(O)</i> |    | <i>gyrA_T86I</i> |    | <i>gyrA_T86I</i><br><i>Cc</i> |        | <i>gyrA_T86I</i><br><i>Cj</i> |        | 23S rRNA_<br>A2075G |    | <i>erm(B)</i> |
|            |       | S             | R  | S                | R  | S                             | R      | S                             | R      | S                   | R  |               |
| DE (food)  | pheno | 32            | 29 | 23               | 38 | 10                            | 10     | 13                            | 28     | 60                  | 1  |               |
|            | geno  | 32            | 29 | 23               | 38 | 10                            | 10     | 13                            | 27 +1* | 60                  | 1  | 0             |
| DE (human) | pheno | 24            | 44 | 13               | 55 | 5                             | 19     | 8                             | 36     | 66                  | 2  |               |
|            | geno  | 24            | 44 | 13               | 55 | 5                             | 19**   | 8                             | 36     | 66                  | 2  | 0             |
| VN (food)  | pheno | 0             | 21 | 0                | 21 | 0                             | 18     | 0                             | 3      | 2                   | 19 |               |
|            | geno  | 0             | 21 | 0                | 21 | 0                             | 17 +1* | 0                             | 3      | 2                   | 9  | 10            |

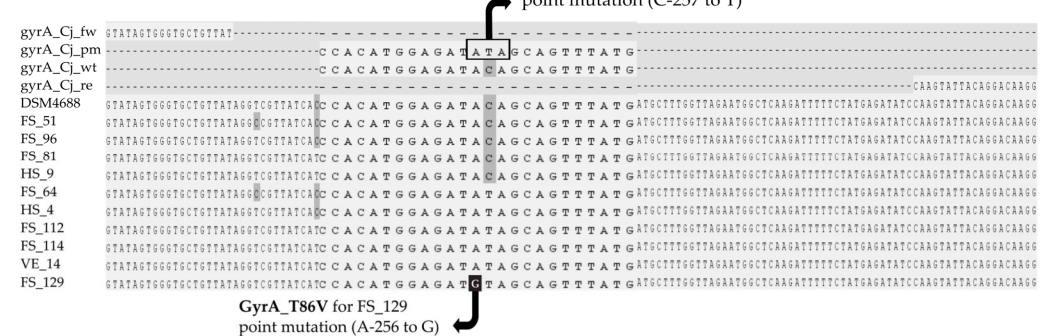
German (DE) and Vietnamese (VN) isolates from food and human origin; pheno, phenotypic result; geno, genotypic in silico result, S, sensitive; R, resistance; +1\* no 100% concordance for two isolates with additional point mutation in *gyrA* (*C. coli* VE\_21 and *C. jejuni* FS\_129 with mutation GyrA\_T86V for valine instead of GyrA\_T86I for isoleucine); \*\* 18 *C. coli* isolates with binding site to probe *gyrA\_T86I\_Cc* pm1 (C) and one isolate with binding site to probe *gyrA\_T86I\_Cc* pm2 (T).

The alignment of *Campylobacter* isolates in Figure 3 shows the binding sites to GyrA\_T86I primers and probe for *C. coli* (a) and *C. jejuni* (b). A degenerated base Y (mixture of C and T) was integrated at position 9 of the probes for *gyrA\_T86I\_Cc*, to account for approximately 6% of *C. coli* isolates available in the NCBI nucleotide database (accession on 10 August 2022, determined by using NCBI Primer Blast Tool) that contain the base T instead of base C. The in silico screening was performed with corresponding alternative bases C (pm1) and T (pm2). In our study, 18 *C. coli* isolates showed base C and one human isolate showed base T (“\*\*” in Table 4).

(a) *C. coli*: GyrA\_T86I and GyrA\_T86V



(b) *C. jejuni*: GyrA\_T86I and GyrA\_T86V



**Figure 3.** Primer binding sites for GyrA\_T86I and GyrA\_T86V of *C. coli* and *C. jejuni* (Aliview 1.2.6.); HS, human isolates; FS, food isolates; VE, Vietnamese food isolates; Cj, *C. jejuni*; Cc, *C. coli*.



Ciprofloxacin-resistant food isolates FS\_129 (*C. jejuni*, Germany) and VE\_21 (*C. coli*, Vietnam) harbored an alternative mutation compared to the common ciprofloxacin resistant isolates resulting in GyrA\_T86V for valine instead of GyrA\_T86I for isoleucine. *C. coli* VE\_21 showed the base triplet GTT (valine) instead of ATT (isoleucine) in *gyrA*. Likewise, *C. jejuni* FS\_129 showed the base triplet GTA (valine) instead of ATA (isoleucine). The designed LNA probes did not account for this additional mutation (A-256 to G) (“+1\*” in Table 4).

#### 3.4. Multiplex Real-Time PCR Assay

The pentaplex real-time PCR assay was developed to detect simultaneously four resistance determinants, including the suitable fragments of the resistance genes *tet*(O) and *erm*(B) as well as the point mutations GyrA\_T86I and A2075G in the 23S rRNA gene. The sequences and final concentrations of primers and probes (IDT and metabion) are listed in Table 3 as well as in Supplementary Material Tables S3 and S4. The designed detection system for *tet*(O) detected all isolates with tetracycline resistance, independently of the *tet*(O) variants (see also Figure 2). For the detection of the resistance to ciprofloxacin in *C. coli* isolates, the designed probes (*gyrA\_T86I\_Cc*) included a degenerated base Y (mixture of C and T) at position 9. As predicted in the in silico screening, all *C. coli* isolates with ciprofloxacin resistance (18 isolates with base C and one human isolate HS\_45 with base T) were detected (see also Table 4, “\*\*\*”).

In addition to the pentaplex real-time PCR assay, a triplex real-time PCR assay combined with a duplex real-time PCR assay consisting of the same primer and probe sequences but labeled with different fluorophores for detection were tested to allow usage of the system in case of limited optical modules in real-time PCR instruments (Supplementary Material Tables S5 and S6). The triplex real-time PCR method included two detection systems—resistance gene *tet*(O) in FAM channel (ZEN<sup>TM</sup>: internal quencher, IABkFQ: Iowa Black<sup>®</sup> FQ quencher) and point mutation GyrA\_T86I in ROX channel (BHQ1: Black Hole Quencher)—combined with IAC in HEX channel (ZEN<sup>TM</sup>: internal quencher, IABkFQ: Iowa Black<sup>®</sup> FQ quencher). The duplex real-time PCR method covered the two resistance determinants for erythromycin resistance: resistance gene *erm*(B) in FAM channel (ZEN<sup>TM</sup>: internal quencher, IABkFQ: Iowa Black<sup>®</sup> FQ quencher) and the point mutation 23S rRNA\_A2075G in HEX channel (BHQ1: Black Hole Quencher).

For evaluation of the real-time PCR assays, the threshold was set at about 10% of the maximum fluorescence of the positive control *C. coli* BfR-CA-15062 (VE\_01) and *C. jejuni* BfR-CA-16092 (VE\_14) for the four detection systems for resistance determinants and at 10% of the maximum fluorescence of the NTC (No Template Control) for the IAC. The triplex and duplex real-time PCR assays showed exactly the same PCR results as the pentaplex assay.

#### 3.5. In-House Validation of the Multiplex Real-Time PCR Assay

##### 3.5.1. Specificity and Selectivity

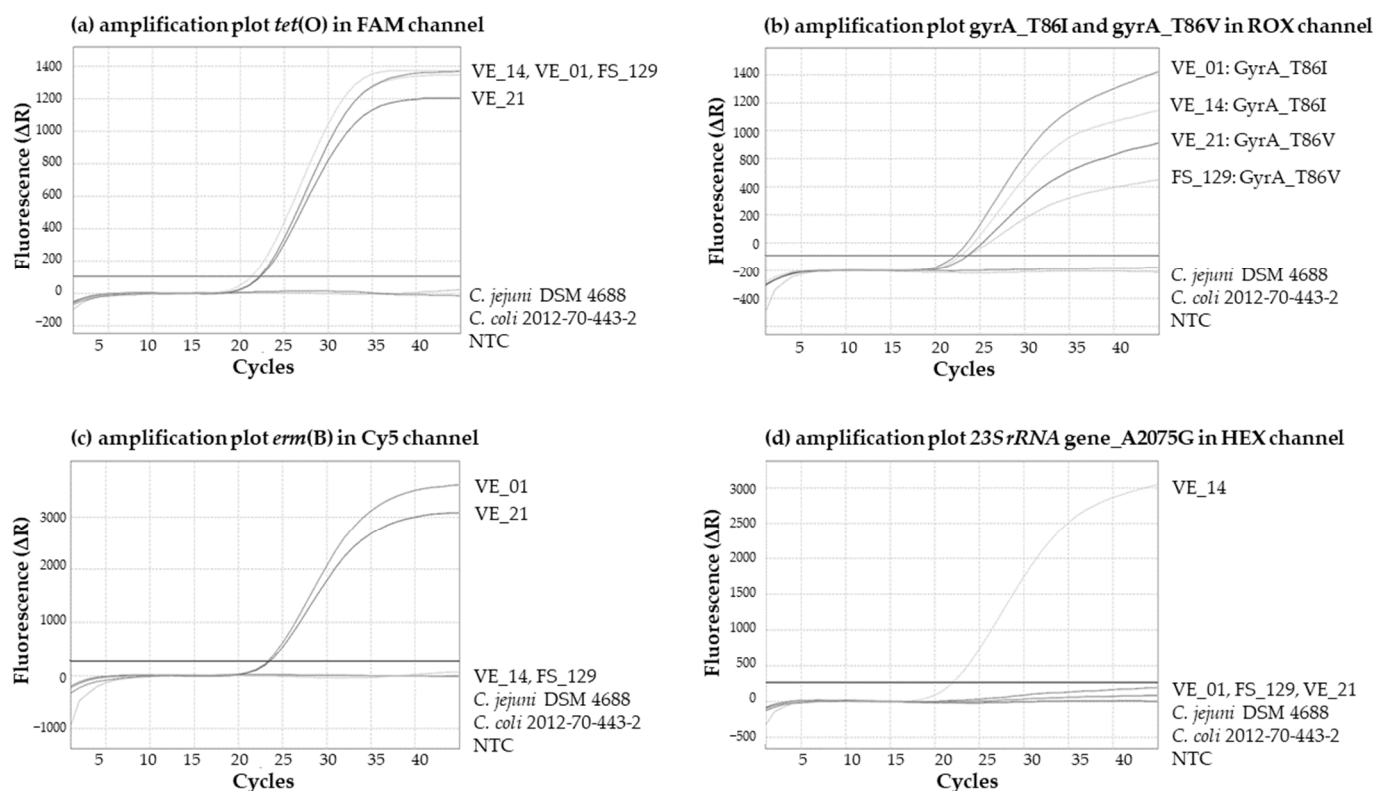
The performance of the pentaplex real-time PCR assay in detecting resistance determinants was tested on all 129 *Campylobacter* isolates from Germany and on 21 *Campylobacter* isolates from Vietnam. The target specificity and selectivity of the real-time PCR were assessed by studying the inclusivity and exclusivity for all four resistance determinants. Isolates, for which antimicrobial resistance was phenotypically determined in the EUCAMP3 panel and resistance determinants were predicted genotypically via sequence analysis (NGS), were also positive for these determinants in the real-time PCR assays, showing 100% inclusivity. The cycle of quantification (C<sub>q</sub>-values) for positive signals detected via real-time PCR on AriaMx equipment is presented in Table 5. Furthermore, isolates with phenotypic susceptibility and absence of resistance-determinants, predicted via NGS, were also negative in the real-time PCR assays, showing 100% exclusivity. No false-positive or false-negative signals were detected.

**Table 5.** Summary of pentaplex real-time PCR results.

| Antimicrobial Resistance       | Resistance Determinant/Gene | Channel | Prevalence of Positive Signals in PCR | Mean Cq-Value ± Standard Deviation | Range of Cq-Values |
|--------------------------------|-----------------------------|---------|---------------------------------------|------------------------------------|--------------------|
| tetracycline                   | <i>tet</i> (O)              | FAM     | <i>n</i> = 94                         | 23.36 ± 1.23                       | 21.06–26.45        |
| ciprofloxacin <i>C. coli</i>   | GyrA_T86I <i>Cc</i>         | ROX     | <i>n</i> = 47                         | 23.87 ± 1.26                       | 22.12–26.92        |
| ciprofloxacin <i>C. jejuni</i> | GyrA_T86I <i>Cj</i>         | ROX     | <i>n</i> = 67                         | 24.05 ± 0.97                       | 21.94–26.14        |
| erythromycin                   | 23S <i>rRNA</i> _A2075G     | HEX     | <i>n</i> = 12                         | 22.48 ± 0.75                       | 21.54–23.92        |
| erythromycin                   | <i>erm</i> (B)              | Cy5     | <i>n</i> = 10                         | 24.10 ± 1.11                       | 23.24–26.62        |
| IAC                            | <i>ntb2</i>                 | ATTO425 | <i>n</i> = 150                        | 31.56 ± 0.46                       | 30.67–33.10        |

Cq, cycle of quantification.

Amplification plots of the pentaplex real-time PCR-assay using AriaMx are presented in Figure 4. The two sensitive control strains *C. jejuni* strain DSM 4688 and *C. coli* strain 2012-70-443-2 were negative for the four detection systems for resistance determinants (Figure 4a–d). The positive control strains VE\_01 and VE\_14 were positive for the resistance determinants *tet*(O) and GyrA\_T86I (Figure 4a,b). In addition, Figures 4c and 4d highlight the difference in the erythromycin resistance determinant, *erm*(B) gene for VE\_01, and the point mutation 23S *rRNA*\_A2075G for VE\_14 respectively.



**Figure 4.** Amplification plots on AriaMx instrument for four resistant determinants. Real-time PCR detection of (a) *tet*(O); (b) both GyrA\_T86I and GyrA\_T86V mutation; (c) *erm*(B); (d) 23S *rRNA*\_A2075G mutation. Test strains harbored the following resistance determinants: BfR-CA-15062 (VE\_01), *tet*(O)-*ermB*-GyrA\_T86I; BfR-CA-16092 (VE\_14), *tet*(O)-GyrA\_T86I-23S *rRNA*\_A2075G; FS\_129, *tet*(O)-GyrA\_T86V; VE\_21, *tet*(O)-*ermB*-GyrA\_T86V; DSM 4688 and 2012-70-443-2 served as negative controls for the four tested resistance determinants.

Two isolates FS\_129 (*C. jejuni*, Germany) and VE\_21 (*C. coli*, Vietnam) with the alternative mutation GyrA\_T86V (see also Section 3.3 and “+1\*” in Table 4) were detected in the ROX channel intended for the resistance determinant GyrA\_T86I (Figure 4b). The base G (instead of A, Figure 3) did not interfere with the detection of the resistance-conferring mutation (GyrA\_T86V).

### 3.5.2. Determination of Efficiency and LOD<sub>95%</sub>

The efficiency of the pentaplex real-time PCR assay was investigated on five DNA concentrations (5000, 1000, 500, 100, and 50 copies/μL DNA) for two isolates. The linear regression analysis was performed, using AriaMx software Version 2.0. With a coefficient of determination  $R^2 \geq 0.98$ , the efficiency was 100% with less than  $\pm 20\%$  deviation from theoretical value. The designed primer–probe systems met the quality criteria of the BVL guidelines [43,44], as well as the Guidelines for validation of qualitative real-time PCR methods [49]. The results of efficiency tests are presented in Supplementary Material Table S7.

The LOD<sub>95%</sub> for the four detection systems for resistance determinants was investigated by measuring 12 independent DNA replicates at eight low-copy-number levels (20, 10, 4, 2, 1, 0.4, 0.2, and 0.02 copies/μL) for two isolates. The LOD<sub>95%</sub>, the 95% confidence interval, and the mean probability of detection (POD) curve with respect to the corresponding 95% confidence range were computed via a web service provided by QuoData (QuoData Web Service [42]). It was observed that the limit of detection for *tet*(O), GyrA\_T86I for *C. coli* and 23S *rRNA*\_A2075G is slightly lower (LOD<sub>95%</sub>  $\leq 5$  copies/μL) compared to *erm*(B) and GyrA\_T86I for *C. jejuni* (LOD<sub>95%</sub>  $\leq 10$  copies/μL) (Table 6).

**Table 6.** Results of LOD<sub>95%</sub>.

|                                    | BfR-CA-16092 (VE_14, <i>C. jejuni</i> ) |                         | BfR-CA-15062 (VE_01, <i>C. coli</i> ) |                         |
|------------------------------------|---|-------------------------|---------------------------------------|-------------------------|
|                                    | LOD <sub>95%</sub>                      | 95% Confidence Interval | LOD <sub>95%</sub>                    | 95% Confidence Interval |
| <i>tet</i> (O)                     | 1.460 cp/μL                             | [0.961, 2.219]          | 2.533 cp/μL                           | [2.028, 5.229]          |
| <i>gyrA</i> _T86I <i>C. jejuni</i> | 6.115 cp/μL                             | [4.134, 9.093]          |                                       |                         |
| <i>gyrA</i> _T86I <i>C. coli</i>   |   |                         | 1.696 cp/μL                           | [1.119, 2.565]          |
| 23S <i>rRNA</i> _A2075G            | 1.214 cp/μL                             | [0.928, 2.265]          |                                       |                         |
| <i>erm</i> (B)                     |   |                         | 5.835 cp/μL                           | [3.938, 8.663]          |

### 3.5.3. Robustness

The robustness of the pentaplex real-time PCR was evaluated by performing efficiency tests for the combination of two parameters, the real-time PCR equipment, and the master mix. Quantstudio5 and CFX96 Touch System as well as HiDi<sup>®</sup> Taq DNA Polymerase master mix gave the same results as the ones obtained using the AriaMx real-time PCR equipment and QuantiNova Multiplex PCR master mix. The detection systems for the four resistance determinants met the quality criteria with an efficiency between 80 and 120% and a coefficient of determination of  $R^2 \geq 0.98$  for all tested combinations. The real-time PCR assay was not influenced by the changes in the tested measurement conditions. The results of efficiency tests for the robustness are presented in Supplementary Material Table S7.

## 4. Discussion

*C. jejuni* and *C. coli* are the predominant *Campylobacter* species in poultry, causing a substantial impact on public health care and leading to most foodborne zoonotic diseases in humans. The prescription of antibiotics can be necessary to treat infections. Yet, the development of antimicrobial resistance (AMR) poses a steadily increasing problem by limiting the number of effective antibiotics. The European Centre for Disease Prevention and Control (ECDC) and the World Health Organization (WHO) have underlined the

threat of antimicrobial resistance to patient safety and the need for global surveillance and concerted action throughout the European Region [50].

As van Belkum [51] presented in 2019, growth-based phenotypic analysis enables reliable antimicrobial susceptibility testing (AST) and ensures appropriate antibiotic therapy for infected patients. In our study, the EUCAMP3 microdilution panel was used for a reliable quantitative determination of the minimal inhibitory concentration (MIC) against relevant antimicrobials in food and human isolates of *C. jejuni* and *C. coli*. Among 129 German isolates, less than 20% were wholly susceptible to the six antibiotics tested. The most widespread antimicrobial resistance was against the fluoroquinolone ciprofloxacin. A high frequency of resistance to ciprofloxacin was also highlighted in the report of 2023 by the European Food Safety Authority (EFSA) and ECDC [29]. Combined resistance to ciprofloxacin and tetracycline was the most frequent resistance patterns observed in German human isolates and food isolates. In contrast, combined resistance to both ciprofloxacin and erythromycin, which is considered critically important for the treatment of campylobacteriosis [29], was not observed in *C. jejuni* and was rare in *C. coli* (8.3% for humans and 5.0% in food). The last two points were further reported in the report of EFSA and ECDC.

Comparing AMR against erythromycin, ciprofloxacin, and tetracycline in food and human isolates, no significant differences were detected in the frequency of antimicrobial resistance in our study. Moreover, due to the limited number of isolates no meaningful conclusion could be taken. This is in line with previous studies. McGill et al. [52] found similar resistance prevalence to erythromycin, ciprofloxacin, and tetracycline between food and human isolates in Ireland from 2001 to 2002. Similarly, in Estonia, Tedersoo et al. [53] found a comparable resistance to antibiotics for broiler chicken meat collected between 2018 and 2019 and human *Campylobacter* isolates. The appearance of resistant *Campylobacter* isolates in humans and animals likely reflects the wide use of antibiotics in poultry production. Yet, a few veterinary isolates (5 *C. jejuni*, 4 *C. coli*) (LGL) were investigated in EUCAMP3 and did not show a major divergence in the resistance profile compared to food and human isolates of this study. The prevalence of *Campylobacter* isolates with similar resistance profiles along the chicken food chain (high resistance rates to (fluoro-)quinolones and tetracycline and relatively low erythromycin resistance rate) was also shown in a German study from 2015 [54]. However, based on poultry data from 2014 to 2016 in Germany, Tenhagen et al. [55] demonstrated that antimicrobial resistance (AMR) and antimicrobial usage (AMU) cannot be systematically associated. Different factors, including animal species, two bacterial species (*C. jejuni* or *C. coli*), the antimicrobial agents, and the usage frequency (increase or decrease), should be further considered for a better understanding of the complex trends of the associations.

Since the early 2000s, genotypic-based methods, such as PCR assays, have been used to explore the determinants of antimicrobial resistance and are available as rapid screening methods to monitor and prevent the emergence of new bacterial antibiotic resistance. The qualitative pentaplex real-time PCR assay was developed based on the specific detection of four determinants in the current study. The elevated occurrence of resistance to ciprofloxacin and tetracycline in EUCAMP3 indicated the necessity to integrate two detection systems. First of all, the point mutation in *gyrA* led to the resistance-conferring amino acid exchange T86I in gyrase subunit A. Secondly, a detection system for the gene *tet(O)* included its mosaic variants *tet(O/M/O)* and *tet(O/32/O)*. The frequency of these two resistance determinants was consistent with a previous study by Ghielmetti et al. [56], who illustrated an increasing prevalence of resistance to quinolones and tetracycline of *C. jejuni* isolates in Switzerland between 2003 and 2020. A combined resistance to both ciprofloxacin and erythromycin, which were considered crucial antimicrobials for the treatment of campylobacteriosis [50], was rarely detected in German isolates of this study (three isolates). Yet, it was frequently observed in isolates from Asian countries [57] and in 19 Vietnamese food isolates of the current study. To cover the resistance to erythromycin, a detection system for *erm(B)* and a detection system for the A2075G substitution in the 23S rRNA gene were implemented in the real-time PCR assay. The gene *erm(B)* was exclusively

detected in Vietnamese food isolates, whereas the A2075G point mutation in the 23S rRNA gene was detected in isolates from Germany and Vietnam. The pentaplex real-time PCR was successfully applied to DNA from all isolates of the test panel. The results of the complete test panel in the pentaplex real-time PCR correlated with the phenotypic results assessed in the EUCAMP3 panel and with genotypic results predicted by NGS data.

Due to the simultaneous detection of four resistance determinants in *C. jejuni* and *C. coli* within a single PCR reaction, the here-developed real-time PCR has an advantage over previously described singleplex conventional PCR systems [13,21,23,24]. Compared to previously described multiplex real-time PCRs [8,22], this PCR is adapted to the current prevalence of antibiotic resistance in human and food isolates from Germany. The pentaplex real-time PCR shows a limitation regarding the point mutations A2074C/G/T in the 23S rRNA gene, which is also associated with erythromycin resistance [12,13,23]. These point mutations could not be tested via the test panel. It can only be proven with appropriate isolates if the pentaplex real-time PCR detects these point mutations. If necessary, a new detection system should be integrated. Furthermore, a real-time PCR for ertapenem might be beneficial, as many German isolates show resistance against this antimicrobial agent (see Table 2). Yet, ertapenem is firstly not included in the priority panel for *Campylobacter* monitoring of human isolates at the EU level [29], and secondly, it exceeds the capability of the real-time PCR machine in the detection of more than five channels.

The developed multiplex PCR assay in this study improved the accuracy of analysis of antibiotics resistance in *Campylobacter*. However, challenges might exist, particularly when applied to the simultaneous detection of point mutations. All four detection systems were optimized for the same annealing temperature and showed similar PCR amplification efficiencies on different PCR machines. Therefore, the accurate detection of each target was not influenced by the other detection systems. These requirements ensured reproducible C<sub>q</sub>-values between 21 and 26 on a fixed amount of DNA. Setting the threshold at around 10% of maximum fluorescence guaranteed comparable results for tested isolates. The developed pentaplex real-time PCR in this study, showed to be robust enough to be transferred to other real-time PCR machines combined with a different master mix. In addition, the developed method was a reliable, sensitive, and easily introducible screening method for the detection of AMR related to ciprofloxacin, tetracycline, and erythromycin resistance on isolates of *Campylobacter jejuni* and *coli*.

Our development can be implemented as a warning tool in routine analysis to detect the spreading of antibiotic resistance. A decisive advantage of real-time PCR assays is that the method can further be developed to detect new incoming resistance determinants. Finally, the real-time PCR assay as rapid qualitative screening tool in combination with EUCAMP as a phenotypic tool for quantifying resistance can be considered as excellent complementary methods.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/microorganisms11122927/s1>. Table S1: Sample overview, including phenotypic resistance determined with EUCAMP3 and resistance determinants relevant for real-time PCR assay. Table S2: Primer binding sites. Table S3: Protocols for pentaplex real-time PCR assay on AriaMx. Table S4: Protocols for pentaplex real-time PCR assay on CFX96 Touch System and Quantstudio5. Table S5: Protocols for triplex real-time PCR assay. Table S6: Protocols for duplex real-time PCR assay. Table S7: Results of efficiency and robustness tests for pentaplex real-time PCR assay.

**Author Contributions:** Conceptualization, M.P., I.H., and K.S.; methodology, V.Z.-P., J.L., and N.H.; software, N.B.; validation, V.Z.-P.; formal analysis, N.B.; investigation, V.Z.-P. and H.Q.L.; resources, U.B. and I.H.; data curation, V.Z.-P., N.B., and H.Q.L.; writing—original draft preparation, V.Z.-P.; writing—review and editing, V.Z.-P., N.B., M.P., I.H., M.Z., and K.S.; visualization, V.Z.-P.; supervision, I.H.; project administration, I.H.; funding acquisition, K.S., H.Q.L., and I.H. All authors have read and agreed to the published version of the manuscript.

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**6.4 Publication 3: Identification of knowledge gaps in whole-genome sequence analysis of multi-resistant thermotolerant *Campylobacter* spp.**

RESEARCH

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# Identification of knowledge gaps in whole-genome sequence analysis of multi-resistant thermotolerant *Campylobacter* spp.

Michael Zarske<sup>1</sup>, Huong Quynh Luu<sup>2</sup>, Carlus Deneke<sup>1</sup>, Marie-Theres Knüver<sup>1</sup>, Maja Thieck<sup>1</sup>, Ha Thi Thu Hoang<sup>3</sup>, Nancy Bretschneider<sup>4</sup>, Ngoc Thi Pham<sup>2</sup>, Ingrid Huber<sup>4</sup> and Kerstin Stingl<sup>1\*</sup>

## Abstract

**Background** *Campylobacter* spp. is the most frequent cause of bacterial food-borne gastroenteritis and a high priority antibiotic resistant bacterium according to the World Health Organization (WHO). European monitoring of thermotolerant *Campylobacter* spp. does not reflect the global burden of resistances already circulating within the bacterial population worldwide.

**Methods** We systematically compared whole genome sequencing with comprehensive phenotypic antimicrobial susceptibility, analyzing 494 thermotolerant *Campylobacter* poultry isolates from Vietnam and Germany. Any discrepancy was checked by repeating the wet lab and improving the dry lab part. Selected isolates were additionally analyzed via long-read Oxford Nanopore technology, leading to closed chromosomes and plasmids.

**Results** Overall, 22 different resistance genes and gene variants (e. g. *erm(B)*, *aph(3')-IIIa*, *aph(2'')-I<sub>f</sub>*, *catA*, *Inu(C)*, *bla<sub>OXA</sub>*, *sat4*) and point mutations in three distinct genes (*gyrA*, 23S rRNA, *rpsL*) associated with AMR were present in the *Campylobacter* isolates. Two AMR genes were missing in the database and one falsely associated with resistance. Bioinformatic analysis based on short-read data partly failed to identify *tet(O)* and *aadE*, when the genes were present as duplicate or homologous gene variants. Intriguingly, isolates also contained different determinants, redundantly conferring resistance to chloramphenicol, gentamicin, kanamycin, lincomycin and streptomycin. We found a novel *tet(W)* in tetracycline sensitive strains, harboring point mutations. Furthermore, analysis based on assemblies from short-read data was impaired to identify full length phase variable *aad9*, due to variations of the poly-C tract within the gene. The genetic determinant responsible for gentamicin resistance of one isolate from Germany could not be identified. GyrT86I, presenting the main determinant for (fluoro-)quinolone resistance led to a rare atypical phenotype of ciprofloxacin resistance but nalidixic acid sensitivity. Long-read sequencing predicted AMR genes were mainly located on the chromosome, and rarely on plasmids. Predictions from long- and short-read sequencing, respectively, often differed. AMR genes were often organized in multidrug resistance islands (MDRI) and partially located in proximity to transposase genes, suggesting main mobilization of resistance determinants is via natural transformation and transposition in *Campylobacter*.

\*Correspondence:  
Kerstin Stingl  
Kerstin.stingl@bfr.bund.de

Full list of author information is available at the end of the article



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**Conclusions** The results of this study suggest that there is frequent resistance gene duplication, mosaicism, and mutation leading to gene variation and truncation in *Campylobacter* strains that have not been reported in previous studies and are missing from databases. Furthermore, there is a need for deciphering yet unknown resistance mechanisms and resistance spread in thermotolerant *Campylobacter* spp. that may pose a challenge to global food safety.

**Keywords** NGS, Susceptibility testing, Antibiotic resistance, Long-read sequencing, Multidrug resistance islands, AMR, Mosaic genes, Resistance monitoring, Southeast Asia

## Background

Spread of multidrug-resistant bacteria is a global public health threat, contributing to more than 670,000 diseases and 33,000 deaths annually in the European Union/European Economic Area (EU/EEA) [1]. Thermotolerant *Campylobacter* species are not yet under strict control through the implementation of a safety criterion but constitute the most common bacterial cause of gastroenteritis in the European Union (EU), with around 220,000 official cases in 2019 [2]. A study estimated the true incidence of campylobacteriosis to be 47 times (95% CI 14–117) higher than reported in the EU but varying considerably between member states [3]. In the EU in 2020, a slightly lower number of campylobacteriosis cases (21%) were hospitalized compared to *Salmonella* infections (29.9%) [4]. Epidemiological data on campylobacteriosis in Vietnam is scarce due to lack of surveillance programs. *Campylobacter* spp. accounted for the largest proportion of all isolates in Vietnamese rural children with diarrheal disease [5]. Furthermore, 20% of stool samples from infants with acute diarrhea in southern Vietnam were tested positive for *Campylobacter* spp. [6].

Acute campylobacteriosis is characterized by watery and bloody diarrhea, abdominal cramps, fever and nausea [7]. In addition, long-term autoimmune sequelae might occur such as the Guillain-Barré syndrome in 0.07%, reactive arthritis in approximately 1–5% and irritable bowel syndrome in around 4% of acute cases [8]. These long-term diseases caused by campylobacteriosis contribute to a public health burden largely underestimated by the public.

A recent study showed that 31% of the reported campylobacteriosis cases were treated with antibiotics, mainly ciprofloxacin and macrolides [9]. According to the World Health Organization (WHO), *Campylobacter* spp. are high-priority antibiotic-resistant pathogens, particularly with regard to their fluoroquinolone resistance [10].

*C. jejuni* and *C. coli* asymptotically colonize the intestinal tract of various animal species, both wild and domestic, which constitutes a potential reservoir for human infections. In particular, poultry is recognized as major source of *Campylobacter* spp. infections in humans, most probably via the consumption of cross-contaminated food during handling of raw meat or direct animal contact [11]. Zoonosis monitoring in Germany

revealed a high prevalence of 51.8% *Campylobacter* spp. positive fresh chicken meat in 2020 [12]. Likewise, 31% of the tested chicken meat from Hanoi was contaminated with thermotolerant *Campylobacter* spp. [13]. Previous studies showed that *Campylobacter* isolates from Vietnam were frequently resistant to (fluoro-)quinolones (62.5–95%) and tetracyclines (71.4–75%), moderately frequent to frequently resistant to streptomycin (21.4–62.5%), and rarely to less frequently resistant to erythromycin (7.4–25%) and gentamicin (7.1–25%) [14–16]. In Germany, recent results from the 2020 zoonosis monitoring program from broiler ceca [12] revealed frequent resistance of *C. spp.* to ciprofloxacin (83.4% for *C. jejuni* and 81% for *C. coli*) and tetracycline (66.4% for *C. jejuni* and 69% for *C. coli*). All broiler isolates from cecal content were sensitive to gentamicin. Resistances to macrolides were only observed in *C. coli* isolates (17.2%). Streptomycin resistance was higher in *C. jejuni* (35%) than in *C. coli* (3.4%), which was a new observation compared to the previous years [17, 18].

Increasing occurrence of antimicrobial resistance (AMR), impeding the effectiveness of antibiotics used for treatment of bacterial diseases, poses a threat to global health [19]. Use of antimicrobials in animal production is recognized as one of the drivers of AMR [20, 21]. In order to reduce the spread of antibiotic resistance in animal production, livestock farms in Germany have been obliged to report and reduce their use of antibiotics since 2011. The overall significant decrease of antibiotic use in all farm animals by 31.6% between mid-2014 compared to mid-2017 was only marginally reflected in the poultry production chain, with a maximum reduction of 3.8% observed in turkey production [22]. From 2017 until 2021, antibiotic use in poultry was significantly reduced by 11.5% in chicken and 13.1% in turkey, while during the same time period antibiotic use in all animals was reduced by 18.2% [23]. In Vietnam, antimicrobial use in livestock accounted for 71.7% (2,751 t) of the total antimicrobials used in 2015. This corresponded to nearly the same amount of antimicrobials per kg of biomass used for human and animal treatment and a 1.6-fold higher use compared to the EU [24]. Some of the antimicrobials used in both countries were among the “highest priority critically important antimicrobials” defined by WHO, i.e.

(fluoro-)quinolones, polypeptide antibiotics and macrolides [25–28].

Systematic analysis and reliable diagnostics of multi-resistant bacterial pathogens are essential to prevent their global spread. A number of studies, delivering whole genome sequencing data with some phenotypic analysis of thermotolerant *Campylobacter* spp. have previously been published [29–35]. However, rigorous in-depth analyses, aiming to identify and solve discrepancies between whole genome sequencing data and phenotypic resistance profiles are scarce for *Campylobacter* spp. Here, we evaluated a common strategy, the prediction of AMR resistant determinants by AMRFinderPlus based on short-read assembly data by recording concordances and experimentally re-analyzing discrepancies between pheno- and genotype of nearly 500 thermotolerant *Campylobacter* spp. from Germany and Vietnam. A selection of isolates was also processed by long-read sequencing using the Oxford Nanopore Technology. The study aimed at identifying knowledge gaps to be addressed in order to use WGS as a tool to reliably predict AMR in *Campylobacter* spp. In particular, it should be clarified, which specific features of AMR in *Campylobacter* spp. still pose problems for current routine WGS analysis and have to be addressed in the future.

## Methods

### Isolates and growth conditions

*C. coli* and *C. jejuni* isolates from Germany were isolated within the zoonosis monitoring program from different poultry matrices from 2013 to 2021 by the federal state laboratories according to EN ISO 10272-1 valid in the respective year [36, 37]. Isolates from Vietnam were isolated from fresh chicken feces from primary production and chicken meat from retail in Hanoi and Haiphong between 11/2016 and 03/2018 by the National Institute of Veterinary Research (Hanoi, Vietnam) by direct streaking on modified charcoal cefoperazone deoxycholate agar (mCCDA, Thermo Fisher Scientific Inc., Waltham, MA, USA) according to EN ISO 10272-1:2017 [37]. At the National Reference Laboratory, isolates were subcultured on Columbia agar supplemented with 5% sheep blood (Oxoid, Thermo Fisher Scientific Inc., Waltham, MA, USA) (ColbA) or passaged in Bolton broth (Oxoid, Thermo Fisher Scientific Inc.) and subcultured on mCCDA in case isolates still exhibited non-*Campylobacter* background flora. Incubation was performed for 48 h under microaerobic conditions (5% O<sub>2</sub>, 10% CO<sub>2</sub>, rest N<sub>2</sub>) at 42 °C. The isolates were stored at –80 °C using the cryobank system (Mast Diagnostica GmbH, Reinfeld, Germany). For DNA extraction and antibiotic susceptibility testing isolates from –80 °C stock cultures were grown on ColbA for 24 h under microaerobic conditions

at 42 °C and subcultured once for another 20±2 h prior to use.

### Species differentiation by PCR

DNA of the isolates was extracted by resuspension of a quarter 10 µL loop of cell material in 400 µL Tris-EDTA buffer (1 mM Tris, 0.1 mM sodium ethylenediaminetetraacetic acid at pH 8.0) followed by 1:100 dilution in 5% Chelex 100 resin (Bio-Rad Laboratories GmbH, Feldkirchen, Germany). Subsequently, thermal lysis was performed for 15 min at 95 °C. After centrifugation at 14,000 x g at 4 °C for 10 min, 2.5 µl of the supernatant was used for real-time PCR analysis, targeting specific fragments of the *C. jejuni mapA*, the *C. coli ceuE* and the *C. lari glyA* genes [38, 39].

### Antibiotic susceptibility testing by microdilution

Broth microdilution susceptibility testing was performed according to M45-A and VET06 [40, 41]. Strains subcultured for 24±2 h at 42°C on ColbA were inoculated in cation-supplemented Mueller-Hinton broth (Thermo Fisher Scientific Inc., Waltham, MA, USA) with 5% fetal calf serum (PAN-Biotech, Aidenbach, Germany) (CAMHB/FCS) at a bacterial concentration of 2–8×10<sup>5</sup> CFU/ml. For this purpose, bacteria were suspended at an OD<sub>600</sub> of 0.2 in buffered peptone water (10 g/L peptone, 5 g/L NaCl, 9 g/L Na<sub>2</sub>HPO<sub>4</sub>×12 H<sub>2</sub>O, 1.5 g/L KH<sub>2</sub>PO<sub>4</sub>, pH 7.0±0.2 at 25°C), which corresponds to approximately 5×10<sup>8</sup> CFU/ml [42]. Upon a 10<sup>-3</sup> dilution in CAMHB/FCS, 100 µl of the resulting 5×10<sup>5</sup> CFU/ml were used as inoculum per well. The inoculum was occasionally controlled by plating 100 µl of a further 10<sup>-3</sup> dilution in duplicate on ColbA in order to obtain approximately 50 CFU per plate. Minimum inhibitory concentrations were determined using the European standardized EUCAMP2 plate (Thermo Fisher Scientific Inc., Waltham, MA, USA). In addition, custom plate formats were prepared with the following antimicrobial agents (Sigma Aldrich, St. Louis, MO, USA) and their concentration ranges: ampicillin (0.5–512 mg/L), chloramphenicol (2–128 mg/L), florfenicol (0.25–16 mg/L), kanamycin (2–1024 mg/L), lincomycin (0.25–128 mg/L), nourseothricin (mixture of streptothricins C, D, E and F; 1–512 mg/L) and spectinomycin (2–512 mg/L). Stock solutions of the antimicrobials were prepared in H<sub>2</sub>O, for florfenicol in dimethyl sulfoxide, and for chloramphenicol in ethanol. The microtiter plates with U-bottom (Greiner Bio-One International GmbH, Frickenhausen, Germany) were prepared one day in advance by adding 50 µl CAMHB/FCS supplemented with the respective double-concentrated antimicrobial per well and stored sealed at 5°C before inoculation. Test strains were prepared as described above except that the inoculum was double-concentrated in a volume of 50 µL (1×10<sup>6</sup> CFU/ml), which was added to each well of

the custom plates, already loaded with 50 µl of double-concentrated antimicrobial per well. Samples were incubated at 37°C for 44 ± 4 h under microaerobic conditions. Minimal inhibitory concentrations (MICs; in mg/L) were semi-automatically analyzed using the Sensititre™ Vizion™ system (Thermo Fisher Scientific Inc., Waltham, MA, USA) and the Sensivizion V2.0 software (MCS Diagnostics BV, Swalmen, The Netherlands). Epidemiological cut-off values (ECOFFs, Table 1) for resistance determination were based on the European Committee on Antimicrobial Susceptibility Testing [43], if available for *Campylobacter* spp. Otherwise, “elevated non-wildtype MICs” were considered based on EUCAST *Campylobacter* spp. MIC distributions and the data obtained in our study for kanamycin (Figure S1). For lincomycin, the “elevated non-wildtype MICs” were based on a previous publication [44]; furthermore, the “elevated non-wildtype MICs” were established based on data from this study for nourseothricin and spectinomycin (Figure S1). For quality assessment of EUCAMP2 plate format, *C. jejuni* strain DSM 4688 (DSMZ - German Collection of Microorganisms and Cell Cultures GmbH, Braunschweig, Germany) and *C. coli* strain 2012-70-443-2 (Technical University of Denmark, Lyngby, Denmark) were included, which displayed sensitive phenotypes.

The correlation of phenotypic resistance against antimicrobials on custom plates and presence of each known AMR gene was experimentally tested by analyzing at least five additional isolates without the resistance marker as negative control. For the frequently observed *bla<sub>OXA</sub>* genes, a portion of *bla<sub>OXA</sub>* positive isolates (139/459) underwent susceptibility testing with ampicillin (Table S1).

### Whole genome sequence analysis

DNA for short-read sequencing was extracted using the PureLink Genomic DNA Mini Kit (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer’s protocol. For this purpose *Campylobacter* isolates were subcultured on ColBA for 20 ± 2 h under microaerobic atmosphere at 42°C and bacteria were harvested from 1 mL of resuspended bacteria at OD<sub>600</sub> of 2 by centrifugation at 14,000 x g for 5 min. The cell pellet was either directly used for DNA extraction or stored at -20°C. DNA for long-read sequencing was extracted using the MagAttract HMW Genomic Extraction Kit (Qiagen N.V., Venlo, The Netherlands) following manufacturer’s instructions, except starting with a cell pellet derived from 1 mL of bacteria at an OD<sub>600</sub> of 2 upon centrifugation, followed by incubation for 1.5 h at 56°C and 900 rpm of agitation. The quality of the DNA was evaluated by spectral analysis (NanoDrop Spectrophotometer, Thermo Fisher Scientific, Waltham, MA, USA) and the concentration was fluorimetrically quantified by Qubit 3.0 Fluorometer (dsDNA HS Assay Kit 0.2–100 ng; Thermo Fisher Scientific, Waltham, MA, USA). DNA extracts for long-read sequencing were analyzed with the 5200 Fragment Analyzer System (Agilent Technologies Corp., Santa Clara, CA, USA) using DNF-464 HS Large Fragment Kit (Agilent Technologies Corp., Santa Clara, CA, USA) to check for DNA degradation/RNA contamination as well as sufficient length (>10,000 bp) of the DNA fragments. DNA libraries for short-read sequencing were prepared using the Illumina DNA Prep, (M) Tagmentation Kit according to the manufacturer’s instructions (Illumina, Inc., San Diego, CA, USA) but using half of the volume of all reagents. Paired-end sequencing was performed on the Illumina MiSeq benchtop sequencer using the MiSeq

**Table 1** Epidemiological cut-off values (ECOFFs, if available) or “elevated non-wildtype MIC values” for evaluation of antibiotic susceptibility testing results of thermotolerant *Campylobacter* spp.

| Antimicrobial   | MIC [mg/L], resistant >, <i>C. jejuni</i> | MIC [mg/L], resistant >, <i>C. coli</i> | Reference                                  |
|-----------------|---|---|--|
| Ampicillin      | 16  | 16                                      | ECOFF for <i>C. spp.</i> [43]              |
| Chloramphenicol | 16  | 16                                      | ECOFF for <i>C. spp.</i> [43]              |
| Ciprofloxacin   | 0.5                                       | 0.5                                     | ECOFF for <i>C. spp.</i> [43]              |
| Erythromycin    | 4   | 8                                       | ECOFF for <i>C. spp.</i> [43]              |
| Florfenicol     | 4   | 4                                       | ECOFF for <i>C. spp.</i> [43]              |
| Gentamicin      | 2   | 2                                       | ECOFF for <i>C. spp.</i> [43]              |
| Kanamycin       | 16  | 16                                      | elevated non-wildtype MICs ([43]; Fig. S1) |
| Lincomycin      | 8   | 8                                       | elevated non-wildtype MICs [44]            |
| Nalidixic acid  | 16  | 16                                      | ECOFF for <i>C. spp.</i> [43]              |
| Nourseothricin  | 4   | 4                                       | elevated non-wildtype MICs (Fig. S1)       |
| Spectinomycin   | 64  | 64                                      | elevated non-wildtype MICs (Fig. S1)       |
| Streptomycin    | 4   | 4                                       | ECOFF for <i>C. spp.</i> [43]              |
| Tetracycline    | 1   | 2                                       | ECOFF for <i>C. spp.</i> [43]              |

reagent kit v3 (600 cycles, Illumina, Inc., San Diego, CA, USA) or on the Illumina NextSeq 500 sequencer using the NextSeq 500/550 mid output kit v2.5 (300 cycles, Illumina, Inc., San Diego, CA, USA) with read lengths ranging between  $2 \times 149$  and  $2 \times 301$ , respectively. DNA libraries for long-lead sequencing (Oxford Nanopore Technology (ONT)) were prepared using the Rapid Barcoding Kit 96 (SQK-RBK110.96, Oxford Nanopore Technologies Limited, Oxford, United Kingdom) according to manufacturer's instructions. Sequencing was performed on the MinION Mk1C instrument using a MinION FlowCell (R9.4.1, Oxford Nanopore Technologies Limited, Oxford, United Kingdom). For verification of truncation of the housekeeping multi-locus sequence typing (MLST) gene *aspA* in BfR-CA-16251, a PCR amplification of *aspA* was performed using the following primers, *aspA*-A9 (5'-AGT ACT AAT GAT GCT TAT CC-3') and *aspA*-A10 (5'-ATT TCA TCA ATT TGT TCT TTG C-3') [45; <https://pubmlst.org/>, last accessed on 05/01/2024]. Subsequently, the PCR fragment was purified using QIAquick PCR Purification Kit (Qiagen, N.V., Venlo, The Netherlands) and suitable amounts of DNA supplemented with either sequencing primer *aspA*-S3 (5'-CCA ACT GCA AGA TGC TGT ACC-3') or *aspA*-S6 (5'-TTC ATT TGC GGT AAT ACC ATC-3') [45; [https://pubmlst.org](https://pubmlst.org/), last accessed on 01/05/2024] were Sanger sequenced (Eurofins Scientific SE, Luxembourg City, Luxembourg).

### Bioinformatic Analysis

Illumina paired-end reads were trimmed and *de-novo* assembled with the AQUAMIS pipeline v1.3.8 [46], which implements e.g. fastp v0.23.2 for read quality control and trimming [47] and shovill v1.1.0 for assembly [48] as well as Quast v. 5.0.2 for assembly quality control. Sufficient quality was defined as base accuracy Q30 (error rate 1:1000) for more than 80% of the reads, and a minimum read coverage of 40. In addition, 10 sequences (Table S2) were also assembled using SKESA assembler using the NCBI Read Assembly and Annotation Pipeline Tool (RAPT at <https://www.ncbi.nlm.nih.gov/rapt>; last accessed on 01/05/2024).

Assembled contigs were analyzed for presence of resistance determinants as well as for plasmid markers using the BakCharak pipeline v3.0.3 [49]. The pipeline is composed of various modules, each serving a specific purpose. It includes the antimicrobial resistance gene finder module which identifies AMR determinants through the use of AMRFinderPlus v3.10.45 [50] and its corresponding AMRFinder database 2023-08-08.2. The Plasmidfinder employs ABRicate v1.0.1 [51] and utilizes the Center for Genomic and Epidemiology (CGE) plasmidfinder database. Default thresholds were applied for both ABRicate and AMRFinderPlus, which included a minimum identity threshold of 80% and 90%, respectively,

and a minimum coverage threshold of 50% for both tools. Furthermore, Platon v1.6 [52] was used to predict putative plasmid location of contigs.

In addition to the BakCharak pipeline, assembled whole genome sequences from isolates showing pheno-genotype discrepancies were analyzed with ResFinder v4.1 [53] using low thresholds of identity (50%) and coverage (40%). This approach not only addressed missing genes in the AMRFinderPlus database but also revealed partial genes and those with reduced homology. Identified AMR gene sequences were extracted from the assembled sequences and analysed via the NCBI Basic Local Alignment Search Tool [54, 55] in order to find the closest AMR gene homolog. The latter search was conducted either using blastn or blastp, with the corresponding databases NCBI nucleotide collection (nr/nt) or non-redundant protein sequences (nr), respectively. Alignments of translated protein sequences were created using UniProt [56]. Subsequently the draft genome assemblies were screened with ABRicate v1.0.1 for their presence/absence of the respective AMR gene homolog (Table S3) using Linux command line. The reference resistance gene and protein sequences representing the most abundant closest relatives are depicted in Table S3. Alignments of nucleotide sequences and mapping of trimmed raw reads to reference resistance genes or the promoter region of *bla<sub>OXA</sub>* genes was performed by Geneious Prime 2020.2.2 (Biomatters Ltd., New Zealand) using default settings. For verification of truncation of the housekeeping MLST gene *aspA* in BfR-CA-16251, *aspA* reference gene Cj0087 of *C. jejuni* NCTC 11168 (NC\_002163.1) was used for mapping of raw reads and additional Sanger sequences were analyzed using SeqMan Pro (Lasergene 17, DNASTAR Inc., WI, USA).

Ridom SeqSphere+v8.4.2 (Ridom, Muenster, Germany) was used to perform phylogenetic analysis on assembled genome contigs from short-read sequencing using either the seven housekeeping genes based MLST or the core genome (cgMLST) scheme of 1343 gene targets previously defined [57]. A threshold of 98% identity and 98% of coverage to one of the respective alleles of the reference sequence NC\_002163.1.gb (*C. jejuni* NCTC 11168) was used. At least 95% "good targets" were found based on cgMLST analysis. In addition, the 7-genes MLST scheme was used to lower the resolution for visualization of isolate diversity [45, [https://pubmlst.org](https://pubmlst.org/)]. New MLST alleles and MLST sequence types were uploaded to PubMLST [58].

The Oxford Nanopore Technology sequencing data was basecalled using Guppy v. 6.0.1 in the "super-accuracy" mode (Oxford Nanopore Technologies, Oxford, UK). Subsequently, ONT reads were assembled and quality was assessed with the MiLongA Pipeline v1.0.1. [59]. This pipeline includes various tools, such as porechop v0.2.4

[60] for trimming and Unicycler v0.4.8 [61] for hybrid assembly. Assembled hybrid genome contigs from short- and long-read sequencing were annotated with Bakta [62] and AMR determinant identification was performed using AMRFinderPlus v3.10.45 [50] and its corresponding database (v. 2023-08-08.2). Raw read sequences and either complete genomes (for those isolates sequenced by ONT) or draft genomes were published within the BioProjects No. PRJNA562653, PRJNA595957, PRJNA648048 and PRJNA872862 at the NCBI sequence read archive (SRA) and Genome database.

### Statistical analyses

*Campylobacter* isolates were categorized into susceptible and resistant using the ECOFFs or elevated non-wildtype MIC values (Table 1). A variable “3–4 resistances” was defined for isolates with three or four resistances based on EUCAMP2 plate format, with nalidixic acid and ciprofloxacin being combined as (fluoro-)quinolones. An odds ratio (OR) with 95% confidence interval (CI) was calculated (Table S4, [63, 64]). *p*-values of less than 0.05 were considered statistically significant.

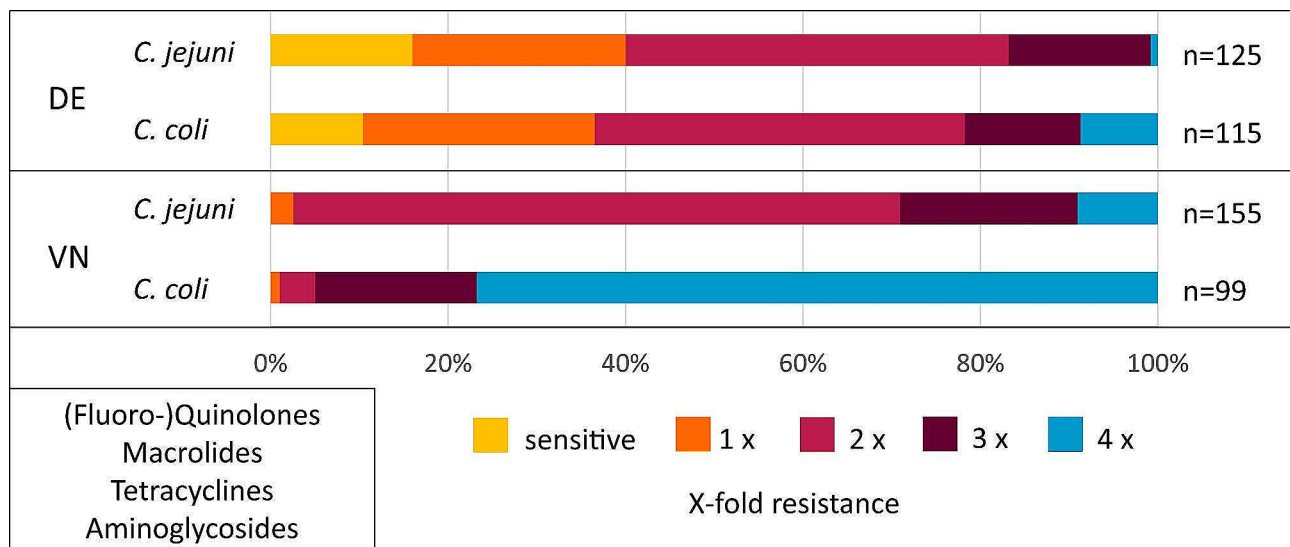
### Results

The 240 *C. coli*<sub>DE</sub> (*n*=115) and *C. jejuni*<sub>DE</sub> (*n*=125) isolates from Germany were taken from the strain collection of the laboratory. They were isolated from different matrices and locations in Germany by federal state laboratories as part of the zoonosis monitoring programs (Table S1, [12, 65]). The 254 *C. coli*<sub>VN</sub> (*n*=99) and *C. jejuni*<sub>VN</sub> (*n*=155) isolates from Vietnam were derived from fresh chicken fecal samples collected between November 2016

and December 2017 from primary chicken production of 26 different chicken farms and from cecum and retail samples in 2018 in the province of Hanoi and Haiphong. The principle size of the farms varied from 100 to 5,000 animals per flock, with only a few samples taken from farms with a flock size of 50,000. When farmers were asked for use of antimicrobials for treatment of chicken during rearing, they reported application of various substances, mostly tetracyclines (chlortetracycline, doxycycline and oxytetracycline), the macrolide tylosin, colistin as polymyxin, the  $\beta$ -lactam amoxicillin and aminoglycosides like gentamicin and neomycin. In total, 254 Vietnamese thermotolerant *Campylobacter* spp. isolates were obtained, of which 155 were identified as *C. jejuni* and 99 as *C. coli*.

### Identification of highly resistant isolates using a standardized microtiter panel

Antimicrobial susceptibility testing was performed for all 494 isolates using the European standardized microtiter plate format EUCAMP2. The panel includes six representative antibiotics from four different antibiotic classes: aminoglycosides, (fluoro-)quinolones, macrolides, and tetracyclines. The proportion of sensitive isolates among the isolates from Germany was 10.4% (*n*=12) for *C. coli* and 16% (*n*=20) for *C. jejuni*; meanwhile, no sensitive isolates were detected among the *Campylobacter* spp. isolates from Vietnam (Fig. 1). In particular, 94.9% (94/99) of *C. coli*<sub>VN</sub> and 29% (45/155) of *C. jejuni*<sub>VN</sub> isolates were resistant to three or four compound classes. In comparison, *C. coli*<sub>DE</sub> and *C. jejuni*<sub>DE</sub> isolates were less



**Fig. 1** Vietnamese *C. coli* isolates displayed highest prevalence of combined resistance to all tested antimicrobial classes. Susceptibility to (fluoro-)quinolones (nalidixic acid, ciprofloxacin), macrolides (erythromycin), tetracycline and aminoglycosides (gentamicin, streptomycin) was tested by microdilution. X-fold resistance, number of antimicrobial classes to which isolates showed resistance (depicted in % of total number of tested isolates per category (n)); DE, German isolates; VN, Vietnamese isolates. Odds ratios are depicted in Table S4

frequently resistant to three or four compound classes (21.7%, 25/115 and 16.8%, 21/125, respectively).

Table 2 provides an overview of the prevalence of resistance to individual antimicrobials tested within the EUCAMP2 plate format. Phenotypic resistance to ciprofloxacin was high in *C. jejuni*<sub>DE</sub> and *C. coli*<sub>DE</sub> isolates (78.4 and 80.9%, respectively) whereas 98.1% of *C. jejuni*<sub>VN</sub> isolates and all *C. coli*<sub>VN</sub> displayed resistance to ciprofloxacin, respectively. Resistance to the erythromycin was low among *C. jejuni*<sub>DE</sub> isolates, with only one resistant *C. jejuni*<sub>DE</sub> isolate identified (0.8%) and moderately frequent among *C. coli*<sub>DE</sub> isolates (18.3%).

In Vietnam, resistance to erythromycin was predominantly found for *C. coli*<sub>VN</sub> isolates (76.8%), while 11% of the *C. jejuni*<sub>VN</sub> isolates showed resistance to this antimicrobial substance. About two-third of *Campylobacter* isolates from Germany were tetracycline resistant (64 and 69.6% for *C. jejuni*<sub>DE</sub> and *C. coli*<sub>DE</sub>, respectively). In comparison, the counterparts from Vietnam were almost completely resistant to this antibiotic ( $\geq 99\%$ ); in fact, only one *C. jejuni*<sub>VN</sub> and one *C. coli*<sub>VN</sub> isolate analyzed in this study were tetracycline sensitive. Resistance to gentamicin was only detected in two *C. coli*<sub>DE</sub> isolates, whereas all *C. jejuni*<sub>DE</sub> were sensitive. In contrast, 78.8% of the *C. coli*<sub>VN</sub> isolates and 21.9% of the *C. jejuni*<sub>VN</sub> were resistant to gentamicin. Resistance to streptomycin was highest in *C. coli*<sub>VN</sub> isolates (85.9%), while in *C. coli*<sub>DE</sub> this resistance was moderately frequent with 13%, which was similar to *C. jejuni*<sub>VN</sub> (12.9%). The *C. jejuni*<sub>DE</sub> isolates were slightly more resistant to streptomycin (18.1%) than the *C. coli*<sub>DE</sub> isolates and the *C. jejuni*<sub>VN</sub> isolates but this was not statistically significant. Overall, the isolates from Vietnam were 5.1 times more likely resistant to three or more antibiotics compared to their counterparts from Germany (OR 5.1, 95% CI 3.4–7.6; Table S4). Taking the same variable of “3–4 resistances,” *C. coli* isolates from Vietnam were far more resistant against the tested antimicrobials than the *C. jejuni* isolates from the same geographic location (OR 46.0, 95% CI 17.5–120.5). The likeliness of displaying 3–4 resistances was not significantly different for *C. coli*<sub>DE</sub> versus *C. jejuni*<sub>DE</sub> ( $p=0.33$ ). However, significantly different acquisition of resistance to erythromycin

was observed for *C. coli* isolates compared to *C. jejuni* not only in Vietnam (OR 26.8, 95% CI 13.5–53.3) but also in Germany (OR 27.7, 95% CI 3.7–209.7).

#### Phylogenetic diversity of strains is a good basis for in-depth AMR analysis

All 494 isolates were subjected to whole-genome sequencing using short-read Illumina technology. To determine phylogenetic relationship of the *Campylobacter* isolates, first multi-locus sequence typing method (MLST) for comparison of the seven housekeeping genes was applied (Fig. 2). For higher resolution, the core gene MLST (cgMLST) scheme based on the comparison of 1343 core genes was used [57] with missing loci pairwise ignored (Ridom SeqSphere+) (Table S5). We identified 15 new MLST allele variants, including an *aspA* allele with a deletion of 19 bases in BfR-CA-16251 (Figure S2) and assigned 191 different sequence types (STs), of which 41 were novel (Fig. 2, Table S1). The *C. jejuni*<sub>VN</sub> subpopulation possessed the greatest diversity of different ST types ( $n=70$ ), followed by the *C. jejuni*<sub>DE</sub> subpopulation ( $n=53$ ). *C. coli* possessed less diversity, since isolates from Germany belonged to 45 different STs, while *C. coli* from Vietnam were attributed to 32 different STs. They were part of the common clonal complexes CC-828 ( $n=148$ ) or CC-1150 ( $n=15$ ) or did not belong to any CC ( $n=51$ ). Although, some isolates from Germany and Vietnam shared the same MLST sequence types ( $n_{ST}=9$ , Fig. 2, circles with dashed line), they were not phylogenetically related on the basis of cgMLST (Table S5). Consistently, resistance patterns were independent of phylogenetic origin, since similar AMR patterns were distributed all over the identified MLST types (Fig. 2).

#### Distribution of resistant determinants in *Campylobacter* spp. from Germany and Vietnam

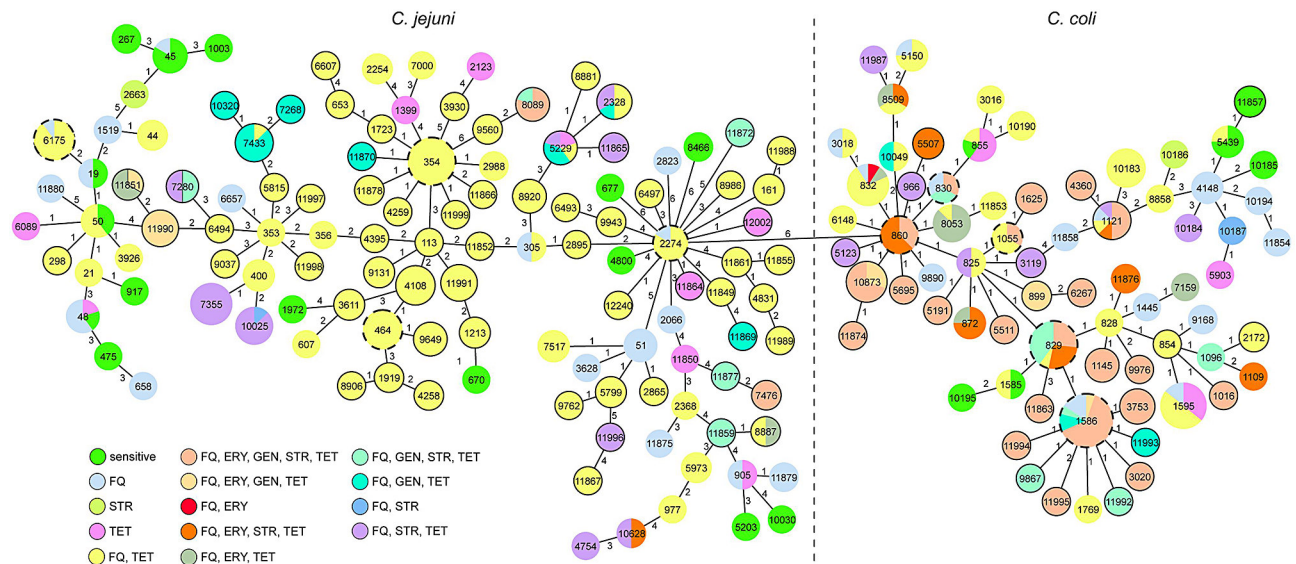
Short-read whole genome sequencing results were processed using the AMRFinderPlus tool [50] for identification of AMR genes. In total, 22 different resistance genes and gene variants (e. g. *erm*(B), *tet*(O), *aadE*, *aph*(3')-IIIa, *aad9*, *catA*, *lnu*(C), *bla*<sub>OXA1</sub>, *sat4*) and point mutations in three distinct genes (*gyrA*, 23S rRNA, *rpsL*) associated

**Table 2** Antimicrobial resistance in *C. coli* and *C. jejuni* from Germany and Vietnam according to EUCAMP2 plates

|                | <i>Campylobacter jejuni</i> |      |                     |      | <i>Campylobacter coli</i> |      |                    |       |
|----------------|-----------------------------|------|---------------------|------|---------------------------|------|--------------------|-------|
|                | Germany ( $n=125$ )         |      | Vietnam ( $n=155$ ) |      | Germany ( $n=115$ )       |      | Vietnam ( $n=99$ ) |       |
|                | n                           | %    | n                   | %    | n                         | %    | n                  | %     |
| Ciprofloxacin  | 98                          | 78.4 | 152                 | 98.1 | 93                        | 80.9 | 99                 | 100.0 |
| Erythromycin   | 1                           | 0.8  | 17                  | 11.0 | 21                        | 18.3 | 76                 | 76.8  |
| Gentamicin     | 0                           | 0.0  | 34                  | 21.9 | 2                         | 1.7  | 78                 | 78.8  |
| Nalidixic acid | 92                          | 73.6 | 149                 | 96.1 | 92                        | 80.0 | 99                 | 100.0 |
| Streptomycin   | 23                          | 18.4 | 20                  | 12.9 | 15                        | 13.0 | 85                 | 85.9  |
| Tetracycline   | 80                          | 64.0 | 154                 | 99.4 | 80                        | 69.6 | 98                 | 99.0  |

ECOFFs (if available) or elevated non-wildtype MICs for resistance evaluation are depicted in Table 1; n, number of tested isolates; odds ratios are depicted in Table S4





**Fig. 2** Test strains showed phylogenetical diversity, with AMR patterns distributed all over the identified MLST types. Minimum spanning tree (MST) based on MLST analysis. Colors indicate different phenotypic resistance profiles obtained with EUCAMP2 plate format. Nodes with numbers represent ST types; node size corresponds to the number of isolates (e.g. ST-267 is only represented by one isolate). Closed circles, Vietnamese isolates; open circles, German isolates; dashed-lined circles, isolates from both countries. FQ, (fluoro-)quinolone resistant; STR, streptomycin resistant; ERY, erythromycin resistant; TET, tetracycline resistant; GEN, gentamicin resistant. Numbers between nodes indicate numbers of allele difference based on 7 housekeeping genes (cgMLST differences are depicted in Table S2). MST was created with Ridom SeqSphere+ software.

with AMR were identified (Fig. 3 and Table S1). The resistance determinants were differently distributed among *Campylobacter* populations from Germany and Vietnam and fewer AMR genes were found in *C. jejuni* compared to *C. coli* (Fig. 3). We first checked whether the identified genes could be associated with the phenotype obtained by the EUCAMP2 plate format (Table 2). In case other resistance genes were identified via WGS analysis, custom plate microdilution for characterization of antimicrobial susceptibility was performed. Hence, the expected phenotypic resistance based on the presence of each AMR gene was experimentally tested. Table 3 summarizes the concordances and discrepancies of phenotypic and genotypic resistance characteristics of the isolates sorted by antibiotic class (detailed in Table S1), which we address in the following sections. As proof of principle, a selection of 14 isolates was also subjected to long-read ONT sequence analysis.

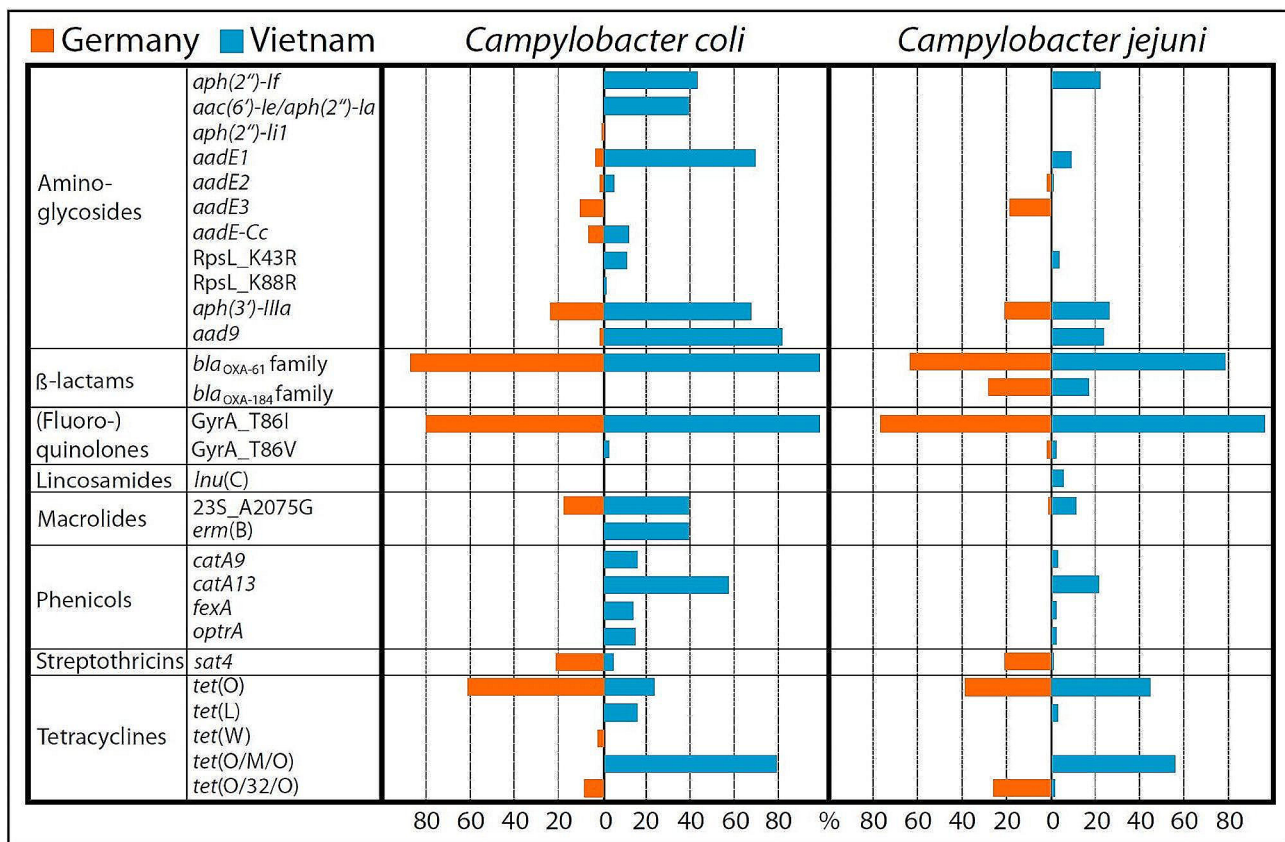
### Resistance to (fluoro-)quinolones

The mutation T86I in the gyrase A subunit was the most prominent mutation found to be associated with resistance to (fluoro-)quinolones. The T86I was found in 98.4% ( $n=436$ ), while the T86V mutation was identified in only 1.6% ( $n=7$ ) of the ciprofloxacin resistant isolates. Nearly all (97%,  $n=246$ ) isolates from Vietnam and 79% ( $n=189$ ) of the isolates from Germany contained this specific resistance mechanism (Fig. 3). Only five isolates from Vietnam and two from Germany showed the mutation T86V and displayed resistance to nalidixic acid and

ciprofloxacin. Three isolates from Vietnam and seven isolates from Germany with the T86I mutation in *GyrA* were resistant to ciprofloxacin ( $MIC_{CIP} = 4\text{--}16$  mg/L) but sensitive to nalidixic acid. Interestingly, six of the seven isolates from Germany susceptible to nalidixic acid had  $MIC$  values  $\leq 2$  mg/L, while being resistant to ciprofloxacin.

### Resistance to macrolides and lincosamides

In all macrolide resistant isolates from Germany ( $n=22/22$ ) and in 59% of the isolates from Vietnam ( $n=55/93$ ) the single point mutation A2075G in the 23S rRNA gene was found, conveying erythromycin resistance. However, 38 *C. coli*<sub>VN</sub> isolates harbored the gene *erm(B)*, encoding an rRNA adenine N-6-methyltransferase, modifying the target binding site for macrolides in the 23S rRNA, thus conferring resistance to macrolides [66]. The MIC distribution of isolates carrying the 23S rRNA A2075G point mutation or the *erm(B)* gene was comparable, ranging from 64 mg/L ( $n_{ermB} = 6$ ;  $n_{23S\_A2075G} = 5$ ) to 128 mg/L ( $n_{ermB} = 5$ ;  $n_{23S\_A2075G} = 4$ ) and exceeding 128 mg/L ( $n_{ermB} = 27$ ;  $n_{23S\_A2075G} = 68$ ) (Figure S3). The translated *erm(B)* genes shared 99–100% amino acid identity to the reference Erm(B) protein WP\_002321849.1, with maximally one conservative mutation (I125V) in three *C. coli* isolates from Vietnam (BfR-CA-16073, BfR-CA-16297, BfR-CA-18879), displaying MIC values  $>128$  mg/L to erythromycin. The resistance gene *lnu(C)*, which codes for a lincosamide nucleotidyltransferase (100% identity shared with



**Fig. 3** Distribution and prevalence of resistance determinants identified by whole genome sequencing in *Campylobacter* spp. Fraction (%) of German (orange bars) and Vietnamese (blue bars) *C. jejuni* ( $n = 280$ ) and *C. coli* ( $n = 214$ ) isolates, carrying the respective resistance determinant are depicted. Resistance determinants are sorted according to antibiotic class. Partial and full-length genes are considered

WP\_002837187.1) was found in eight *C. jejuni* isolates from Vietnam. In four of the eight isolates, the point mutation A2075G in 23S rRNA was also present, which is sufficient for resistance to lincomycin. However, the other four isolates, harboring the *lnu(C)* gene in absence of the 23S rRNA A2075G point mutation, were sensitive to erythromycin but resistant to lincomycin (MIC of 64 to >128 mg/L), indicating *lnu(C)* as the determinant for lincomycin resistance in these isolates. The point mutation A103V in the L22 ribosomal protein was identified in 124 macrolide sensitive isolates, from which 103 isolates displayed MICs of  $\leq 1$  mg/L erythromycin. Furthermore, the point mutation was identified in three lincomycin sensitive isolates. Hence, this mutation alone did not confer resistance to macrolides nor lincomycin.

### Resistance to tetracyclines

Tetracycline resistance of *Campylobacter* isolates was associated with the presence of either the ribosomal protective protein-encoding genes *tet(O)*, mosaic variants (*tet(O/M/O)*, *tet(O/32/O)*, the latter missing in the AMRFinder database) or *tet(W)*, or the efflux transporter-encoding gene *tet(L)*. ResFinder enabled the detection of the Tet(O) protein variants, which
















share  $\geq 92.3\%$  identity with each other, while Tet(W) shows  $\sim 67\%$  identity to the Tet(O) proteins (Fig. 4).

In some resistant isolates, *tet(O)* and/or its mosaic variants were either partially found at the end of contigs ( $n = 54$ ) or were falsely absent ( $n = 8$ ). These isolates were reanalyzed by mapping raw reads to reference tetracycline resistance genes using Geneious Prime software (exampled in Figure S4). As a result the presence of multiple different variants of *tet(O)*, including isolates exhibiting unique variants of *tet(O/M/O)* with varying degree and localisation of *tet(M)* sequence introgression, could be detected (Figure S5, visualized using [67], Table S1). However, as expected, mapping of reads to template *tet(O)* gene variants did not provide information about the presence of multiple identical full-length and/or partial gene copies. We confirmed the presence of multiple copies of identical or *tet(O)* variants by employing AMRFinderPlus on hybrid assemblies obtained from long-read sequencing of selected isolates. Consistently, except for one isolate (BfR-CA-17105), only long-read sequencing was capable of identification of multiple identical copies of *tet(O)* genes. Long-read sequencing also detected different truncated versions of *tet(O)* variants (in BfR-CA-15991, BfR-CA-18842, BfR-CA-16077,

**Table 3** Correlation and discrepancies between genotype and phenotype of *Campylobacter* spp. resistance profile using AMR FinderPlus

| Antibiotic class     | Antibiotic tested | #Isolates tested | #Isolates resistant | Resistance determinant  | Correlation between pheno- and genotype | Reason for discrepancy†   |
|----------------------|-------------------|------------------|---------------------|---|---|---|
| Aminoglycosides      | GEN               | 494              | 114                 | <i>aph(2'')-li</i> , <i>aph(2'')-lf</i> , bifunctional <i>aac(6'')-le/aph(2'')-la</i>   | 99.1% (113/114)                         | isolate with yet unknown resistance determinant (MIC > 16 mg/L; GEN-R; <i>n</i> = 1),   |
|                      | STR               | 494              | 143                 | <i>aadE1-3</i> , <i>aadE-Cc</i> , RpsL_K43R, RpsL_K88R                                  | 78.1% (114/146)                         | <i>aadE3</i> gene not found (missing in database (WP_057035408.1); STR-R; <i>n</i> = 29), partial <i>aadE1</i> genes (STR-R; <i>n</i> = 2), isolate slightly resistant (MIC = 8 mg/L), but harboring none of the known resistance determinants ( <i>n</i> = 1)                                      |
|                      | KAN               | 157              | 139                 | <i>aph(2'')-lf</i> , bifunctional <i>aac(6'')-le/aph(2'')-la</i> , <i>aph(3'')-IIIa</i> | 100% (139/139)                          | n.a.  |
|                      | SPC               | 139              | 118                 | <i>aad9</i>   | 30.5% (36/118)                          | partial genes found due to frame-shift within poly-C tract (SPC-R; <i>n</i> = 82)   |
| β-Lactams            | AMP               | 139              | 134                 | <i>bla</i> <sub>OXA-61</sub> and <sub>-184</sub> family genes                           | 98.5% (132/134)                         | Isolate susceptible (MIC = 16 mg/L), but harboring <i>bla</i> <sub>OXA-193</sub> ( <i>n</i> = 1); isolate slightly resistant (MIC = 32 mg/L), but harboring no <i>bla</i> <sub>OXA</sub> gene ( <i>n</i> = 1)   |
| (Fluoro-) Quinolones | CIP               | 494              | 442                 | GyrA_T86I, GyrA_T86V  | 100% (442/442)                          | n.a.  |
|                      | NAL               | 494              | 432                 | GyrA_T86I, GyrA_T86V  | 97.7% (432/442)                         | CIP-R/NAL-S phenotype, yet unknown mechanism ( <i>n</i> = 10)   |
| Lincosamides         | LCM               | 44               | 35                  | <i>Inu(C)</i> , 23S_A2075G, <i>erm(B)</i>   | 88.6% (31/35)                           | isolates slightly resistant (MIC = 16 mg/L), but harboring none of the known resistance determinants ( <i>n</i> = 4)  |
| Macrolides           | ERY               | 494              | 115                 | 23S_A2075G, <i>erm(B)</i>   | 100% (115/115)                          | n.a.  |
|                      |                   |                  |                     | 50S_L22_A103V   | 20% (31/155)                            | point mutation not associated with resistance to erythromycin; 20% correlation due to additional presence of either 23_A2075G or <i>erm(B)</i>  |
| Nourseothricin       | NTC               | 98               | 56**                | <i>sat4</i>   | 100% (56/56)                            | n.a.  |
| Phenicols            | CHL               | 130              | 93                  | <i>catA9</i> , <i>catA13</i> , <i>fexA</i> , <i>optrA</i>                               | 100% (94/94)                            | n.a.  |
|                      | FLO               | 53               | 29                  | <i>fexA</i> , <i>optrA</i>  | 62.1% (18/29)                           | isolates slightly resistant (MIC = 8–16 mg/L) in absence of known phenicol resistance determinants ( <i>n</i> = 11)   |
| Tetracyclines        | TET               | 494              | 412                 | <i>tet(O)</i> , <i>tet(O/32/O)</i> , <i>tet(W)</i> , <i>tet(O/M/O)</i> , <i>tet(L)</i>  | 84.6% (351*/415)                        | partial (mosaic-) <i>tet(O)</i> genes found (TET-R; <i>n</i> = 54), (mosaic-) <i>tet(O)</i> genes not found, but phenotypic resistance expressed (under coverage threshold; TET-R; <i>n</i> = 8), <i>tet(W)</i> with two point mutations (G511A/G1736A leading to D171N/G579D; TET-S; <i>n</i> = 2) |

AMP, ampicillin; CHL, chloramphenicol; CIP, ciprofloxacin; ERY, erythromycin; FLO, florfenicol; GEN, gentamicin; KAN, kanamycin; LCM, lincomycin; NAL, nalidixic acid; NTC, nourseothricin; SPC, spectinomycin; STR, streptomycin; TET, tetracycline; n.a., not applicable (100% correlation); \* *tet(O/32/O)* found as *tet(O)* with reduced identity (93.4%; missing in database; TET-R; *n* = 41) not depicted here, since pheno- and genotype were consistent with only incorrect nomenclature; \*\*Considering a cut-off value of > 4 mg/L as resistant; †Based on the prediction of resistance determinants obtained via the BakCharak pipeline (comprises the AMRFinderPlus tool and its corresponding database version 2023-08-08.2)

| AMR | Name         | Protein Acc. No. | length | % identity |      |      |      |      | VN/DE   |
|-----|--------------|------------------|--------|------------|------|------|------|------|---|
| CHL | CatA13       | WP_040564913.1   | 207    | 100        | 43.7 | 42.7 |      |      |    |
|     | CatA9        | WP_001010387.1   | 216    | 43.7       | 100  | 94.0 |      |      |    |
|     | Cat-TC       | WP_032488880.1   | 238    | 42.7       | 94.0 | 100  |      |      |    |
| GEN | Aph(2'')-li1 | WP_052776520.1   | 297    | 100        | 82.5 | 77.1 |      |      |    |
|     | Aph(2'')-lf  | WP_021424053.1   | 297    | 82.5       | 100  | 77.4 |      |      |    |
|     | Aph(2'')-la  | WP_001028144.1   | 291    | 77.1       | 77.4 | 100  |      |      |    |
| STR | AadE-Cc      | WP_002785795.1   | 299    | 100        | 31.1 | 32.5 | 31.8 |      |    |
|     | AadE 1       | WP_001255868.1   | 288    | 31.1       | 100  | 62.2 | 60.8 |      |    |
|     | AadE 2       | WP_001255866.1   | 302    | 32.5       | 62.2 | 100  | 83.8 |      |    |
|     | AadE 3       | WP_057035408.1   | 302    | 31.8       | 60.8 | 83.8 | 100  |      |    |
| TET | Tet(L)       | WP_002294500.1   | 458    | 100        | 20.4 | 21.5 | 21.5 | 20.4 |    |
|     | Tet(W)       | WP_000691721.1   | 639    | 20.4       | 100  | 66.8 | 67.0 | 67.1 |    |
|     | Tet(O/M/O)   | WP_002872163.1   | 639    | 21.5       | 66.8 | 100  | 93.3 | 92.3 |   |
|     | Tet(O)       | WP_063856405.1   | 639    | 21.5       | 67.0 | 93.3 | 100  | 93.6 |  |
|     | Tet(O/32/O)  | WP_215475009.1   | 639    | 20.4       | 67.1 | 92.3 | 93.6 |      |  |

**Fig. 4** Visualization of the protein variants found in thermotolerant *Campylobacter* spp. Closest NCBI matches with accession number (Acc. No.), amino acid length and percentage of protein identity to each other are depicted (computed with UniProt Align tool (Release 2023\_02, [56])). Country-specific prevalence is highlighted with national flags, whose sizes correspond to the magnitude of prevalence (detailed in Fig. 3). CHL, chloramphenicol; GEN, gentamicin; STR, streptomycin; TET, tetracycline; VN, Vietnam; DE, Germany. Percent sequence identity is colored as follows: 100%, black; 80–99%, dark blue; 60–79%, blue; ≤ 59%, light blue

BfR-CA-16088, BfR-CA-16297 and BfR-CA-19087) or a mutated *tet(O)* leading to a premature stop codon and a truncated protein (BfR-CA-16040) (Table 4). Those seven isolates with tetracycline resistance also harbored one or two additional copies of *tet(O)* or gene variants.

Within the tested concentration range (0.5–64 mg/L), we did not find differences in the degree of resistance associated with a single copy of *tet(O)* or its variant genes or with multiple copies of *tet* genes. The predominant resistance gene (full-length and/or partial) among the isolates tested was *tet(O)* (119 and 91 isolates from Germany and Vietnam, respectively). *tet(O/M/O)* was exclusively found in isolates from Vietnam ( $n=164$ ) and *tet(O/32/O)* predominantly in isolates from Germany ( $n_{DE}=42$ ,  $n_{VN}=2$ ). Thus, different *Campylobacter* populations harbored distinct gene variants. One of the *C. coli*<sub>DE</sub> isolates (BfR-CA-17078) carrying the *tet(O/32/O)* gene was sensitive to tetracycline and carried a point mutation introducing a stop codon (G1475A; p.W492Ter).

The correlation of *tet(L)* presence and tetracycline resistance in *Campylobacter* was only shown in isolates also carrying *tet(O)*. Three isolates from Germany harbored the *tet(W)* gene, yet two of them were sensitive to tetracycline and showed the same amino acid substitutions (D171N and G579D) (Figure S6).

#### Resistance to the aminoglycosides gentamicin and kanamycin

Gentamicin resistance in *Campylobacter* was rare in Germany, with only two identified resistant *C. coli*. One of the two isolates harbored the resistance gene *aph(2'')-li<sub>1</sub>*, which encodes an aminoglycoside phosphotransferase [68]. For the second isolate, the genetic determinant for gentamicin resistance was not detected but phenotypic resistance was repeatedly observed by microdilution assays (MIC > 16 mg/L). Here, further studies are needed to decipher the underlying mechanism of gentamicin resistance. In 112 gentamicin resistant isolates

**Table 4** Long-read sequencing found AMR genes often near transposase genes and on the chromosome

| Isolate No.  | VN/DE | Spec | predictions short-read assembly |                   |                                |                               |                           |                 | predictions based on ONT/hybrid assembly  |  |
|--------------|-------|------|---------------------------------|-------------------|--------------------------------|-------------------------------|---------------------------|-----------------|---|--|
|              |       |      | plasmids contigs                | plasmids circular | plasmids mobilization elements | plasmids conjugation elements | circ-conjugation elements | plasmid contigs | Point mutations and AMR genes on chromosome (in bold indicates location in proximity to transposase genes)  | AMR genes on plasmid                                       |
| BFR-CA-15687 | DE    | Cc   | 1 (-)                           | 0                 | 1                              | 6                             | 2                         | 1               | <i>bla</i> <sub>OXA-489</sub> ;tet(O);GyrA_T86I   | <i>tet</i> (O)   |
| BFR-CA-15991 | VN    | Cc   | 0                               | 0                 | 0                              | 0                             | 1                         | 0               | <b>tet(O/M/O)-catA9-tnp</b> <sub>IS1216</sub> family- <b>fexA-optrA-tnp</b> <sub>IS1216</sub> family- <b>tet(L);aac(6')-le/aph(2'')-la-aadE1-tet(O)</b> <sub>X<sub>ΔC</sub>-terminus</sub> ; <i>bla</i> <sub>OXA-193</sub> ;23S_A2075G; 23S_A2075G; 23S_A2075G;GyrA_T86I  |  |
| BFR-CA-16040 | VN    | Cc   | 1 (-)                           | 0                 | 0                              | 0                             | 1                         | 0               | <b>tnp</b> <sub>ISCo2</sub> family- <b>catA13-aph(3')-IIIa-aad9</b> ;tet(O/M/O)- <i>aad9-erm</i> (B)- <i>aadE1</i> ;aac(6')-le/aph(2'')-la-aadE1-tet(O) <sub>X<sub>ΔC</sub>-terminus</sub> ; <i>bla</i> <sub>OXA-489</sub> ;GyrA_T86I   |  |
| BFR-CA-16046 | VN    | Cc   | 2 (-)                           | 1                 | 1                              | 6                             | 2                         | 1               | <b>tnp</b> <sub>ISCo2</sub> family- <b>catA13-aph(3')-IIIa-aad9-aph(2'')-If-<i>bla</i><sub>OXA-193</sub></b> ;tet(O/M/O)- <i>aad9-erm</i> (B)- <i>aadE1</i> ;aacE-Cc;GyrA_T86I  |  |
| BFR-CA-16077 | VN    | Cj   | 1 (-)                           | 0                 | 0                              | 0                             | 1                         | 0               | <b>tnp</b> <sub>ISCo2</sub> family- <b>catA13-aph(3')-IIIa-aad9-aph(2'')-If</b> ;tet(O/M/O)- <i>aadE1-tet</i> (O) <sub>X<sub>ΔN</sub>-terminus</sub> ; <b>aph(3')-IIIa-tnp</b> <sub>ISCaJe6</sub> family; <i>bla</i> <sub>OXA-184</sub> family; <b>tnp</b> <sub>ISCo2</sub> family- <b>lnu(C)</b> ;GyrA_T86I                                  |  |
| BFR-CA-16088 | VN    | Cj   | 1 (-)                           | 0                 | 0                              | 0                             | 1                         | 0               | <b>tnp</b> <sub>ISCo2</sub> family- <b>catA13-aph(3')-IIIa-aad9-aph(2'')-If</b> ;tet(O/M/O)- <i>aadE1-tet</i> (O) <sub>X<sub>ΔN</sub>-terminus</sub> ; <b>aph(3')-IIIa-tnp</b> <sub>ISCaJe6</sub> family; <i>bla</i> <sub>OXA-184</sub> family; <b>tnp</b> <sub>ISCo2</sub> family- <b>lnu(C)</b> ;GyrA_T86I;23S_A2075G;23S_A2075G;23S_A2075G |  |
| BFR-CA-16110 | VN    | Cc   | 0                               | 0                 | 0                              | 0                             | 1                         | 0               | <i>tet</i> (O)- <i>aad9-erm</i> (B)- <i>aadE1</i> ; <i>bla</i> <sub>OXA-193</sub> ;GyrA_T86I  |  |
| BFR-CA-16201 | VN    | Cc   | 0                               | 0                 | 0                              | 0                             | 2                         | 1               | <i>tet</i> (O)- <i>aad9-erm</i> (B)- <i>aadE1</i> ; <b>tnp</b> <sub>ISCo2</sub> family- <b>catA13-aph(3')-IIIa-aad9-aph(2'')If-<i>bla</i><sub>OXA-193</sub></b> ;tet(O);GyrA_T86I   |  |
| BFR-CA-16258 | VN    | Cc   | 1 (+)                           | 1                 | 0                              | 0                             | 2                         | 1               | <i>tet</i> (O)- <i>aad9-erm</i> (B)- <i>aadE1</i> ;tet(O); <i>bla</i> <sub>OXA-193</sub> ;GyrA_T86I   |  |
| BFR-CA-16297 | VN    | Cc   | 0                               | 0                 | 0                              | 0                             | 1                         | 0               | <i>tet</i> (O/M/O)- <i>aad9-erm</i> (B)- <i>aadE1</i> ; <b>aph(2'')-If-aph(3')IIIa-tnp</b> <sub>ISCaJe6</sub> family; <i>aac</i> (6')-le/aph(2'')-la-aadE1-tet(O) <sub>X<sub>ΔC</sub>-terminus</sub> ; <b>tet(O)-tnp</b> <sub>IS607</sub> family; <i>bla</i> <sub>OXA-193</sub> ;GyrA_T86I  |  |
| BFR-CA-16737 | DE    | Cj   | 2 (+)                           | 0                 | 1                              | 6                             | 2                         | 1               | <i>bla</i> <sub>OXA-185</sub> like;tet(O);GyrA_T86I   | <i>tet</i> (O/32/O)- <i>aadE2_Δ1-415-sat4-aph(3')-IIIa</i> |
| BFR-CA-18842 | VN    | Cc   | 0                               | 0                 | 0                              | 0                             | 1                         | 0               | <i>aac</i> (6')-le/aph(2'')-la-aadE1-tet(O) <sub>X<sub>ΔC</sub>-terminus</sub> ; tet(O/M/O); <i>bla</i> <sub>OXA-193</sub> ;GyrA_T86I   |  |
| BFR-CA-19087 | DE    | Cc   | 3 (+)                           | 0                 | 0                              | 0                             | 2                         | 1               | <b>tnp</b> <sub>IS607</sub> family- <b>tet(O/32/O)-aph(2'')-li-aph(3')-IIIa-aad9-aadE1-tet(O)</b> <sub>X<sub>ΔN</sub>-terminus</sub> ;tet(O/32/O);aacE-Cc;GyrA_T86I   |  |
| BFR-CA-19301 | VN    | Cj   | 0                               | 0                 | 0                              | 0                             | 1                         | 0               | <b>aadE3-sat4-aph(3')IIIa-tnp</b> <sub>IS1216</sub> family;tet(O/32/O); <i>bla</i> <sub>OXA-193</sub> ;GyrA_T86I  |  |

Bold numbers, plasmid predictions based on short-read sequence data are consistent with ONT data; (+), true; (-), false prediction of AMR gene localization on plasmids compared to ONT data. Genes in bold depict AMR determinants located on the chromosome in proximity to transposase genes. 50S\_L22\_A103V mutation was omitted due to absence of resistance phenotype; VN, Vietnam; DE, Germany; Spec, Species; Cj, *C. jejuni*; Cc, *C. coli*

from Vietnam, the aminoglycoside phosphotransferase gene *aph(2'')-If* ( $n=76$ ) and the gene *aac(6')-Ie/aph(2'')-Ia* ( $n=38$ ) were found, the latter coding for a bifunctional enzyme combining a phosphotransferase with an N-acetyltransferase. Both resistance determinants also confer resistance to kanamycin.

Kanamycin resistance was further associated with the presence of the aminoglycoside phosphotransferase *aph(3')-IIIa*. In total 160 isolates contained this gene ( $n_{VN}=106$ ,  $n_{DE}=54$ ) and were phenotypically resistant to kanamycin. Among them were 97 isolates ( $n_{VN}=96$ ,  $n_{DE}=1$ ) with a combination of *aph(3')-IIIa* and either *aph(2'')-If* ( $n_{VN}=73$ ) or the bifunctional gene ( $n_{VN}=21$ ), both conferring gentamicin and kanamycin resistance, or *aph(2'')-li<sub>1</sub>* ( $n_{DE}=1$ ). Furthermore, two additional *C. coli*<sub>VN</sub> (BfR-CA-16297, BfR-CA-18728) harbored a combination of *aph(3')-IIIa*, *aph(2'')-If* and the bifunctional gene and, thus, acquired two genetic determinants redundantly encoding a gentamicin and kanamycin modifying enzyme and a further enzyme for kanamycin inactivation. Intriguingly, long-read sequencing even revealed two isolates (BfR-CA-16077, BfR-CA-16088) with two copies of *aph(3')-IIIa* in combination with *aph(2'')-If*. Within the test ranges of gentamicin (0.12–16 mg/L) and kanamycin (2–1024 mg/L), we could not observe increased MIC values for isolates containing multiple redundant resistance determinants compared to isolates only harboring a single gene.

#### Resistance to the aminoglycoside streptomycin

Four variants of *aadE* genes (*aadE-Cc* and *aadE* 1, 2, 3, Fig. 4), coding for aminoglycoside 6-adenylyltransferases and two additional point mutations in the *rpsL* ribosomal gene were associated with streptomycin resistance in the *Campylobacter* spp. isolates. The predominant streptomycin resistance gene in Vietnam was *aadE1* (WP\_001255868.1,  $n_{VN}=82$ ,  $n_{DE}=2$ ). AMRFinderPlus identified a partial *aadE1* gene (88.9% protein sequence coverage) in two of these isolates from Vietnam (BfR-CA-19112, BfR-CA-19119), which displayed resistance to streptomycin. Mapping of raw reads to reference gene *aadE1* revealed the presence of the full-length gene, thus indicating an assembly error. Both isolates additionally carried a partial *aadE2* ( $\Delta 1-109$  bp) as verified by extraction of the Bakta annotated coding sequences and subsequent alignment to a reference gene (NG\_047393.1, Table S3). This observation explained streptomycin resistance in these two isolates. Hence again, the presence of redundant homologous genes resulted in contig breaks during the assembly process, impeding the accurate reconstruction of genes from short-read sequences. In total, mapping of reads to template *aadE2* revealed eight isolates displaying truncated non-functional AadE2 (WP\_001255866.1), among them three streptomycin

sensitive isolates from Germany (BfR-CA-16737, BfR-CA-16834, BfR-CA-19311), confirming loss of function of AadE2 due to truncation (*aadE2* $\Delta 1-415$ ). The five isolates from Vietnam also contained full length *aadE1*, consistent with their streptomycin resistant phenotype. The AadE3 variant (WP\_057035408.1), exclusively found in isolates from Germany ( $n=29$ ), is missing in the AMRFinderPlus database and was, thus, only found by manual ABRicate search using the *aadE3* reference nucleotide sequence (Table S3). The AadE-Cc variant (WP\_002785795.1) was detected in *C. coli*<sub>VN</sub> ( $n=11$ ) and *C. coli*<sub>DE</sub> ( $n=8$ ). While three isolates from Vietnam and one from Germany in addition contained the *aadE1*, one isolate from Germany displayed streptomycin sensitivity, corresponding to a *aadE-Cc* with a point mutation ( $\Delta A558$ ; p.A187LfsTer188) leading to early termination of translation, correctly annotated by AMRFinderPlus.

A point mutation in the RpsL ribosomal protein was rare and only observed in isolates from Vietnam. The RpsL K43R point mutation was present in 10 *C. coli* and 5 *C. jejuni* isolates, while one *C. coli* harbored the RpsL K88R mutation (BfR-CA-18880). Isolates carrying either RpsL K43R or RpsL K88R were resistant to streptomycin (MIC > 16 mg/L). One of these isolates (BfR-CA-18738) additionally carried the *aadE1* gene.

#### Resistance to the aminoglycoside spectinomycin

Spectinomycin resistance was widespread among isolates from Vietnam ( $n=116$ ) and rare among isolates from Germany ( $n=2$ ). In our study, the presence of a gene encoding the spectinomycin adenylyltransferase Aad9 (WP\_002578722.1) was associated with high-level resistance (MIC of 256 to >512 mg/L) and was carried by 80.8% *C. coli*<sub>VN</sub> and 23.2% *C. jejuni*<sub>VN</sub> isolates, as well as by the two *C. coli*<sub>DE</sub> isolates (Fig. 3).

In the majority of spectinomycin resistant isolates ( $n=82/118$ ), the AMRFinderPlus identified the presence of a truncated version of *aad9* (69.8 to 88.0% gene coverage to WP\_002578722.1). Again, this was partially due to an inability of correct identification of full-length *aad9* genes from short-read sequence data caused by the presence of multiple copies of *aad9*, confirmed by long-read sequencing (e.g. BfR-CA-16040 and BfR-CA-16046). Additionally, we observed frameshifts within a putative poly-C tract present in the resistance gene, leading to a truncated Aad9 protein. However, all isolates, carrying *aad9* showed phenotypic resistance. We wondered whether *aad9* inactivation by poly-C was only present in a subpopulation of the bacterial suspension and/or whether frame-shifting can lead to restoration of a full-length protein. Indeed, when we mapped raw reads to an *aad9* *C. coli* reference gene linked to the reference protein WP\_057031337.1 (Acc. NZ\_CP091310.1:1,750,066–1,750,845), we detected a variable number of cytosines

in the frame shift region in some of the sequences, suggesting that *aad9* undergoes phase variation. To identify potential reversal of the correct number of cytosines in the poly-C tract associated with phenotypic resistance, we subjected one of the isolates, BfR-CA-15987, to selection pressure on ColB<sub>A</sub> plates supplemented with 128 mg/L spectinomycin, followed by whole genome sequencing analysis. Analysis of sequence data before and after selection on spectinomycin showed that spectinomycin selected for BfR-CA-15987 clones with one additional cytosine within the poly-C tract, restoring the full-length gene (Figure S7).

### Resistance to chloramphenicol and florfenicol

Resistance to antibiotics from the phenicol group was only observed in the isolates from Vietnam. About 58% ( $n=57$  of the *C. coli* and 23% ( $n=36$ ) of the *C. jejuni* isolates carried one or multiple phenicol modifying enzymes and showed resistance to chloramphenicol (MIC 32 to >128 mg/L) (Fig. 3 and Table S1). The most common resistance determinant was a gene (*catA13*) coding for a type A-13 chloramphenicol O-acetyltransferase (WP\_040564913.1; Fig. 4). This resistance gene was present in all except five chloramphenicol resistant isolates, either alone or in combination with *catA9* ( $n=14$ ), encoding a type A-9 chloramphenicol O-acetyltransferase (WP\_001010387.1). The *catA9* determinant was also present in the residual five chloramphenicol resistant isolates. The point mutation in *catA9* observed in two chloramphenicol resistant *C. coli* isolates (BfR-CA-16261, BfR-CA-18728), leading to a single amino acid substitution (p. A197T) in CatA9, was falsely annotated as *catTC* gene with an internal stop codon by AMRFinderPlus.

One sensitive *C. coli* (BfR-CA-16259) displayed a *catA* gene, which corresponded to a protein of 262 amino acids and was N-terminally identical to CatA13 until P179 (CatA13\_p.L180-K207delins180-262). The C-terminus was different from CatA proteins. The gene was “correctly” found as a partial *catA13* gene by AMRFinderPlus.

Furthermore, 15.2% *C. coli* ( $n=15$ ) and 1.9% *C. jejuni* ( $n=3$ ) were highly resistant to florfenicol (MIC values of >16 mg/L; Table S1). The two resistance genes coding for a florfenicol exporter protein A (*fexA*) and an ABC-F type ribosomal protection protein (*optrA*), respectively, were found to be associated with high level florfenicol resistance. In the majority of highly resistant isolates, both genes were present ( $n_{C.coli}=12$ ,  $n_{C.jejuni}=3$ ); just three *C. coli* isolates either harbored *fexA* (BfR-CA-15989) or *optrA* (BfR-CA-16261, BfR-CA-18728), indicating that either gene might be sufficient for high level florfenicol resistance. Medium-level resistance (MIC 8–16 mg/L) could not be attributed to the presence of a genetic determinant ( $n=11$ ; Table 3 and S1).

### Resistance to $\beta$ -Lactams

Genes encoding oxacillinases (class D  $\beta$ -lactamases) of the OXA-61- or -184-like family were identified in 215 (89.6%) and 244 (96.1%) isolates from Germany and Vietnam (Table S1 and S3) by AMRFinderPlus, respectively. The predominant variant found was *bla*<sub>OXA-193</sub>, which accounted for 61.2% of identified *bla*<sub>OXA</sub> genes (281/459). Other variants that were found more frequently were *bla*<sub>OXA-489</sub>, *bla*<sub>OXA-184</sub>, and *bla*<sub>OXA-460</sub>. Overall, 21 different *bla*<sub>OXA</sub> genes were identified and further variants with yet unknown point mutations, belonging to either *bla*<sub>OXA-61</sub> or *bla*<sub>OXA-184</sub> family genes. Genes of the OXA-184-like family were only detected in *C. jejuni* isolates. Susceptibility to ampicillin was tested in approximately 30% of the isolates, demonstrating resistance to ampicillin with MIC values ranging from 32 to >512 mg/L in the presence of a *bla*<sub>OXA</sub> gene, except for one strain. This *C. jejuni* from Germany (BfR-CA-14940) displayed a MIC of 16 mg/L ampicillin, just below the ECOFF for resistance, but carried a *bla*<sub>OXA-193</sub> gene. We analyzed the promoter of the *bla*<sub>OXA</sub> gene in this isolate using the Geneious software. It was found previously that a transversion (G to T) at position -57 restored the Pribnow box, leading to up-regulation of *bla*<sub>OXA</sub> and high-level  $\beta$ -lactam resistance [69]. Indeed, this point mutation was missing in BfR-CA-14940, thus potentially explaining the low observed MIC for ampicillin. Consistently, isolates carrying *bla*<sub>OXA</sub> with lower MIC values between 32 and 64 mg/L also did not harbor the optimal Pribnow box for increased *bla*<sub>OXA</sub> transcription. There was one exception to the rule (BfR-CA-16023), carrying a *bla*<sub>OXA</sub> gene with the non-optimal Pribnow box but displaying a MIC value of 256 mg/L. Furthermore, one *C. coli*<sub>VN</sub> isolate was detected, which did not harbor a *bla*<sub>OXA</sub> gene, but showed slight ampicillin resistance just above the ECOFF (MIC=32 mg/L).

### Resistance to Nourseothricin

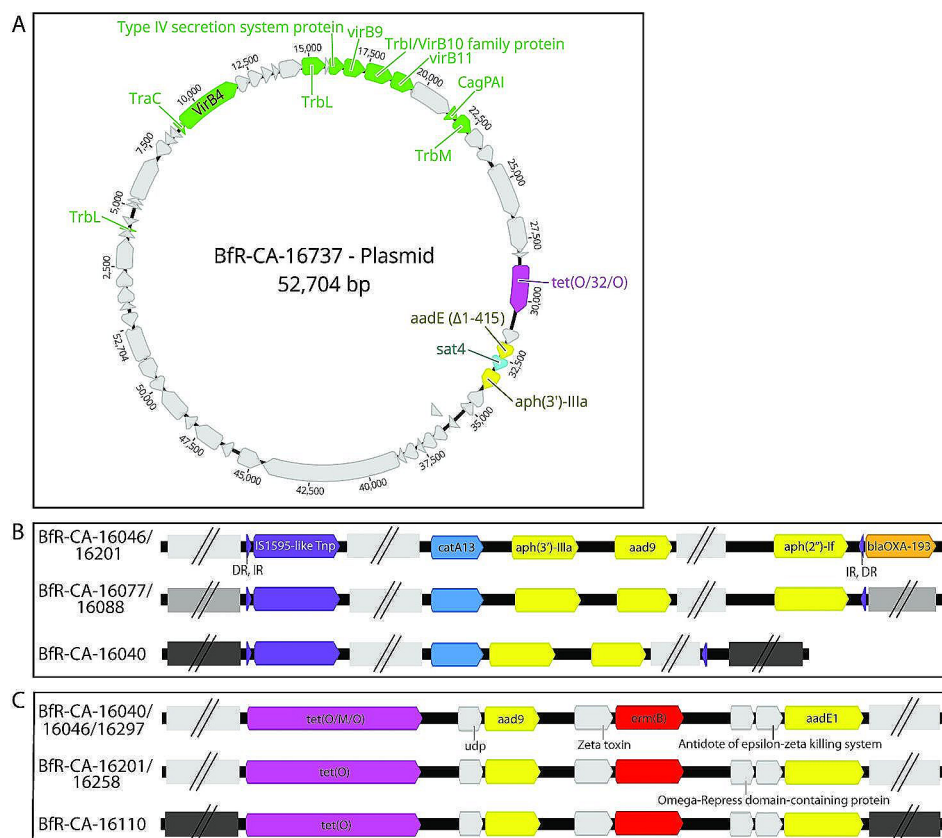
The resistance determinant *sat4*, encoding a streptothricin N-acetyltransferase, accounted for resistance to nourseothricin, a mixture of streptothricins C, D, E and F. Isolates carrying *sat4* showed MIC values between 8 and 512 mg/L nourseothricin, while the respective sensitive isolates without *sat4* had MICs of  $\leq 1$ –2 mg/L. Although an ECOFF value is not yet officially published, we defined >4 mg/L as elevated non-wildtype MICs for our study to categorize sensitive and resistant isolates (Table 1). The *sat4* resistance determinant was more common in Germany with 21.7% *C. coli* ( $n=25/115$ ) and 20.8% *C. jejuni* ( $n=26/125$ ) harboring *sat4* compared to only five isolates from Vietnam. The translated protein sequences showed high similarity to the reference protein WP\_000627290.1.

### Prediction of localization and mobilization of AMR genes

The tool Platon v1.6 was used for annotation of plasmid localization of AMR genes based on short-read data. For verification, selected isolates were also processed by Oxford Nanopore long-read technology (n=14). All fourteen genomes could be closed using the Unicycler hybrid assembler and the chromosomes displayed a size between 1.62 and 1.82 Mb, while six isolates carried an additional circular plasmid of 3.3 kb to 52 kb (Table 4). AMR genes in these isolates were mostly found on the chromosome. Only two plasmids carried either a *tet(O)* gene (BfR-CA-15687) or an operon containing *tet(O/32/O)* – *aadE2\_Δ1-415* – *sat4* – *aph(3')-IIIa* (BfR-CA-16737). We asked whether the current plasmid prediction from short-read data using the Platon tool corresponded to the closed genomes/plasmids upon long-read sequencing within our dataset. We observed that the annotation of “plasmid contigs” by Platon overestimated plasmid existence in three isolates (false positives), while missing the plasmid in one isolate (false negative, Table 4).

The prediction of the presence of either/and (i) a circular plasmid, (ii) mobilization or (iii) conjugation elements (Table S1, column BC, BD, BE) led to missing two plasmid-containing isolates. However, false positive results were lacking. If this conservative filter was applied for all short-read data (Table S1, column BF>0), 183 of the total 494 isolates were predicted to carry one or multiple plasmid/s. However, prediction of plasmid-location of AMR genes seemed to be inaccurate based on the Platon tool optimized for other bacteria such as *Escherichia coli*: only one out of the two plasmids, which contained AMR gene/s, was detected by Platon and a further four isolates were falsely annotated as carrying AMR genes on plasmids based on short-read data.

Based on long-read sequencing data and hybrid assemblies using Unicycler, we further investigated the localization of AMR gene clusters and their mobilization potential using AMRFinderPlus. In principle, we found three types of AMR gene localizations that suggest different mobilization of AMR genes (Fig. 5). As mentioned



**Fig. 5** Mobilizable MDRI clusters and AMR genes in *Campylobacter* spp. identified by long-read sequencing. Localization of AMR clusters on **A**, plasmid, **B** and **C**, the chromosome, with **B** in proximity to transposase genes. All elements are mobilizable by natural transformation in *Campylobacter* spp. In addition, conjugative transfer (**A**) and transposition (**B**) is likely to occur. In **A**, genes associated with type IV secretion/conjugation are depicted in green; in **B**, transposase genes and associated direct (DR) and inverted repeats (IR) are marked in purple. AMR genes from different antimicrobial classes are depicted in different colors; blue, *catA* genes, yellow, aminoglycoside resistance genes; orange, *bla<sub>OXA</sub>* gene; light purple, *tet(O)* variant genes; red, *erm(B)* genes. Grey arrows, non-AMR related genes; grey boxes with two vertical lines indicate clusters of non-AMR related genes, with homology indicated using identical shading



above, plasmid localization of AMR genes was rare. Only one *tet(O)* gene in a *C. coli* isolate and the operon structure *tet(O/32/O) – aadE2\_Δ1-415 – sat4– aph(3')-IIIa* in a *C. jejuni* were plasmid-located within the long-read sequenced isolates (Fig. 5A), thus, being transferable via conjugation. Chromosomal AMR genes, like e. g. the gene cluster *tet(O) – aad9 – erm(B) – aadE1* can be transferred by natural transformation, depending on homologous recombination. Likewise, we observed that the gene context of this MDRI was rather stable in the analyzed long-read sequenced isolates, with five out of six isolates displaying homologous gene context flanking the MDRI (Fig. 5C). Also the chromosomal *aac(6')-Ie/aph(2'')-Ia– aadE1 – tet(O)<sub>ΔC-terminus</sub>* gene cluster was embedded in a highly conserved genomic region in the analyzed four isolates, which is expected for mobilization via natural transformation. However, we frequently found chromosomal MDRI in proximity to a transposase gene. For example the *catA13 – aph(3')-IIIa – aad9* MDRI was situated in three different chromosomal contexts with and without additional adjacent AMR genes in five analyzed isolates (Fig. 5B). Hence, this MDRI was putatively disseminated by natural transformation and transposition, thereby enhancing the movement within a bacterial chromosome but also among the bacterial population. A similar mechanism of transfer might be predicted for other MDRI as well as for single AMR genes in proximity to transposase genes, e. g. *aph(2'')-If– aph(3')-IIIa* in *C. coli* BfR-CA-16297, *aadE3 – sat4– aph(3')-IIIa* in *C. jejuni* BfR-CA-19301, *lnu(C)* or a second copy of *aph(3')-IIIa* in *C. jejuni* BfR-CA-16077 and BfR-CA-16088, *tet(O/M/O) – catA9 – fexA – oprA – tet(L)* in *C. coli* BfR-CA-15991 and *tet(O/32/O) – aph(2'')-li<sub>1</sub> – aph(3')-IIIa – aad9 – aadE1 – tet(O)<sub>ΔN-terminus</sub>* in *C. coli* BfR-CA-19087 (Figure S8).

## Discussion

The study aimed to improve AMR diagnostics of thermotolerant *Campylobacter* spp. by elucidating the reliability of predictions for antimicrobial resistances from whole genome sequence data. Within nearly 500 investigated isolates, whole genome cgMLST results suggested a broad diversity of isolates, constituting a suitable data source for in-depth AMR analysis. We detected 14 different resistance genes and genes with point mutations in isolates from Germany and 22 different AMR determinants associated with antibiotic resistance in the *Campylobacter* spp. population from Vietnam. Each identified resistant determinant was correlated to phenotypic resistance against the respective antimicrobial. Any discrepancies were re-analyzed. Our study showed high rates of aminoglycoside, (fluoro-)quinolone, macrolide, phenicol and tetracycline resistance in isolates from Vietnam, which is likely related to the extensive use of

antibiotics on farms [24] and comparable to those previously reported in other Asian countries such as China, Malaysia, and the Philippines [69–72]. Resistance to (fluoro-)quinolones and tetracycline was also frequent in isolates from Germany, while resistances to aminoglycosides and macrolides were comparably low, which is in line with recent data from Germany [73].

Our main question was whether current next generation sequence data analysis pipelines are prepared for appropriate detection of potential worldwide spread of multi-resistant *Campylobacter* spp. With our systematic approach we observed five principle discrepancies between pheno- and genotype in thermotolerant *Campylobacter* spp.

### Missing or falsely annotated AMR genes in databases

First, certain AMR genes were either missing in the AMRFinderPlus and ResFinder databases (*aadE3*), although previously published [74] or falsely annotated to confer resistance in *Campylobacter* spp. in the AMRFinderPlus database (ribosomal L22 protein A103V mutation). Despite the previous suggestion that the point mutation A103V in the ribosomal protein L22 may confer resistance to macrolides [66], our findings do not show a correlation between this mutation and erythromycin resistance. This is consistent with the conclusion reached by others, who also found no association between A103V and resistance to macrolides [30, 75]. Furthermore, the mosaic gene variant *tet(O/32/O)* is also missing in the AMRFinderPlus database (version 2023-08-08.2) and was identified by the pipeline as *tet(O)* with reduced identity (~93%), thus, at least not causing a pheno-/genotype discrepancy. However, ResFinder database 2.1.0 includes this variant. The above mentioned inconsistencies can be easily addressed by curation and harmonization of the databases.

### Detection of *tet(O)*, *aadE* genes and *aad9* partially failed due to frequently observed presence of multiple copies or variant genes

Second, short-read sequencing eventually failed or falsely detected partial (inactive) AMR genes, if multiple copies and/or homologous mosaic genes were present. This was, in particular, the case for *tet(O)* but also for *aadE* gene variants and multiple copies of *aad9*. In the *Campylobacter* population from Vietnam, there was a high prevalence of isolates with either two identical or two distinct variants of the (mosaic) tetracycline resistance genes (Table S1). The assembler used may have encountered difficulties in generating complete resistance gene sequences from raw reads due to regions of ambiguity within the assembly process. Consequently, either incomplete genes were identified in these isolates, or the sequencing reads were inadequate in length and did not

meet the pipeline's coverage threshold, which resulted in "absence" of AMR gene detection (Table 3). As proof of principle SKESA as alternative assembler was used for the assembly of short-read sequencing data of 10 isolates, for which detection of some AMR genes failed using the shovill assembler (Table S2). However, the results were similar, except that in one isolate the full-length copy of *aad9* was, in addition, falsely detected as "partial" upon SKESA assembly. In another isolate, in which *tet(O)* was missing upon shovill assembly, SKESA assembly led to the detection of a partial *tet(O)*. In a study with commensal *E. coli*, short-read sequencing was capable of detecting only one copy of each duplicated resistance gene, yet the authors did not observe partial or unidentified genes arising from allelic variants [76].

Under our test conditions, we did not observe any functional differences between the *tet(O)* or *aadE* variants, nor enhanced resistance levels were detected, if isolates carried multiple copies of resistant gene variants (verified by long-read sequencing). Thus, so far the impact or purpose for redundant genetic determinants in *Campylobacter* spp. remains unknown. It might be speculated that redundant genes are located in a gene context with essential/other AMR genes, thereby, being co-transferred. Most initial discrepancies from AMRFinder with its default thresholds were resolved by manual search of missing genes via ABRicate and by mapping of raw reads to reference genes using Geneious Prime (as exemplified in Figure S4). Mosaic tetracycline genes such as *tet(O/M/O)* and *tet(O/32/O)* variants were previously found [77, 78] and in this study, we showed differential distribution of these variant genes in different *Campylobacter* populations. Yet, the complexities arising from the diverse recombinant forms of *tet(O)* within *Campylobacter* isolates from Vietnam could not be conclusively resolved unless long-read sequencing was applied. Long-read sequencing by Oxford Nanopore Technology deciphered multiple copies of AMR genes (including multiple identical genes and or partial genes) and, furthermore, revealed AMR gene localization (Table 4), which was frequently inconsistent with predictions from short-read sequencing. Hence, a combination of short- and long-read sequencing may circumvent inconsistencies caused by the presence of multiple AMR gene (variants) with the additional benefit of identification of AMR gene location.

#### **Novel point mutations in *tet(W)* led to AMR gene inactivation, while *aad9* was identified as phase variable gene**

Third, while some partial genes harboring point mutations were correctly identified by the pipeline, we identified novel point mutations D171N/G579D in *Tet(W)*, leading to a tetracycline sensitive phenotype

(BfR-CA-16942 and BfR-CA-18353). Furthermore, in case of the *aad9* gene, around 70% of the isolates were annotated to display a truncated inactive version of *aad9*, but those isolates were indeed resistant to spectinomycin. Next to assembly problems due to multiple copies of *aad9*, we revealed weakness of the assembling process to correctly identify the poly-C tract variant of functional *aad9*. This was probably due to a mixture and ambiguity of raw reads with different number of cytosines within this novel phase variable gene (Figure S7). As proof of principle we reselected an isolate annotated as harboring an inactive *aad9* gene on spectinomycin and after re-sequencing, we were able to correctly identify the full-length *aad9* gene. This observation is in agreement with reversion to a functional gene by insertion/deletion of cytosines, explaining the phenotypic resistance observed in the antimicrobial sensitivity tests. Thus, we concluded that *aad9* is frequently inactivated by frameshifting, but the isolates keep resistance to spectinomycin as a bacterial population due to the reversion of the frameshift. Phase variation was proposed an important mechanism for regulation of several genes in *Campylobacter* spp., in particular for host response, like e.g. the *flgR/S* system, essential for motility [79, 80]. Here, it might balance the cost for AMR gene carriage and suggests prolongation of persistence of the AMR gene.

#### **MIC values just above the cut-off probably display non-specific resistance due to enhanced efflux and/or decreased inward diffusion**

Fourth, discrepancies were identified for isolates with MIC values close to the cut-off value. Most frequently, we found isolates without any known resistance determinant but with slight resistance according to the current ECOFF or elevated non-wildtype MICs. This was the case for four lincosamide and eleven florfenicol resistant isolates and for one isolate resistant to ampicillin (Table 3). Low level resistance without known gene determinants might be promoted by increased efflux or decreased influx mechanisms [80–83]. It has been previously reported that inactivation of the ABC-efflux transporter CmeABC led to increased sensitivity to a variety of antimicrobials such as (fluoro-)quinolones, macrolides, phenicols and tetracyclines [84, 85]. Low level ampicillin resistance was due to the presence of the non-optimal Pribnow box in the promotor region, if *bla<sub>OXA</sub>* genes were present (see above and [69, 86]). We identified a further exceptional isolate (BfR-CA-16023) carrying a *bla<sub>OXA</sub>* gene with the non-optimal Pribnow box but displaying a MIC value of 256 mg/L. As for the slightly ampicillin resistant isolate BfR-CA-19104 without *bla<sub>OXA</sub>*, there might be additional efflux and/or decreased influx mechanisms, which await further investigations.

### Unknown resistance mechanisms in *Campylobacter* spp. remain elusive

Fifth, we found isolates harboring unknown resistance mechanisms. One gentamicin resistant isolate from German turkey cecum (BfR-CA-15687) did not harbor any of the known resistance determinants but repeatedly showed high level resistance to gentamicin (MIC > 16 mg/L). The isolate was also resistant to (fluoro-)quinolones (GyrAT86I) and tetracycline (*tet*(O)) and carried a *bla*<sub>OXA-489</sub> gene. Further studies are needed to identify the unknown gentamicin resistance mechanism.

As previously confirmed by other studies and reiterated by our WGS results, the single point mutation T86I in the “quinolone resistance determining region” (QRDR) of the gyrase subunit A confers resistance to (fluoro-)quinolones in *Campylobacter* spp. [33, 87, 88]. This widespread resistance in *Campylobacter* isolates is likely due to the demonstrated fitness advantage that it confers at least in some *C. jejuni* isolates [89]. However, we found several isolates, harboring the point mutation GyrA T86I and displaying high-level resistance to ciprofloxacin but complete sensitivity to nalidixic acid (Table 3 and S1). This phenomenon was previously found by others [29, 90] but the underlying mechanism is yet unsolved. We conclude that the point mutation alone is not sufficient for both resistances to ciprofloxacin and nalidixic acid and the overall (fluoro-)quinolone resistance mechanism in *Campylobacter* remains enigmatic.

### Fitness costs of AMR and Impact of AMR gene localization on transfer and spread

ONT sequencing and hybrid assembly of long- and short-reads led to closure of circular contigs, the chromosome and potential plasmids. Hybrid assembly data resulted in improved identification of multiple AMR gene(s) variants, which were non-resolvable by only short-read analysis. Interestingly, *Campylobacter* populations in Germany and Vietnam showed distinct patterns of gene variants, e.g. *tet*(O/M/O) in Vietnam and *tet*(O/32/O) in Germany. The reason for the acquisition of redundant resistance mechanisms by the isolates is uncertain. In our analysis we could not find enhanced levels of resistance due to multiple resistance determinants, since the presence of one copy already led to high level resistance of the AMR investigated. However, if selection is exerted on AMR genes situated within AMR gene clusters, also neighboring AMR genes are co-selected and transferred from one isolate to another. We conclude that AMR genes in *C. jejuni* and *C. coli* were frequently organized in mobilizable MDRI next to transposase genes and different MDRI harbored multiple AMR genes with analogous function. Hence, these isolates appear to be perfectly prepared for a changing selective environment

and additionally harbored transiently non-functional AMR genes, which might be restored under selection pressure.

Interestingly, most AMR genes appeared to be chromosomally located, frequently in association with transposase genes (Fig. 5). Plasmid prediction from short-read data was limited, while long-read data identified 43% strains carrying a plasmid (n = 6/14). From these isolates, only two plasmids were identified with AMR genes, one harbored *tet*(O), the other *tet*(O/32/O) – *aadE2* Δ1-415 – *sat4* – *aph*(3′)-IIIa. This is consistent with previous findings in the literature, where plasmids containing tetracycline resistance genes were reported in *Campylobacter*, such as the self-transferable plasmid pTet and *tet*(O) associated AMR gene clusters [91, 92]. Previous research has demonstrated the existence of the resistance gene cluster *aadE* – *sat4* – *aph*(3′)-IIIa, located on both the chromosome and plasmids, in *C. jejuni* and *C. coli* isolates [74, 92–95]. These findings align with our results from long-read sequencing. It is noteworthy that the use of streptothricin was restricted to the former German Democratic Republic, and ceased by 1989 at the latest, while therapeutic use in humans has been halted due to its nephrotoxicity [96]. It is possible that the *sat4* gene is conserved to some degree as it is co-flanked within the aminoglycoside resistance conferring genes *aadE* and *aph*(3′)-IIIa, that might provide an advantage to *Campylobacter* in Germany and explain the observed preferential presence of *sat4* in isolates from Germany.

Spread of macrolide resistance is of great concern, since in particular macrolides are the drug of choice to treat campylobacteriosis in humans [9]. The point high level resistance conferring mutation A2075G in the 23S rRNA was shown to result in a substantial decrease in bacterial fitness among *C. jejuni* [97, 98]. This fact may explain its low prevalence in areas with a comparably low selection pressure. In regions with high selection pressure, such as Vietnam [25, 99] this mutation was more frequently found (Fig. 3). Additionally, high-level resistance to macrolides and/or lincosamides is also conferred by the emerging resistance gene *erm*(B), which was first described in a *C. coli* strain isolated from swine in China [100]. We showed in our study that phenotypic resistance testing with erythromycin cannot distinguish the presence of the 23S rRNA point mutation from that of *erm*(B), since the MIC distribution of both resistant determinants was comparable (Figure S3). As also observed in our study (Fig. 5), *erm*(B) has already been shown to be part of different MDRI [98, 101, 102] and probably derived from Gram-positive bacteria [103]. In the ONT-analyzed isolates, *erm*(B) was located on the chromosome with a rather conserved gene context, suggesting mobilization via natural transformation (Fig. 5).

We previously showed that natural transformation in *C. jejuni* was a highly efficient process and occurred most frequently under microaerobic conditions at neutral pH, found in the natural hosts [104].

The *catA13-aph(3')-IIIa-aad9* cluster was one of the AMR clusters found in proximity to transposase genes (Fig. 5; Table 4). As expected for transposable elements, the AMR cluster context was rather diverse, with occasional acquisition of additional nearby located AMR genes, like *aph(2'')-If* and *bla<sub>OXA-193</sub>*. Interestingly, in another study from China the two resistance genes *fxaA* and *optrA* were found together as part of an MDRI, which aligns with the data we collected [105]. Given that the two genes were also identified in close proximity to transposases within operon structures among isolates from Vietnam, it is highly probable that they will continue to disseminate. Although chloramphenicol is not commonly used in human medicine due to its bone marrow toxicity, it is still reserved for the treatment of severe infections such as certain types of meningitis, rickettsiae, or typhoid fever [105–109].

The high prevalence and frequent redundant presence of multiple homologous and analogous resistance genes, e. g. *aph(2'')-If*, *aac(6')-Ie/aph(2'')-Ia*, *aph(3')-IIIa* and *aadE* in particular, in the isolates from Vietnam, may reflect regular selection of MDRI, resulting in AMR accumulation. In general, high resistance to aminoglycosides should be regarded as concerning as they are considered a high-priority critically important antimicrobial class according to the World Health Organization [110].

## Conclusion

Our results highlight the extensive presence of various AMR genes and gene variants, as well as point mutations associated with AMR in the investigated *Campylobacter* population. The approach corroborated the necessity for continuous update of databases with respect to novel AMR gene (variants), point mutations leading to (transient) inactivation of AMR and for including long-read sequencing for improved detection of redundant AMR genes and AMR gene locations. Limitations of gene detection from short-read assemblies can partially be dealt with by lowering required coverage thresholds and complementing analysis with read mapping approaches. Furthermore, yet unknown mechanisms for gentamicin and (fluoro-)quinolone resistance, transiently inactive AMR genes and mobilization of MDRI await further investigation. The findings showed elevated levels of resistance depending on the origin of isolation, emphasizing the need for improved surveillance and diagnostics of AMR in thermotolerant *Campylobacter* spp. along the food production chain globally.

## Abbreviations

|           |   |
|-----------|---|
| AMR       | Antimicrobial Resistance  |
| CAMHB/FCS | Cation-supplemented Mueller-Hinton broth with 5% fetal calf serum |
| CC        | Clonal Complex  |
| CI        | Confidence Interval   |
| ColbA     | Columbian Blood Agar  |
| ECOFF     | Epidemiological Cut Off   |
| EDTA      | Ethylenediaminetetraacetic Acid                                   |
| FCS       | Fetal Calf Serum  |
| mCCDA     | Modified Charcoal-Cefoperazone Deoxycholate Agar                  |
| MDRI      | Multidrug Resistance Island                                       |
| MIC       | Minimum Inhibitory Concentration                                  |
| MLST      | Multi-locus sequence typing                                       |
| MST       | Minimum Spanning Tree   |
| ONT       | Oxford Nanopore Technology  |
| OR        | Odds Ratio  |
| QRDR      | Quinolone resistance determining region                           |
| SRA       | Sequence Read Archive   |
| WGS       | Whole-Genome Sequencing   |
| WHO       | World Health Organization   |

## Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12864-024-10014-w>.

Supplementary Material 1

Supplementary Material 2

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## Author contributions

Conceptualization: KS; Methodology, MZ, MK, MT; Investigation, MZ, HQL, HTTH, MK, MT; software, CD; formal analysis, MZ, NB, KS, IH; Writing– original draft, MZ, KS; Writing– review and editing, HQL, CD, PTN, IH; Funding acquisition, HQL, KS. All authors reviewed and agreed upon the final version of the manuscript.

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## Data availability

Raw read sequences and either complete annotated genomes or draft genomes were published within the BioProjects No. PRJNA562653, PRJNA595957, PRJNA648048 and PRJNA872862. All data supporting the findings of this study are provided within the paper and its supplementary information. All additional information are available upon request from the authors.

## Declarations

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Competing interests

The authors declare no competing interests.

### Author details

<sup>1</sup>Department of Biological Safety, Federal Institute for Risk Assessment (BfR), Diederichsstraße 1, Berlin D-12277, Germany

<sup>2</sup>National Institute of Veterinary Research (NIVR), 86 Truong Chinh Street, Hanoi, Dong Da District, Vietnam

<sup>3</sup>Department of Bacteriology, National Institute of Hygiene and Epidemiology (NIHE), 1 Yersin Street, Hanoi, Trung District, Vietnam

<sup>4</sup>Department of Molecular Biology and Gene Technology, Bavarian Health and Food Safety Authority, Oberschleissheim D-85764, Germany

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**6.5 Publication 4: The point mutation A1387G in the 16S rRNA gene confers aminoglycoside resistance in *C. jejuni* and *C. coli***

**The point mutation A1387G in the 16S rRNA gene confers aminoglycoside resistance in *Campylobacter jejuni* and *Campylobacter coli***

Michael Zarske<sup>a</sup>, Christiane Werckenthin<sup>b</sup>, Julia C. Golz<sup>a</sup>, Kerstin Stingl<sup>a,#</sup>

<sup>a</sup>German Federal Institute for Risk Assessment (BfR), Department of Biological Safety, National Reference Laboratory for *Campylobacter*, Berlin, Germany

<sup>b</sup>Lower Saxony State Office for Consumer Protection and Food Safety (LAVES), Food and Veterinary Institute, Oldenburg, Germany

Running Title: Novel aminoglycoside resistance in *Campylobacter* spp.

#correspondence to: Kerstin.Stingl@bfr.bund.de.

**Abstract**

Thermotolerant *Campylobacter* spp. are the most frequent cause of foodborne bacterial diarrhea and high-priority antibiotic-resistant pathogens according to the World Health Organization (WHO). Monitoring revealed current low prevalence of gentamicin resistance in European *Campylobacter* spp. isolates but substantial presence of gentamicin-modifying genes circulating globally.

Using a combined approach of natural transformation and whole genome sequencing, we revealed a novel gentamicin resistance mechanism, namely the point mutation A1387G in the 16S rRNA gene, originally identified in a *C. coli* isolate from turkey caecal content. The

transformation rate of the resistance using genomic DNA of the resistant donor to sensitive recipient *C. jejuni* and *C. coli* was  $\sim 2.5 \log_{10}$  lower compared to the control *rpsL*-A128G point mutation conferring streptomycin resistance. Antimicrobial susceptibility tests showed cross-resistance to apramycin, kanamycin and tobramycin, with transformants exhibiting more than 4- to 8-fold increased MICs to apramycin and tobramycin and over 64-fold higher MICs to kanamycin compared to wildtype isolates. Although transformants showed 177-1235 variations relative to the recipient, only the A1387G point mutation in the 16S rRNA was in common. This mutation was causal for resistance, as transformation of a 16S rRNA\_A1387G PCR fragment into susceptible isolates also led to resistant transformants. Sanger sequencing of the 16S rRNA genes and Oxford nanopore whole genome sequencing of transformants identified clones harboring either all three copies with A1387G or a mixed population of wildtype and mutated 16S rRNA gene alleles. Within 15 passages on non-selective medium, transformants with mixed populations of the 16S rRNA gene copies partially reverted to wildtype, both geno- and phenotypically. In contrast, transformants harboring the A1387G point mutation in all three 16S rRNA gene copies kept full resistance within at least 45 passages. We speculate that partial acquisition and rapid loss of the point mutation limited its spread among *C. spp.* isolates. In-depth knowledge on resistance mechanisms contributes to optimal diagnosis and preventative measures.

## **Introduction**

In 2022, the leading cause of bacterial gastroenteritis in the European Union was again *Campylobacter* spp. with 137,000 reported Campylobacteriosis cases, i. e. more than twice the number of reported *Salmonella* infections (1). Patients with acute campylobacteriosis show symptoms like watery and/or bloody diarrhea, abdominal cramps, fever and nausea (2).

Furthermore, there is a potential for the development of long-term autoimmune sequelae following an acute infection, such as Guillain-Barré syndrome, reactive arthritis and irritable bowel syndrome (3). While antibiotic therapy may not be required for the majority of cases of food-borne campylobacteriosis, patients with severe or persistent infections necessitate antimicrobial treatment (4). Fluoroquinolones and macrolides are the preferred pharmaceutical agents applied in clinics for the treatment of campylobacteriosis (5, 6). However, globally emerging antimicrobial resistances (AMR) are impeding the effectiveness in treatment with these agents, in particular for (fluoro-)quinolones (7-9). Thus, in cases of systemic infection, aminoglycoside antibiotics, specifically gentamicin, persist as the recommended therapeutic option given the susceptibility of *Campylobacter* isolates to this particular class of antibiotics (10).

Aminoglycosides are a class of potent broad-spectrum antibiotics derived from actinomycetes, which have been in use since the 1940s (11). Their primary mode of action involves inhibition of bacterial protein synthesis via blocking elongation or directly inhibiting initiation, with the exact mechanism varying by chemical structure (12-15). Aminoglycosides can be subdivided into two main classes based on the core structure of the aminocyclitol moiety: those derived from streptidine (e.g. streptomycin) and those derived from 2-deoxystreptamine (e.g. gentamicin, kanamycin). The 2-deoxystreptamine-derived aminoglycosides further divide into subclasses based on the specific substitution pattern of their side chains. These structural differences are crucial for their mechanisms of action and susceptibility to various aminoglycoside-modifying enzymes (16). Resistance to gentamicin is rare in Europe (17, 18) but more frequently encountered in China (19, 20), Vietnam (21) and the Philippines (22). Aminoglycoside resistance in *Campylobacter* spp. is mainly attributed to enzymatic inactivation of the aminoglycoside by enzymes. These enzymes include aminoglycoside N-

acetyltransferases (AAC), O-phosphotransferases (APH), and O-nucleotidyltransferases (ANT), which are the predominant mechanisms of resistance (23-25). Resistance to gentamicin, kanamycin and tobramycin is conveyed by the presence of aminoglycoside 2''-phosphotransferase genes (*aph(2'')*) with several distinct variants identified in *Campylobacter* (19, 26-31). Kanamycin resistance is also attributed to the presence of 3'-phosphotransferase genes, like *aph(3')-IIIa* (32) and *aph(3')-VIIa* (33). A recent study found the presence of the resistance gene *apmA* in a *C. coli* isolate which may encode an acetyltransferase for inactivating apramycin (30).

In this study, we report the identification of a novel point mutation in the bacterial A-site of the 16S rRNA in a German *C. coli* isolate that causes resistance to aminoglycosides with a 2-deoxystreptamine structure. We investigated the transferability of this mutation among thermotolerant *Campylobacter* spp. through natural transformation and evaluated its stability under non-selective conditions. Our results highlight the importance of in-depth investigation of the mechanisms of antimicrobial resistance in food-borne pathogens in order to evaluate their spread and persistence. This will improve prediction of resistances using current diagnostics of whole genome sequencing.

## **Results**

### **Identification of a *C. coli* isolate from turkey caecum with an unknown gentamicin resistance mechanism**

Routine resistance monitoring of thermotolerant *Campylobacter* spp. isolated from German turkey in 2018 revealed the presence of a *C. coli* isolate (BfR-CA-15687) displaying gentamicin

resistance with a MIC value >16 mg/L (21). This isolate displayed ST type 10049, which is not yet assigned to a clonal complex. The antimicrobial microdilution assay using the EU-wide harmonized EUCAMP3 plate format was conducted in three independent experiments, all confirming high level gentamicin resistance. In addition, the isolate was resistant to ciprofloxacin and tetracycline (Table 1, Suppl. Figure S1A). Whole genome sequencing based on Illumina short-reads was carried out to identify known resistance determinants. However, no known resistance determinant associated with gentamicin resistance was detected in the assembly or in trimmed reads. As expected for ciprofloxacin and tetracycline resistant *C. spp.*, the presence of the T86I point mutation in gyrase subunit A and two copies of the *tet(O)* resistance gene - one located on the chromosome and the other on the plasmid - were detected. The isolate additionally harbored a *bla*<sub>OXA-489</sub> gene with a G to T transversion at position -57 in the promoter region, expected to restore the Pribnow box and to confer resistance to ampicillin (34), which was not phenotypically characterized. The short-read sequencing was repeated, again leading to lack of any known resistance determinants for the observed gentamicin resistance.

Table 1. MIC values of BfR-CA-15687, including test ranges and resistance evaluation

| Antimicrobial   | Plate format | Test range (mg/L) | resistant > MIC (mg/L) | MIC of BfR-CA-15687 | Evaluation (R, S) |
|-----------------|--------------|-------------------|------------------------|---------------------|-------------------|
| Apramycin       | custom       | 0.03-32           | 16*                    | >32                 | R                 |
| Chloramphenicol | EUCAMP3      | 2-64              | 16                     | 4                   | S                 |
| Ciprofloxacin   | EUCAMP3      | 0.12-32           | 0.5                    | 32                  | R                 |
| Ertapenem       | EUCAMP3      | 0.12-4            | 0.5                    | 0.12                | S                 |
| Erythromycin    | EUCAMP3      | 1-512             | 4 (Cj), 8 (Cc)         | 2                   | S                 |
| Gentamicin      | EUCAMP3      | 0.25-16           | 2                      | >16                 | R                 |
| Kanamycin       | custom       | 1-1024            | 16*                    | >1024               | R                 |
| Streptomycin    | custom       | 0.25-16           | 4                      | 1                   | S                 |
| Tetracycline    | EUCAMP3      | 0.5-64            | 1 (Cj), 2 (Cc)         | >64                 | R                 |
| Tobramycin      | custom       | 0.06-64           | 16*                    | >64                 | R                 |

For resistance evaluation ecological cut-off values (ECOFF) were used, if available or – indicated with \* - elevated non-wildtype MIC. R, resistant; S, sensitive; MIC, minimal inhibitory concentration; Cj, *C. jejuni*; Cc, *C. coli*.

The known gentamicin phosphorylating Aph(2'') enzyme variants not only confer resistance to gentamicin but also to the aminoglycosides kanamycin (KAN) and tobramycin (TOB) (21, 25). Hence, BfR-CA-15687 was also tested for susceptibility to these two aminoglycosides as well as to apramycin (APR) and streptomycin (STR) using custom created plates (Table 1, Suppl. Figure S1B, C). BfR-CA-15687 additionally demonstrated elevated non-wildtype MIC values for APR (>32 mg/L), KAN (>1024 mg/L) and TOB (>64 mg/L) (Table 2). In comparison, the tested wildtype isolates (*C. jejuni* BfR-CA-14430; *C. coli* BfR-CA-14856) and reference strains (81-176; NCTC 11168) displayed sensitivity to GEN and notably lower MIC values for the aminoglycosides APR (4-8 mg/L), KAN (8-16 mg/L) and TOB (4-8 mg/L). Given the absence of determinants linked to APR, KAN or TOB resistance, these results indicated that the unidentified gentamicin resistance determinant might be a potential factor also contributing to the observed elevated MIC values to APR, KAN and TOB (Table 2).

**Table 2.** MIC values of the donor *C. coli* BfR-CA-15687, wildtype recipient isolates and transformant strains to aminoglycosides

|   | Species          | Sample               | MIC [mg/L] |      |       |     |     |
|---|------------------|----------------------|------------|------|-------|-----|-----|
|   |                  |                      | APR        | GEN  | KAN   | STR | TOB |
| <b>Donor</b>  | <i>C. coli</i>   | BfR-CA-15687         | >32        | >16  | >1024 | 1   | >64 |
| <b>Wildtype recipient isolates</b>                      | <i>C. coli</i>   | BfR-CA-11057         | 4          | 0.5  | 16    | 1   | 8   |
|   |                  | BfR-CA-14856         | 8          | 0.5  | 16    | 1   | 8   |
|   | <i>C. jejuni</i> | BfR-CA-14430         | 4          | 0.5  | 8     | 1   | 4   |
|   |                  | NCTC 11168           | 4          | 0.25 | 8     | 0.5 | 8   |
|   |                  | 81-176               | 4          | 0.25 | 8     | 1   | 8   |
| <b>TF using gDNA<sub>BfR-CA-15687</sub></b>             | <i>C. coli</i>   | BfR-CA-11057-TF15687 | >32        | >16  | >1024 | 1   | >64 |
|   |                  | BfR-CA-14856-TF15687 | >32        | >16  | >1024 | 2   | >64 |
|   | <i>C. jejuni</i> | 81-176-TF15687       | >32        | >16  | >1024 | 1   | >64 |
| <b>TF using 16SrRNA fragment<sub>BfR-CA-15687</sub></b> | <i>C. coli</i>   | BfR-CA-11057-TF16S   | >32        | >16  | >1024 | 1   | >64 |
|   |                  | BfR-CA-14856-TF16S   | >32        | >16  | >1024 | 2   | >64 |
|   | <i>C. jejuni</i> | BfR-CA-14430-TF16S   | >32        | >16  | >1024 | 0.5 | >64 |
|   |                  | NCTC 11168-TF16S     | >32        | >16  | >1024 | 1   | >64 |
|   |                  | 81-176-TF16S         | >32        | >16  | >1024 | 1   | >64 |

MIC, minimal inhibitory concentration; TF, transformant; TF16S, transformant after transformation of the 16S rRNA PCR fragment; APR, apramycin, GEN, gentamicin, KAN, kanamycin, STR, streptomycin, TOB, tobramycin.

### **Natural transformation experiments showed that the unknown APR-GEN-KAN-TOB resistance is transferable among isolates**

To explore the transferability of the observed APR-GEN-KAN-TOB resistance through natural transformation, genomic DNA from isolate BfR-CA-15687 was used to naturally transform different recipient strains, which were sensitive to gentamicin and displayed low MICs for apramycin, kanamycin and tobramycin (Table 2). The *C. coli* and *C. jejuni* transformants were selected on 16 and 8 mg/L TOB, respectively, in order to provide a selective pressure at the MIC or maximally 2-fold MIC of the respective recipient strains. As a control, genomic DNA from a streptomycin-resistant *Campylobacter jejuni* transformant BfR-CA-14430-strep, harboring the *rpsL*<sub>A128G</sub> point mutation, was used and transformants were selected on 16 mg/L STR (35). This allowed for the quantification of natural transformation capacity and normalization of the transformation rate.

When transforming gDNA of BfR-CA-15687 into recipient strains *C. jejuni* 81-176, *C. coli* BfR-CA-11057 and *C. coli* BfR-CA-14856, we observed transformation rates of approximately  $10^{-7}$  per CFU after 48 hours of incubation (Figure 1). As control, transformation of the *rpsL*<sub>A128G</sub> point mutation in the same recipient strains was around 2.5 log<sub>10</sub> more efficient, with transformation rates ranging from  $2.23 \times 10^{-5}$  to  $1.26 \times 10^{-4}$  per CFU (Figure 1). Additionally, when using a PCR fragment of the 16S rRNA\_A1387G as substrate, transformation rates were in mean  $4.51 \times 10^{-6} \pm 1.48 \times 10^{-6}$  per CFU (overall mean of the strains, n=5).



## **Whole-genome sequencing analysis elucidates a correlation between aminoglycoside resistance and a novel 16S rRNA gene mutation**

In order to gain insight into the genetic determinant of the APR-GEN-KAN-TOB resistance in BfR-CA-15687, whole genome sequencing of the sensitive BfR-CA-11057 recipient and nine of its isogenic APR-GEN-KAN-TOB resistant transformants derived from two independent transformation experiments using gDNA of BfR-CA-15687 were compared. For this purpose, the donor isolate BfR-CA-15687 and the recipient isolate BfR-CA-11057 also underwent Oxford Nanopore long-read sequencing in addition to short-read sequencing to obtain a closed genome upon Unicycler hybrid assembly. Mapping of trimmed reads derived from each transformant to the hybrid assembly of BfR-CA-11057 revealed 177 to 1235 sequence nucleotide variants (SNPs) relative to the recipient strain (Table S1B). “Unused reads”, which did not map to the recipient, were subsequently mapped to the donor strain BfR-CA-15687 hybrid assembly sequence. However, those reads did not map to any sequence region common to all transformants (Table S2). Moreover, the transformants exhibited no similarities among genetic regions with low coverage, putatively representing deleted regions compared to the recipient (Table S3). Intriguingly, the consensus of all examined variations in transformants relative to the recipient was a mutation in all three copies of the 16S rRNA gene, located in the aminoacyl tRNA decoding A-site (A1387G; *E. coli* numbering A1408G; Table S1A, B and Figure 2), which was also present in the donor strain BfR-CA-15687 (NCBI; Genome Accession CP126367-CP126368, BioSample Accession SAMN34728731).

***In vitro* transformation experiments using a PCR fragment of the 16S rRNA of BfR-CA-15687 verified A1387G point mutation in 16S rRNA gene as a novel mechanism for aminoglycoside resistance in *C. jejuni* and *C. coli***

In order to ascertain that the phenotypic resistance was indeed solely attributed to this novel point mutation, we amplified the majority of the 16S rRNA gene (10-1514 bp) of BfR-CA-15687 by PCR (Figure 2A). In order to render the PCR fragment mobilizable via natural transformation in *C. jejuni* and *C. coli*, a 5'-*EcoRI* motif was introduced at both ends of the PCR fragment and the fragment was methylated by an *EcoRI* methylase before use as DNA substrate in the natural transformation experiments (35, 36).

For all five aminoglycoside sensitive recipient strains, BfR-CA-11057, BfR-CA-14856, BfR-CA-14430, NCTC 11168 and 81-176, APR-GEN-KAN-TOB resistant transformants were obtained by transformation of the 16S rRNA PCR fragment of BfR-CA-15687 (Table 2). Subsequent short-read sequencing unveiled the A1387G mutation within the 16S rRNA gene of all five analyzed transformants (one per parental strain), providing corroborative evidence for the causal association between this specific mutation in the 16S rRNA gene and phenotypic resistance to APR-GEN-KAN-TOB in *C. jejuni* and *C. coli*.

We investigated whether the 16S rRNA gene point mutation could be found in previously published sequences of *C. coli*. Using the Basic Local Alignment Search Tool (BLAST) at NCBI, we screened over 32,000 publicly available *C. coli* whole-genome sequences. However, we did not find any instance of this mutation.

### **Transformants harbor different numbers of 16S rRNA\_A1387G gene copies per chromosome**

We intended to know, whether all three 16S rRNA gene copies, present in *C. jejuni* and *C. coli* chromosomes, displayed the A1387G point mutation in the transformants, leading to TOB resistance. Hence, just after natural transformation with gDNA, single TOB resistant transformant colonies of BfR-CA-11057-TF15687 and 81-176-TF15687 were once subcultured on ColbA. Subsequently, DNA was extracted from this first passage and the 16S rRNA fragment

comprising position 1387 was Sanger sequenced. We observed two different genotypes of transformants on non-selective ColbA – either a G was detected at position 1387 (Figure 3, CFU 2, no selection) or a double peak in the chromatogram of the Sanger sequence, corresponding to a mixed population of A and G at position 1387 was identified (Figure 3, CFU 1, no selection).

Furthermore, single colonies just after transformation were streaked on plates with varying concentrations of TOB (4, 8 and 16 mg/L) and without TOB supplementation (Figure 3). The colonies from non-selective plates with only G at position 1387 maintained this genotype independent of the TOB concentration the colony was characterized from. However, colonies from non-selective plates, showing a mixed population of A and G at position 1387 in the 16S rRNA gene, switched to a “pure” G at position 1387 at either 8 or 16 mg/L TOB (Figure 3). Hence, the relative ratio of transformants with a distinct number of 16S rRNA gene copies with A1387G appeared to be dynamic, unless all three copies displayed the resistance determining mutation.

We further wanted to decipher, whether the mixed A/G population of 16S rRNA gene sequences at position 1387 was caused by a mixture of 16S rRNA gene variants in the same bacterium. For this purpose, Oxford Nanopore Technologies (ONT) sequencing was performed on a freshly obtained transformant of 81-176-TF15687 only once passaged on non-selective ColbA and displaying a mixed population of A/G at position 1387 in the Sanger sequence. The same DNA was subjected to Illumina short-read sequencing. Using long-read and short-read sequences a hybrid assembly was created using Unicycler, leading to a closed chromosome and one plasmid of 44.8 kb. To rule out assembly errors, the trimmed long-reads of the 81-176-TF15687 transformant were mapped to its hybrid assembly. All three copies of the 16S rRNA gene located in the three ribosomal RNA (rrn) operons at positions 39,157 - 44,986 bp

(rrn I), 395,917 – 401,610 bp (rrn II) and 693,077 – 698,770 bp (rrn III) of the chromosome were visualized (Figure S2). Indeed, in this transformant, the 16S rRNA gene at rrn I and rrn II displayed the A1387G point mutation, whereas the copy at position rrn III maintained wildtype base A at position 1387.

### **The copy number of 16S rRNA genes with A1387G is important for persistence of aminoglycoside resistance**

Furthermore, we intended to evaluate the stability of the newly acquired resistance. For this purpose, fresh transformant colonies of 81-176-TF15687 were first serially diluted to form new single colonies. Subsequently, representative colonies were repeatedly subcultured on non-selective ColbA. Upon passage 1 the genotype at position 1387 in the 16S rRNA genes was initially analyzed by Sanger sequencing (Figure 4). As mentioned above, either a mixed A/G genotype at position 1387 in 16S rRNA or only G at this position was observed (81-176-TF15687<sub>16SrRNA\_A/Gmix1387</sub> or 81-176-TF15687<sub>16SrRNA\_G1387</sub>, respectively). After 15 passages on non-selective ColbA, the resistance to tobramycin was reassessed in transformants by agar dilution, that had initially been shown to harbor the mixed A/G genotype. Colonies of the subcultured transformants, that were stamped, i. e. transferred by velvet cloth on ColbA plates with different concentrations of TOB, revealed loss of resistance to 8 and 16 mg/L TOB after passaging. Thus, colonies were only observed on non-selective medium and, in a smaller quantity, in the presence of 4 mg/L TOB (Figure 4A). The reversion to a sensitive genotype (only A at position 1387 in the 16S rRNA gene) was confirmed by Sanger sequencing for a colony, which only grew on non-selective ColbA (Figure 4A). After passaging 81-176-TF15687<sub>16SrRNA\_G1387</sub> for 45 times, which initially only displayed G at position 1387, the phenotypic assay revealed stability of resistance to TOB. In particular, the number of colonies

with and without TOB was stable, independent on the concentration of TOB (Figure 5B). Here, Sanger sequencing of a representative colony showed that the G base at position 1387 was still the only base present after passaging, thus, suggesting stable G1387 presence in all three copies of the 16S rRNA gene.

## Discussion

According to regular zoonosis surveillance of thermotolerant foodborne *Campylobacter* spp., resistance to gentamicin remains rare in Europe (17). In 2022, gentamicin resistance was observed in 2 % of the *C. coli* isolates and 0.1 % of *C. jejuni* isolates from broiler, and in 3 % of *C. coli* and 0.5 % of *C. jejuni* isolates from infected humans. Thus, this antibiotic substance can still be considered effective for treatment of campylobacteriosis. However, in China, 15.6 % of *C. jejuni* and 79.9 % of *C. coli* isolates from chicken and swine collected in 2014 and 13 % of *C. jejuni* as well as 50 % of *C. coli* isolated from humans in 2017-2018 showed gentamicin resistance (19, 20). Likewise, a high proportion of *C. spp.* isolates from chicken were gentamicin resistant in Vietnam (21.9 % and 78.8 % in *C. jejuni* and *C. coli* isolated from broiler, respectively (21)) and on the Philippines (65.2 %, (22)). Although point mutations in the 16S rRNA gene have been linked with 2-deoxystreptamine aminoglycoside resistance in other organisms (37-40), this has never been observed before in *C. spp.*. In this study, we have demonstrated that the A1387G mutation occurring at the bacterial A-site (aminoacyl tRNA binding site; Figure 2) confers resistance to the aminoglycosides APR, GEN, KAN and TOB in *C. coli* and *C. jejuni*. Target site modifications, involving genetic mutations and enzymatic methylation predominantly occur at the bacterial A-site, which serves as the binding site for most aminoglycosides (36, 41, 43).

The aminoglycosides used in this study, except streptomycin, all have a 2-deoxystreptamine backbone in common. While GEN, KAN and TOB belong to the 4,6-disubstituted class with an ammonium group ( $\text{NH}_3^+$ ) at position 6' of ring I, APR differs in its structural appearance by belonging to the 4-monosubstituted subclass, while having a hydroxy group (OH) at position 6' of ring I. Nevertheless, these four aminoglycosides have rings I and II in common and either residue ( $\text{NH}_3^+$ , OH) of ring I is able to build a Watson-Crick pseudo pair with 16S rRNA A1408 (numbering in *E. coli*, corresponding to A1387 in *C. spp.*), thereby inhibiting the decoding step of protein biosynthesis of the bacterium (16).

We wondered, why we were the first to describe this point mutation in *Campylobacter* and if there were sequences published that harbor this specific 16S rRNA gene point mutation. Thus, we conducted analyses utilizing the NCBI BLAST tool and screened the database of whole genome sequences publicly available. However, we could not find the point mutation in any of the more than 32,000 whole-genome sequencing datasets of *C. coli*, suggesting that the resistance determinant identified in the field isolate from caecum of a turkey in Germany during routine monitoring is rare. Likewise, its transferability via horizontal gene transfer was inefficient, with a  $\sim 2.5 \log_{10}$  lower transformation rate compared to the control *rpsL*<sub>A128G</sub> point mutation. In addition, we only observed transformants when using relatively low initial concentrations of selective aminoglycoside, just above the MIC of the respective wild-type recipients. If transformant colonies were picked initially and streaked on increasing concentrations of selective aminoglycoside, we observed adaptation of colonies with initial mixed 16S rRNA gene copies with and without A1387G mutation to only G at position 1387 at higher concentrations (Figure 3). This likely stems from a gradual transition from a sensitive to a fully resistant phenotype. Hence, we concluded that colonies that have undergone only partial transition are still impeded in growth due to the selective pressure at higher

concentration of aminoglycoside, potentially resulting in a reduced growth rate. Likewise, loss of resistance was observed upon few passages on non-selective medium (Figure 4A), while the resistance was stable if all three copies of the 16S rRNA genes harbored G at position 1387 (Figure 4B). The combined results suggest that complete resistance is only evident when all three copies of the 16S rRNA gene have acquired the A1387G point mutation but that acquisition might be the limiting factor. Interestingly, previous studies reported that thermotolerant *C. spp.* carry aminoglycoside modifying enzymes, such as Aph(2'') phosphotransferase variants (19, 28, 29). Thus, we speculate that although the presence of all three copies of the 16S rRNA gene provides high level gentamicin resistance in *C. spp.*, the gradual acquisition of mutated 16S rRNA gene(s) with a low level of resistance may not provide sufficient advantage under selection pressure. In Germany, gentamicin resistance in *C. spp.* is very low, with the isolation of only single isolates per year during zoonosis monitoring. From our analysis, it is tentative to speculate that the 16S rRNA\_A1387G gene in the *C. coli* from caecal content of turkey may have emerged by sublethal concentrations of selective agent over time, which remains to be further investigated for potential future practical consequences. A similar phenomenon of gradual acquisition of point mutations in multiple copies of the 16S rRNA gene was observed in *Nocardia farcinica* exposed to amikacin for 24 hours (38). Here, the three 16S rRNA genes showed increasing copy number containing the A1408G point mutation, leading to all copies with a G at position 1408 after prolonged incubation under selective conditions.

In the absence of selective pressure, we did not detect any growth deficiencies on blood agar in transformants, which stably acquired G in all copies of the 16S rRNA gene. However, easy loss of the point mutation upon passage on non-selective blood agar in transformants with mixed copies of A and G at position 1387 indicated fitness costs *in vitro* in *C. spp.*. In other

bacterial pathogens like *Mycobacteria* (41, 42) and in *Borrelia burgdorferi* (43) the point mutation in the 16S rRNA gene is frequently found as resistance determinant for gentamicin in clinical isolates, demonstrating principle toleration and full function of the mutated 16S rRNA gene *in vivo* at least in some bacterial species.

Macrolide resistance in *Campylobacter* is primarily attributed to the A2075G point mutation in the 23S rRNA gene (44). In contrast to the 16S rRNA\_A1387G gene mutation, the macrolide resistance conferring mutation was stable upon passaging on non-selective medium, even in isolates harboring only 2 out of 3 copies of the 23S rRNA gene with A2075G (45). This enhanced stability might be a factor contributing to its frequent occurrence in macrolide resistant *Campylobacter*, while antibiotic resistant mutations in the 16S rRNA gene have not been observed before. However, also the macrolide conveying 23S rRNA mutations have been shown to lead to fitness costs in *C. jejuni* in chicken (46). Nevertheless, further investigations are required to evaluate the potential loss of fitness of the 16S rRNA\_A1387G gene mutation also *in vivo*. Here, strains with all three 16S rRNA gene copies harbouring the A1387G mutation might be tested over time for colonization capacity in a chicken model. In particular, it would be interesting to challenge the resistant strain in competition with an isogenic strain, carrying all three wildtype 16S rRNA gene copies with A1387.

## **Conclusion**

The novel point mutation A1387G in the 16S rRNA gene of *C. jejuni* and *C. coli* was revealed as novel causative determinant for APR, GEN, KAN and TOB resistance. However, acquisition in less than all three copies of the three 16S rRNA genes in *C. spp.* led to rapid loss and return to a sensitive phenotype. This phenomenon putatively contributed to *C. coli* BfR-CA-15687 being the first and to our knowledge yet only isolate, harboring this resistance. Understanding the



molecular mechanism of resistances as well as their acquisition and persistence in pathogens is crucial for combatting the spread of resistances globally.

## **Methods**

### **Strains and growth conditions**

*Campylobacter coli* BfR-CA-11057, BfR-CA-14856 and BfR-CA-15687 were isolated in Germany in the years 2012, 2016 and 2018 from raw cow milk, raw goat milk and caecum of turkey, respectively. *Campylobacter jejuni* isolate BfR-CA-14430 was obtained from fresh chicken meat in Germany in 2016 (47). Isolation was conducted by the federal state laboratories according to EN ISO 10272-1 valid in the respective year (48, 49). In addition, reference strains *C. jejuni* NCTC 11168 (50), 81-176 (51) and DSM 4688 (DSMZ - German Collection of Microorganisms and Cell Cultures GmbH, Braunschweig, Germany) as well as *C. coli* strain 2012-70-443-2 (Technical University of Denmark, Lyngby, Denmark) were used. If not stated otherwise, incubation of all cultures was performed under microaerobic conditions with 5 % O<sub>2</sub>, 10 % CO<sub>2</sub> and 85 % N<sub>2</sub> (Binder, Tuttlingen, Germany). Cultures derived from -80 °C stock cultures (MAST Group Ltd., Bootle, UK) were cultivated on Columbia blood agar plates containing 5 % defibrinated sheep blood (ColbA, Oxoid, Thermo Fisher Scientific Inc., Waltham, MA, USA) at a temperature of 42 °C for 24 h. Subsequently, the bacteria were subcultured on ColbA for 20 ± 2 h before use. For the selection of transformants, ColbA was supplemented with either tobramycin (8 - 16 mg/L) or streptomycin (16 mg/L) (Sigma Aldrich, St. Louis, MO, USA, respectively).

### **PCR Amplification and methylation of 16S rRNA**

For the amplification of the 16S rRNA of target sequence BfR-CA-15687, forward primer C127 (5'-CTA GCG AAT TCA GAG TTT GAT CCT GGC TCA G-3') and reverse primer C128 (5'-GGA CTG

AAT TCA AGG AGG TGA TCC AAC CGC A-3') were used, carrying each an *EcoRI* motif at the 5' end. The PCR amplification was performed using a Q5 High Fidelity DNA Polymerase (New England Biolabs, Ipswich, MA, USA), following PCR fragment purification using the QIAquick PCR Purification Kit (Qiagen, N.V., Venlo, The Netherlands). Subsequently, the PCR fragment was methylated using an *EcoRI* methyltransferase (New England Biolabs, Ipswich, MA, USA) for 1 h at 37 °C, followed by heat inactivation at 65 °C for 15 min. This procedure is mandatory for mobilization of the PCR fragment for DNA uptake by *C. spp.* (36, 52).

### **Sanger sequencing analysis of 16S rRNA genes**

The primers 16SrRNA-F1 (5'-AGA GTT TGA TCC TGG CTG AG-3') and 16SrRNA-R1 (5'-AAG GAG GTG ATC CAG CCG CA-3') (53) were used for amplification applying the Q5 High Fidelity DNA Polymerase (New England Biolabs, Ipswich, MA, USA). The PCR fragment was purified using the QIAquick PCR Purification Kit (Qiagen, N.V., Venlo, The Netherlands) and 1.5 µg of the DNA was supplemented with the sequencing primer 16SrRNA-S4 (5'-AGT CCC GCA ACG AGC GCA AC-3') (54) for Sanger sequencing at Eurofins Scientific SE, Luxembourg City, Luxembourg.

### **DNA uptake assay and transformation**

Recipient strains from a 20 h ± 2 h preculture on ColbA was resuspended in 5 mL of brain heart infusion (BHI, Oxoid, Thermo Fisher Scientific Inc., Waltham, MA, USA) and adjusted to an optical density (OD) at 600 nm of 0.3. Subsequently, the strains were cultured at 140 rpm and 37 °C in an atmosphere containing 3.5 % H<sub>2</sub>, 6 % O<sub>2</sub>, 7 % CO<sub>2</sub> and rest N<sub>2</sub> for 6 hours. Cultures were passaged to fresh BHI and grown over night at the same conditions (16-18 h), using a suitable inoculum assuming doubling times of 1-1.5 hours. Cells were harvested in exponential growth phase at OD<sub>600nm</sub> = 0.05-0.6 by centrifugation at 14,000 x g for 5 min. The pellet was resuspended in fresh BHI supplemented with 1 µg/mL DNA, either genomic DNA of

BfR-CA-15687, BfR-CA-14430-strep (35) or methylated 16S rRNA gene fragment. DNA uptake, recombination and outgrowth of phenotypic resistance was accomplished by incubation for 4 h at 37 °C. After incubation, cell suspensions were serially diluted in BHI, plated on ColbA with and without tobramycin or streptomycin and incubated for 48 h at 37 °C. The transformation rate was calculated as the ratio of the number of transformants grown on ColbA supplemented with the respective antimicrobial and the total number of colonies on non-selective plates.

### **Antimicrobial susceptibility testing using microdilution**

The susceptibility testing using broth microdilution method followed the guidelines outlined in M45-A and VET06 (55, 56). Isolates subcultured on ColbA at a temperature of 42 °C for  $20 \pm 2$  h were inoculated into cation-supplemented Mueller-Hinton broth (Thermo Fisher Scientific Inc., Waltham, MA, USA) with 5 % fetal calf serum (PAN-Biotech, Aidenbach, Germany) (CAMHB/FCS) at a bacterial concentration ranging from 2 to  $8 \times 10^5$  CFU/ml. The minimum inhibitory concentrations (MICs) were determined using the European standardized EUCAMP3 plate (Thermo Fisher Scientific Inc., Waltham, MA, USA). In addition, custom plate formats were prepared, incorporating the subsequent antimicrobial agents (Sigma Aldrich, St. Louis, MO, USA) and their concentration ranges depicted in Table 1. Stock solutions of the antimicrobials were dissolved in H<sub>2</sub>O. The U-bottom microtiter plates (Greiner Bio-One International GmbH, Frickenhausen, Germany) were prepared by adding 50 µL of CAMHB/FCS supplemented with the corresponding double-concentrated antimicrobial agent per well. Before use, the sealed plates were stored at 4 °C for 24 h. The isolates were prepared following the described method above, with the exception that the inoculum was double concentrated in a volume of 50 µL, which was added to each well of the pre-prepared customized plates. Samples were incubated at 37 °C for 48 h. The determination of minimal inhibitory

concentrations (MICs; in mg/L) was performed using the semi-automated Sensititre™ Vizion™ system (Thermo Fisher Scientific Inc., Waltham, MA, USA) and the Sensivizion V2.0 software (MCS Diagnostics BV, Swalmen, The Netherlands). The determination of antimicrobial resistance in *Campylobacter* was based on the guidelines established by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) for Epidemiological cutoff Values (ECOFFs) (57). “Elevated non-wildtype MICs” were previously defined for kanamycin resistance (21). For apramycin and tobramycin “elevated non-wildtype MICs” were defined as >16 mg/L. All ECOFFs and elevated non-wildtype MICs are depicted in Table 1. For quality control, *C. jejuni* strain DSM 4688 and *C. coli* strain 2012-70-443-2 were included, which displayed sensitive phenotypes.

#### **Assessment of stability of acquired antibiotic resistance**

To assess the stability of the acquired resistance determinant, fresh transformant colonies were first serially diluted to form new single colonies on non-selective ColbA plates. Subsequently, representative colonies were repeatedly subcultured on non-selective ColbA at 42 °C for 20 ± 2 h. Following the indicated number of passages, bacteria were resuspended in 1 mL buffered peptone water (10 g/L peptone, 5 g/L NaCl, 9 g/L Na<sub>2</sub>HPO<sub>4</sub> × 12 H<sub>2</sub>O, 1.5 g/L KH<sub>2</sub>PO<sub>4</sub>, pH 7.0 ± 0.2 at 25 °C), serially diluted to approximately 200 colonies in 100 µL and plated on ColbA. After incubation at 37 °C for 48 hours under microaerobic conditions, colonies were transferred by stamping with velvet cloth on a series of ColbA plates containing 4, 8, and 16 mg/L tobramycin and, as the last plate, ColbA without antimicrobial. The orientation of the plates during stamping was carefully marked, ensuring comparison of plate images, which were taken after 48 h of incubation at 37 °C using the G:BOX CHEMI XX6 imaging system (Synoptics Ltd, Beacon House, Nuffield Road, Cambridge) controlled by the Genesys V1.8.5.0 software (Synoptics Ltd, Beacon House, Nuffield Road, Cambridge).

## Whole genome sequencing

*Campylobacter* isolates were subcultured on ColbA for  $20 \pm 2$  h at 42 °C. Bacteria were harvested from 1 ml cells at OD<sub>600nm</sub> of 2 by centrifugation at 14,000 x g for 5 min. DNA extraction for short-read sequencing was performed using either the Maxwell RSC Cultured Cells DNA Kit (Promega Corporation, Fitchburg, WI, USA) or the PureLink Genomic DNA Mini Kit (Thermo Fisher Scientific, Waltham, MA, USA). For long-read sequencing, DNA extraction was performed using either the MagAttract HMW Genomic Extraction Kit (Qiagen N.V., Venlo, The Netherlands) according to the protocol but with a 1.5 h of 56 °C incubation step or the MasterPure Complete DNA & RNA Purification Kit (Biozym Scientific GmbH, Hessisch Oldendorf, Germany) following the manufacturer's protocol but using a more concentrated RNase A solution (100 mg/mL; Qiagen N.V., Venlo, The Netherlands).

The DNA quality was assessed through spectral analysis using a NanoDrop Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), while the concentration was determined using a Qubit 3.0 Fluorometer with the dsDNA BR Assay Kit (4–2000 ng; Thermo Fisher Scientific, Waltham, MA, USA). DNA libraries for short-read sequencing were prepared using the Illumina DNA Prep, (M) Tagmentation Kit (Illumina, Inc., San Diego, CA, USA), with the modification of using half the volume of all reagents. The Illumina MiSeq benchtop sequencer, equipped with the MiSeq reagent kit v3 (600 cycle, Illumina, Inc., San Diego, CA, USA), or the Illumina NextSeq 500 sequencer utilizing the NextSeq 500/550 mid output kit v2.5 (300 cycle, Illumina, Inc., San Diego, CA, USA) were employed for paired-end sequencing. The read lengths were set from 2 x 149 to 2 x 301, depending on the instrument used.

DNA libraries for long-read sequencing were prepared using the Rapid Barcoding Kit 96 (SQK-RBK110.96, Oxford Nanopore Technologies Limited, Oxford, United Kingdom). The sequencing

process was performed on the MinION Mk1C instrument, using either the MinION R9.4.1 or R10.4.1 FlowCell (Oxford Nanopore Technologies Limited, Oxford, United Kingdom). To process the Oxford Nanopore Technology sequencing data, the Guppy basecaller v. 6.4.8 (Oxford Nanopore Technologies, Oxford, UK) was used in the "super-accuracy" mode.

### **Bioinformatic analysis**

The Illumina paired-end reads were subjected to trimming and *de novo* assembly using the AQUAMIS pipeline, version 1.3.8 (58). For data quality assessment, reads were considered satisfactory if they exhibited a base accuracy of Q30 (error rate 1:1000) for over 80 % of the reads, and if the minimum read coverage was at least 40. For ONT reads, quality control and assembly was conducted using the MiLongA Pipeline v1.0.1 (59). This pipeline includes various tools, such as porechop v0.2.4 (60) for trimming and Unicycler v0.4.8 (61) for hybrid assembly. Quality was considered sufficient if the filtered median fragment length (N50 value) was >10,000 and the read coverage exceeded the minimum of 30.

The assembled contigs underwent analysis using the BakCharak pipeline v3.0.4 (62), which incorporates the antimicrobial resistance gene finder module. This module utilizes AMRFinderPlus v3.10.45 (63) and the corresponding AMRFinder database 2023-04-17.1 to identify resistance determinants, applying default thresholds. Furthermore, ResFinder v4.1 (64) was employed on assembled and trimmed read data with lowest thresholds applied (30 % identity, 20 % coverage) to complement AMRFinderPlus results. Assembled genome contigs were annotated with Bakta (65).

Geneious Prime 2023.2.1 (Biomatters Ltd., New Zealand) was employed to conduct mapping of trimmed reads to assemblies and to analyse sequence variations of the transformants relative to the recipient (Figure 5). In detail, the trimmed short-reads obtained from the

resistant transformants were mapped to the bakta annotated Unicycler hybrid assembly of the recipient isolate. Deletions in the transformants relative to the recipient were identified by displaying low coverage regions (maximum coverage of 5 reads). Variations/SNP's were identified using a minimum coverage of 40 and a minimum variant frequency of 0.8. The paired unused reads were subsequently mapped to the bakta annotated Unicycler hybrid assembly sequences of the donor isolate, which had been concatenated (chromosome and plasmid), to investigate the transfer of larger sequences, such as putative genes or antimicrobial resistance islands (minimum coverage regions ( $\geq 20$  reads)). Finally, the consensus of the SNPs was found using the "Compare Annotations" function (Table S1). Putative deletions in common among the various resistant transformants were detected by tracking "low coverage" regions in the recipient (Table S2). Potential insertions were identified by detection of "high coverage" regions of "unused reads" in the donor, which did not map to the recipient (Table S3). Nucleotide sequences of 16S rRNA genes were aligned using Geneious Prime software with the Geneious Alignment algorithm.

The results retrieved from Sanger sequencing were analyzed with SeqMan Pro 17 (DNASTAR Lasergene 17, DNASTAR, Inc., Madison, WI, USA).

A small fragment (position 1357-1433) of the 16S rRNA of BfR-CA-15687 comprising the A1387G transition was used to search against NCBI's Nucleotide collection (nr/nt) and Whole-genome shotgun contigs (wgs) databases using BLASTN 2.15.0+ (66). Limitations were set for the latter database as *Campylobacter coli* (taxid:195).

### **Data availability**

The complete sequence of the BfR-CA-15687 genome (incl. plasmid) can be found at the National Center for Biotechnology Information (NCBI; Genome Accession CP126367-

CP126368, BioSample Accession SAMN34728731). The 16S ribosomal RNA gene sequence of BfR-CA-15687 is additionally published as GeneBank file, Accession No. PQ227239.

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### **Author contributions**

Conceptualization: K.S. and M.Z.; Formal analysis: M.Z. and K.S.; Investigation: M.Z. and C.W. Methodology: M.Z. and J.G. Writing—original draft: M.Z. and K.S. Writing—review & editing: J.G. and C.W. All authors reviewed and agreed upon the final version of the manuscript.



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## Figure Legends

**Figure 1.** The transformation rates of APR<sup>R</sup>-GEN<sup>R</sup>-KAN<sup>R</sup>-TOB<sup>R</sup> resistance determinant using gDNA of BfR-CA-15687 was ~2.5 log<sub>10</sub> lower (blue bars) than transformation of the control *rpsL*<sub>A128G</sub> point mutation using gDNA of BfR-CA-14430-strep leading to STR<sup>R</sup> (turquoise bars). The sensitive wildtype strains *C. jejuni* 81-176, *C. coli* BfR-CA-11057 and *C. coli* BfR-CA-14856 were transformed with 1 µg/ml gDNA. Transformation rates were assessed from the ratio of resistant transformants relative to CFU on non-selective Columbia blood agar. The data stem

from at least three independent experiments, with error bars representing standard deviation.

**Figure 2.** Primary sequence of the 16S rRNA of *C. coli* BfR-CA-15687 (A) and proposed secondary structure of *Campylobacter* spp. 16S rRNA (B, modified from (67)) with the location of the point mutation highlighted with blue frames in A and B, leading to APR<sup>R</sup>-GEN<sup>R</sup>-KAN<sup>R</sup>-TOB<sup>R</sup> resistance phenotype. Blue frame in B, sequence of the aminoacyl tRNA decoding region (A-site) of sensitive (1387A in green) and resistant (1387G in red) phenotypes. Numbers indicate positions in the *C. spp.* 16S rRNA sequence. Forward and reverse primers (grey boxes) flanked with 5'-*Eco*RI motifs are depicted in A, which were used for amplification of a 16S rRNA gene fragment of BfR-CA-15687, transformed into sensitive recipient strains.

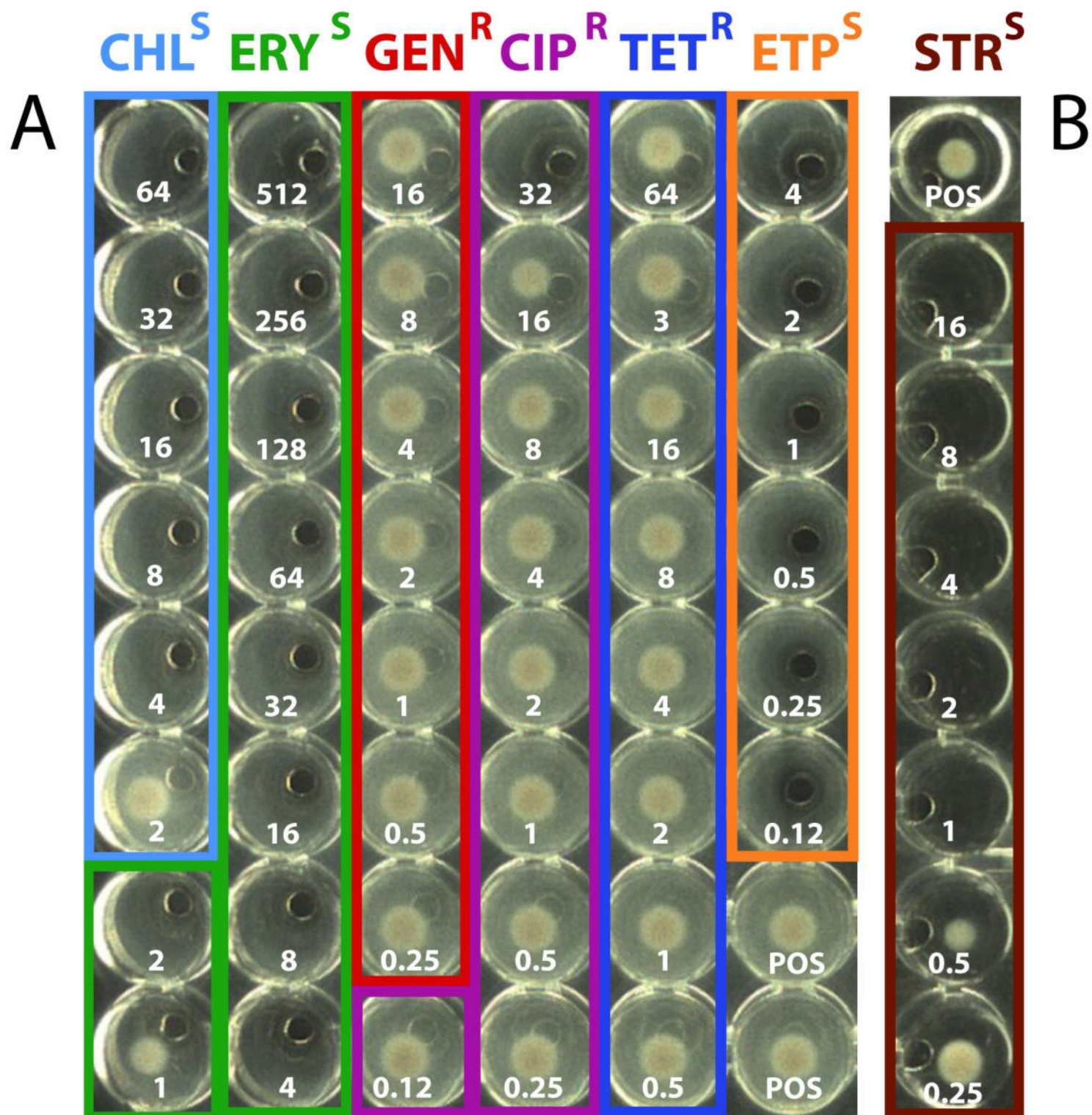
**Figure 3.** Single colonies of transformants switched from a mixed A/G genotype at position 1387 in 16S rRNA to only G at higher TOB concentrations. Two representative transformant colonies (CFU 1 and CFU 2) of each *C. jejuni* 81-176-TF15687 and *C. coli* BfR-CA-11057-TF15687 after transformation were transferred to different concentrations of TOB and in parallel on non-selective ColbA. Sanger sequencing revealed two populations of resistant transformants – either harboring base G upon transformation or a mixture of bases A and G at position 1387 in the 16S rRNA genes (marked with black arrows), which changed to only G under higher TOB concentrations. The base color code of the Sanger sequences is indicated below the chromatograms. TOB, tobramycin; wt, wildtype.

**Figure 4.** A transformant harboring a mixed A/G genotype at position 1387 in 16S rRNA reverted to a sensitive phenotype after 15 passages (A). In contrast, a transformant with only G at position 1387 maintained resistance even after 45 passages (B). Transformants of *C. jejuni* 81-176-TF15687 were passaged on non-selective ColbA. After the indicated number of

passages, the transformant culture was diluted and spread on non-selective ColbA plates in order to obtain single colonies. Subsequently, colony material was transferred on plates with different concentrations of TOB by stamping with velvet cloth. Photographs of colony patterns on each plate were captured after the indicated number of passages. Sanger sequences are shown after passage 1 (fresh transformant) and after repeated subculturing. A colony, which did not grow on TOB supplemented plates, was taken from non-selective ColbA for Sanger sequencing after passaging.

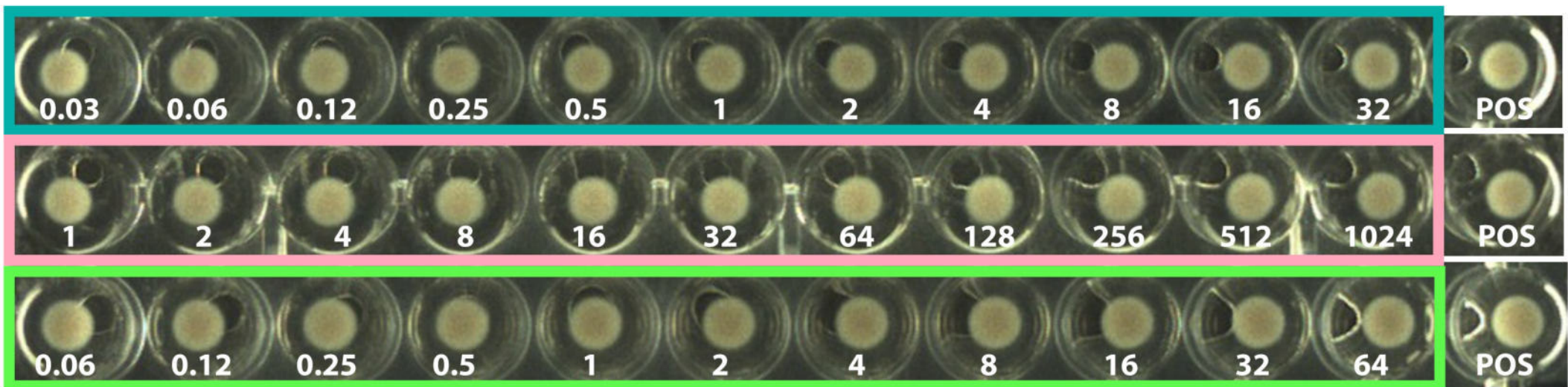
**Figure 5.** Schematic illustration of the strategy for identification of the novel resistance determinant. Trimmed short-reads from resistant transformants were mapped to the Unicycler hybrid assembly of the recipient. Variants/SNPs and deletions were revealed per transformant. For each transformant, unused reads were subsequently mapped to the Unicycler hybrid assembly of the donor isolate in order to find AMR gene transfer. The consensus of SNPs, deletions and or insertions of all transformants was verified to be present in the donor, but absent in the recipient isolates.



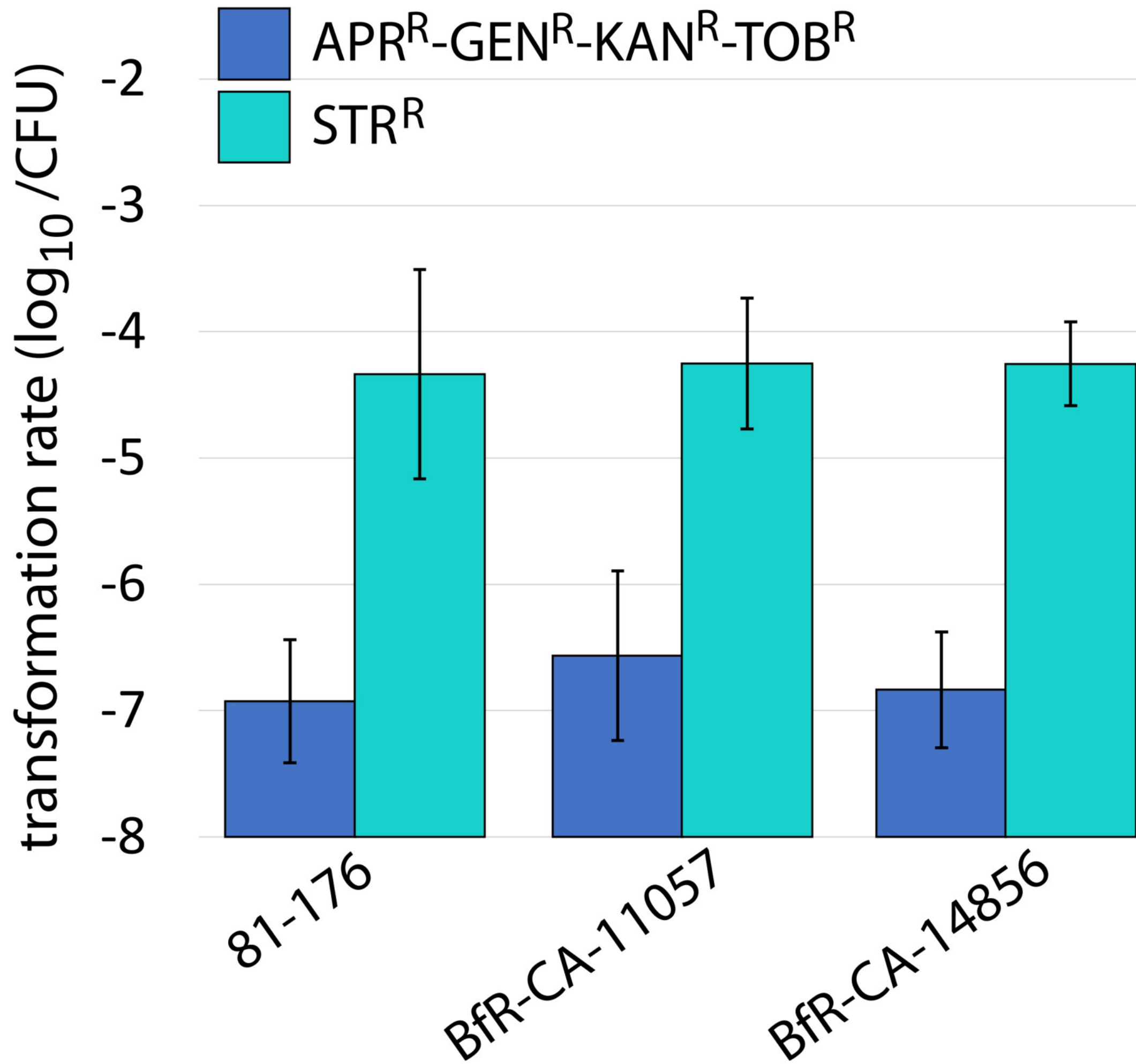


**C**

**APR<sup>R</sup> KAN<sup>R</sup> TOB<sup>R</sup>**





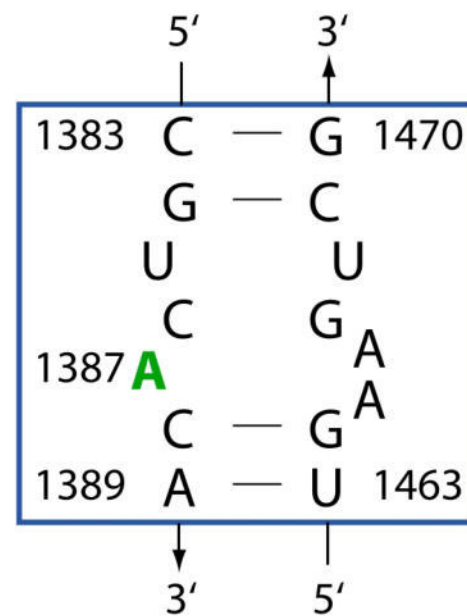
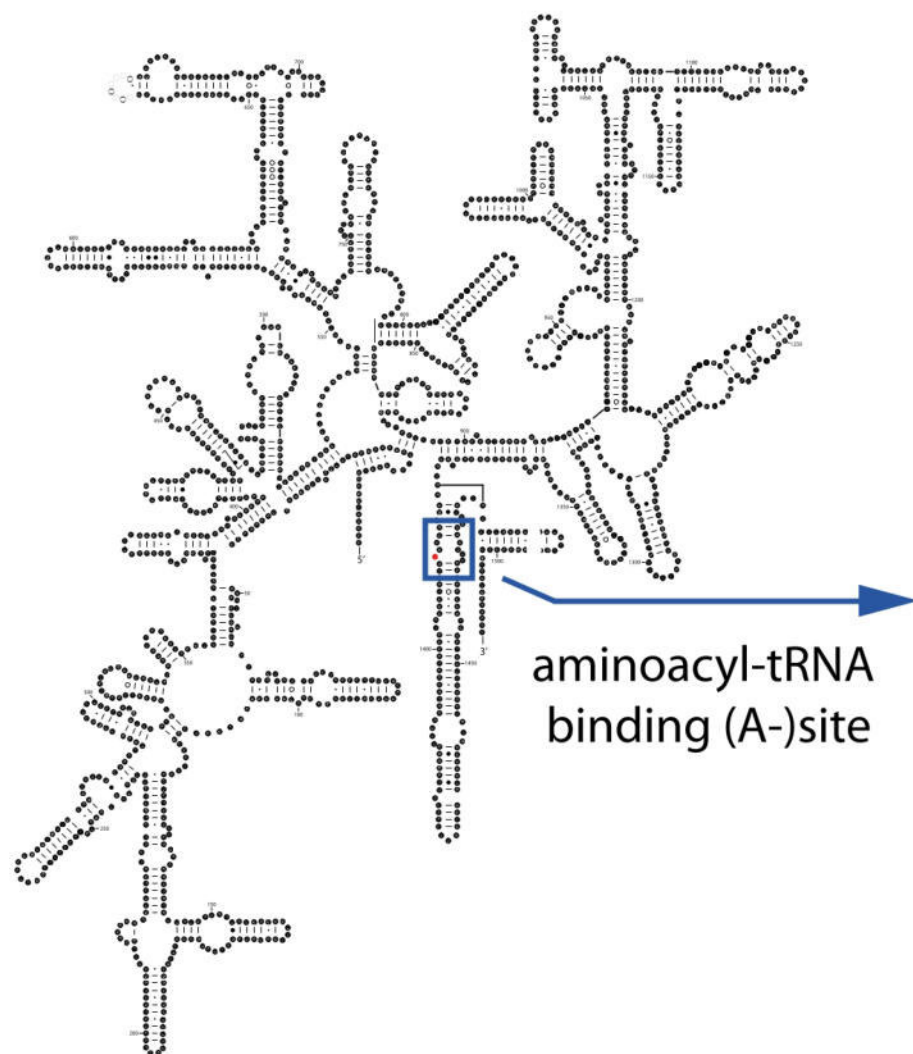
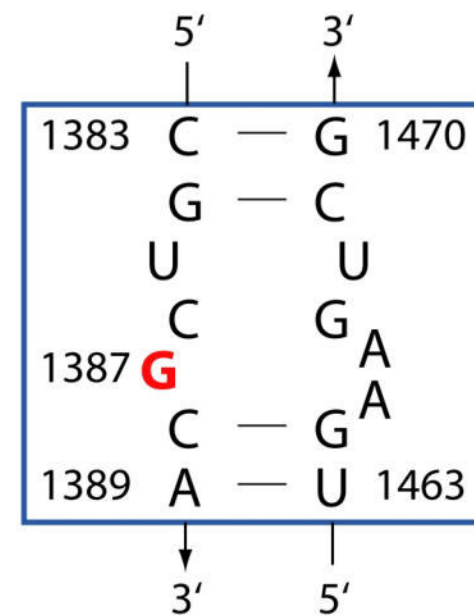


**A**EcoRI- **Primer C127**

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**A1387G****Primer C128** -EcoRI**B****sensitive****APR<sup>R</sup> GEN<sup>R</sup> KAN<sup>R</sup> TOB<sup>R</sup>**

*C. jejuni* 81-176-TF15687

*C. coli* BfR-CA-11057-TF15687

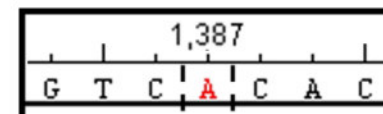
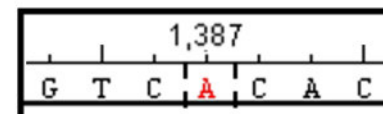
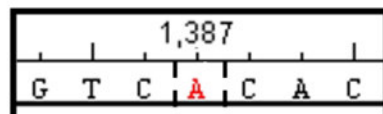
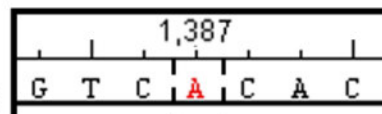
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CFU 2

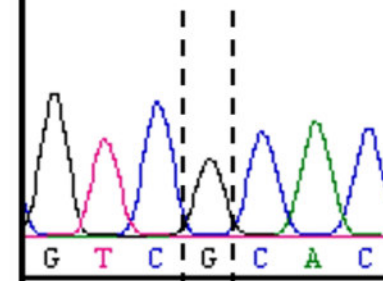
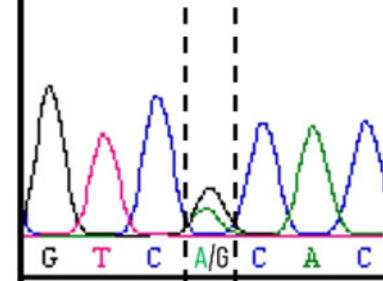
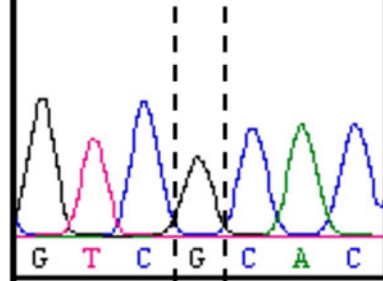
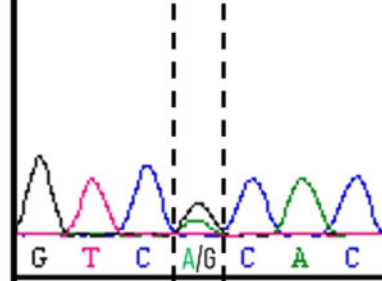
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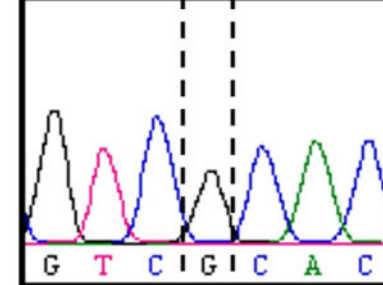
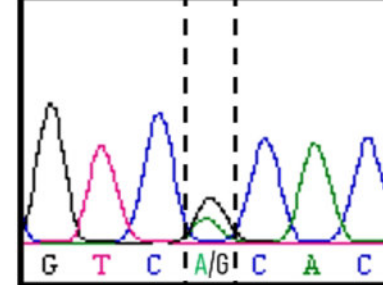
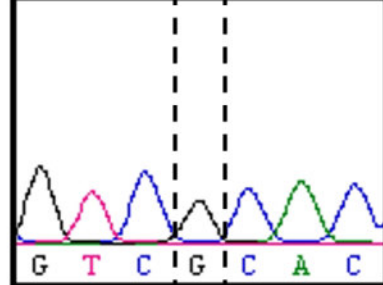
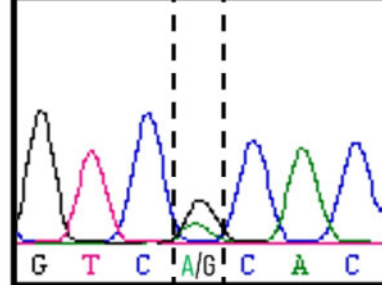
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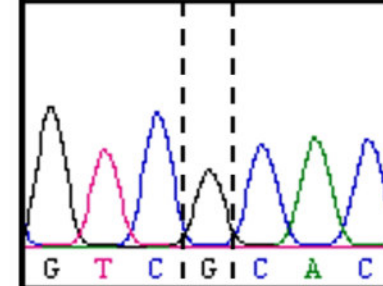
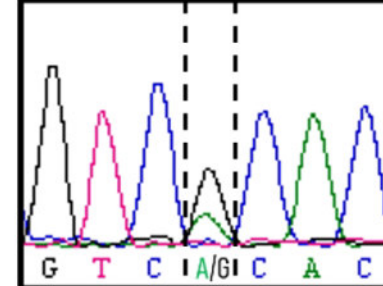
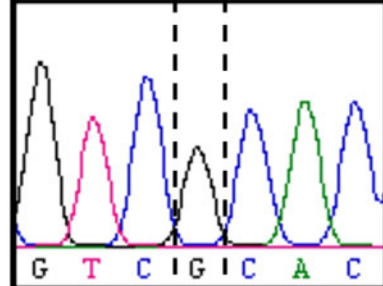
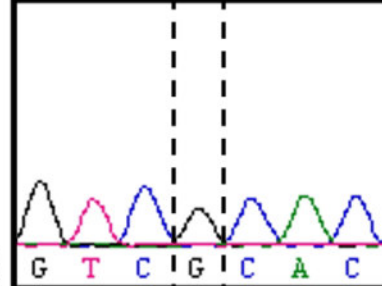
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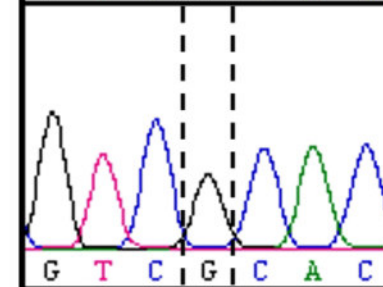
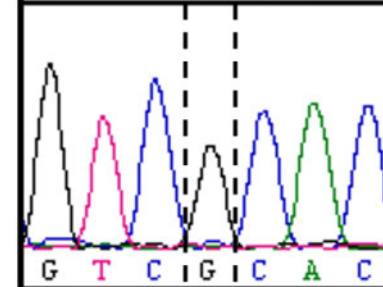
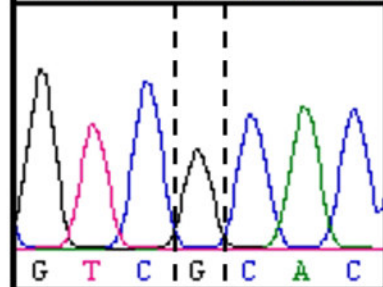
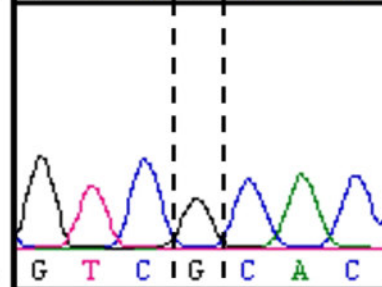
4 mg/L TOB



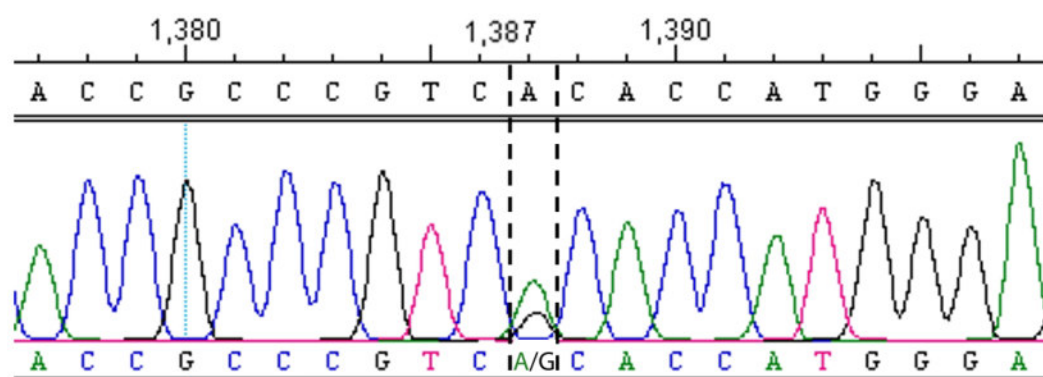
8 mg/L TOB



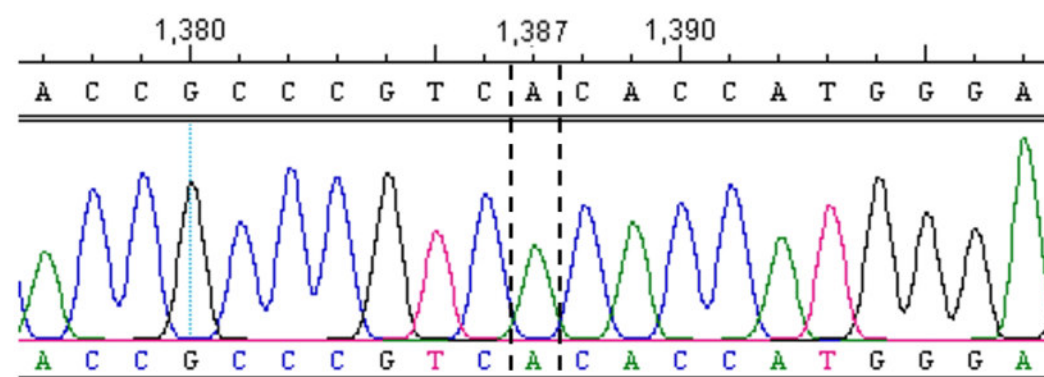
16 mg/L TOB



passage 1



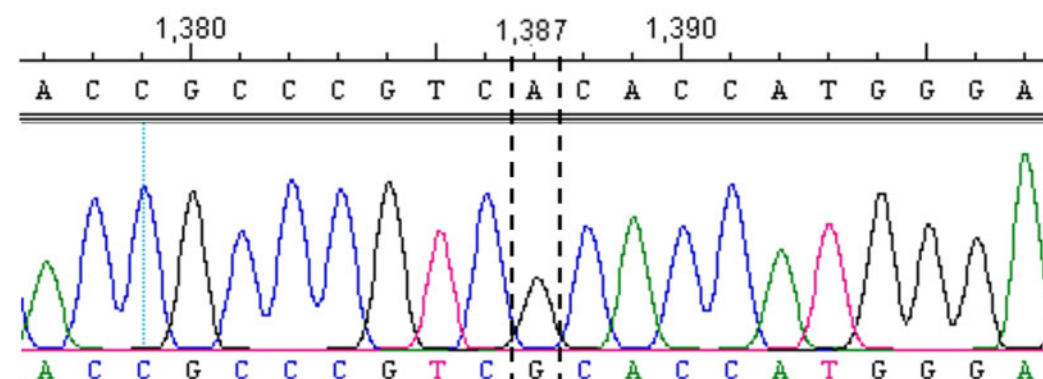
passage 15



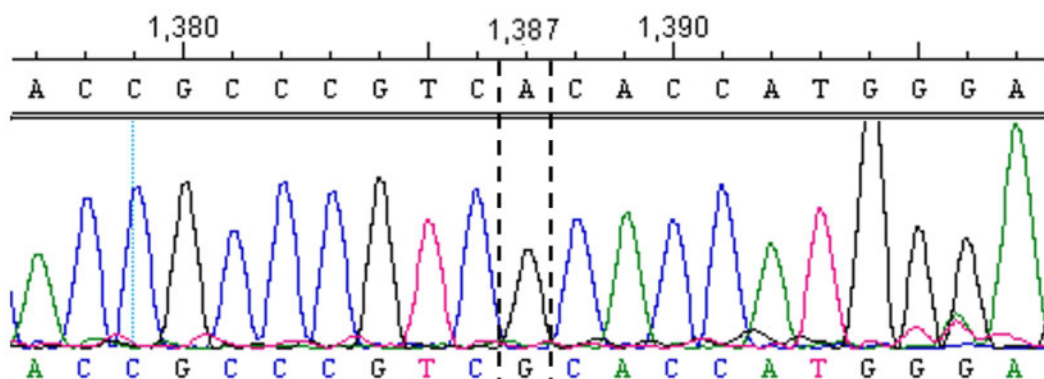
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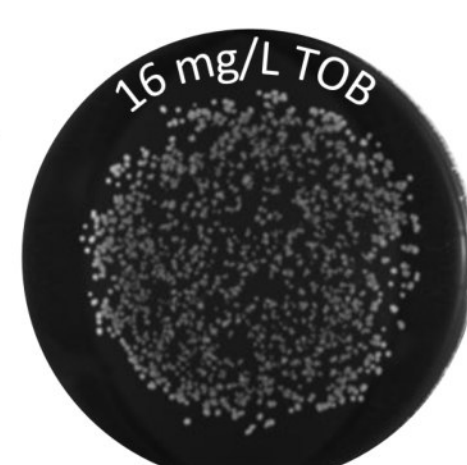
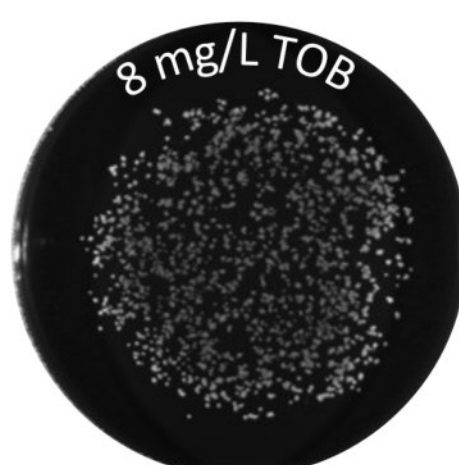
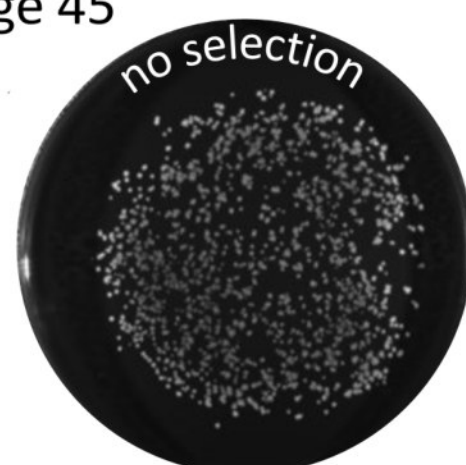
passage 1

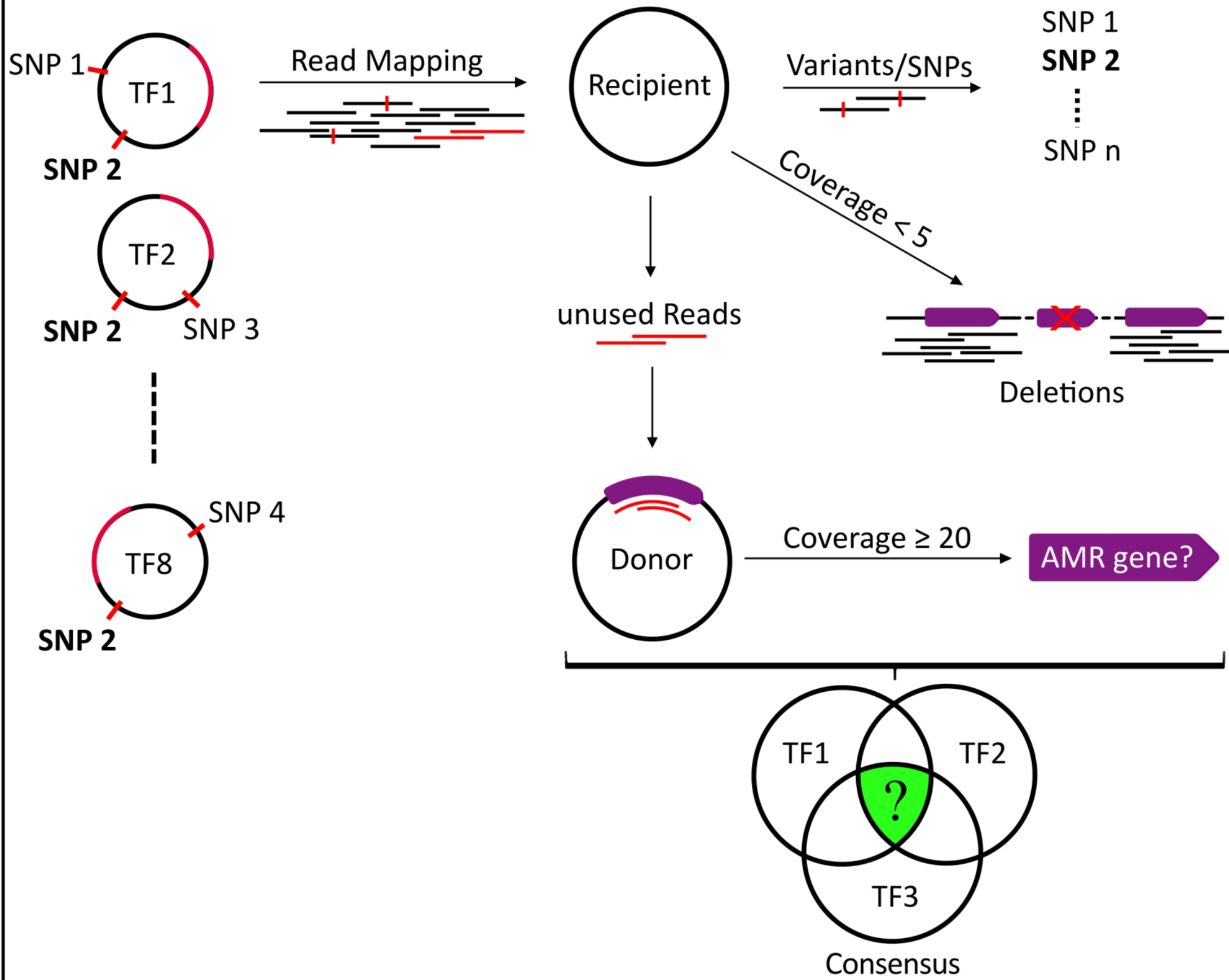


passage 45



passage 45





## 7 Discussion

*C. jejuni* and *C. coli* stand out as the primary agents associated with campylobacteriosis in humans and thus have a major impact on public health globally. Although antibiotics are crucial for treatment in human and veterinary medicine, their use have led to resistances arising and circulating in bacterial populations. This poses a serious threat as it limits antibiotic treatment options. Hence, The European Centre for Disease Prevention and Control (ECDC) and the World Health Organization (WHO) have emphasized on the need for better surveillance of antimicrobial resistance (AMR) worldwide (159). *Campylobacter* was classified as a high-priority resistant bacterium regarding its fluoroquinolone resistance (58) and was identified by the Centers for Disease Control and Prevention (CDC) as a serious threat to public health (57).

### 7.1 Phenotypic evaluation reveals regional variation of resistance profiles in *Campylobacter* spp.

The conducted studies comprising *Campylobacter jejuni* and *C. coli* isolates from different geographical regions within Europe and Asia showed that antimicrobial resistance and associated resistance determinants vary depending on origin of isolation. Our study on *Campylobacter* spp. in Georgia provided the first data on antimicrobial resistance for this pathogen in the country (89). Likewise, prevalence of antimicrobial resistance in *Campylobacter* from Vietnam was scarce. Although there are some studies regarding antimicrobial resistance among Vietnamese *Campylobacter* isolates, there are either only a limited number of isolates tested (149, 195) or the study does not reflect the recent situation (153). Hence, our study comprising 254 *Campylobacter* spp. isolates from the poultry production chain and retail markets in Vietnam is the most comprehensive evaluation of antimicrobial resistance in *Campylobacter* in Vietnam to date.

The antibiotic resistance profiles of *Campylobacter* spp. isolates from poultry in Georgia and Germany showed similarities with the antimicrobial resistance data profiles of *Campylobacter* spp. in EU member states. In particular, both *C. jejuni* and *C. coli* from poultry sources in the EU displayed notable resistance to fluoroquinolones and tetracycline, aligning with the findings of our studies (121). Resistance to the aminoglycoside gentamicin was similarly low in isolates from both countries. It was also intriguing to find that all human and chicken isolates from Georgia were sensitive to macrolides, whereas German *C. coli* isolates from chicken meat from retail had a prevalence of 12.8% macrolide resistance in 2022 (160). Similarly, our study of *C. coli* isolates from poultry obtained in Germany from 2013 to 2021 found an 18.3% prevalence of macrolide resistance (67). In Germany, the implementation of mitigation strategies has mandated farmers to reduce antibiotic use (144, 145). This was reflected by a decrease of antibiotic use in poultry by 11.5% in chickens and a 13.1% in turkeys from 2017 to 2021. Although the overall use of antimicrobials has decreased, there has been a shift from the use of polypeptide antibiotics, sulfonamides, and fluoroquinolones toward a preference for aminoglycosides and lincosamides. Additionally, lincosamides are gradually replacing some of the macrolides used. As a result, the use of lincosamides in chickens increased by 2.3%, while macrolide use decreased by 2.2% between 2017 and 2021 (161). The continued use of macrolides and lincosamides in German poultry farming may be a factor contributing to the occurrence of macrolide-resistant isolates. Nevertheless, temporal trends from 2014 to 2022 showed a significant decrease in macrolide resistance at least in *C. jejuni* from broilers and fattening turkeys (121). Meanwhile, due to the lack of data on macrolide use in veterinary settings in Georgia, no conclusions can be drawn, and it remains unclear why all tested isolates were susceptible.

The Vietnamese *Campylobacter* isolates differed as they were comparably more resistant for all tested substances, except that German *C. jejuni* were more frequently

resistant to streptomycin compared to Vietnamese *C. jejuni* (18.4% vs. 12.9%). Next to both *C. jejuni* and *C. coli* being nearly fully or fully resistant to fluoroquinolones and tetracyclines, respectively, especially the *C. coli* isolates also showed high-level resistance to aminoglycosides, macrolides and phenicols (67). This is probably due to the large quantities of antibiotics used for rearing of food animals in Vietnam. For instance, in 2015, Vietnam utilized a total of 3,838 t of antimicrobials, with 71.7% allocated for veterinary purposes. Compared with EU data from 2014, Vietnam's use of antimicrobials for animals was 1.72 times higher (162). Moreover, among the used antimicrobials in Vietnam there are substances that are regarded “highest priority critically important” for human health as stated by the WHO (163-167). This was also reflected in a questionnaire that was handed to farmers where they stated that they used e.g. tetracyclines, tylosin (macrolide), colistin (polymyxin), amoxicillin ( $\beta$ -lactam) and gentamicin during rearing of chicken. A study on antimicrobial use in household, semi-industrialized, and industrialized pig and poultry farms in Vietnam found that farmers with higher education levels and larger farm sizes were more likely to follow recommended dosages, withdrawal times, and manufacturer guidelines. Consequently, household farmers were less likely to adhere to these recommended guidelines (168). In 2013, Vietnam was the first country from WHO's Western Pacific region to approve a national action plan to fight antimicrobial resistance (169). This action plan is thought to establish better surveillance of resistances circulating in bacterial populations and to improve laboratory capacity. Furthermore, the addition of antimicrobials to animal feed for growth promotion has been banned since 2018 (168). Macrolides and fluoroquinolones are the preferred pharmaceuticals to treat severe and persisting campylobacteriosis (27, 51, 53). Hence, combined resistance to both substance classes is regarded critically important for treatment. As there were no erythromycin resistant isolates obtained in Georgia, treatment with macrolides would still be applicable. From our study comparing antimicrobial resistance in



*Campylobacter* isolates from Germany and Vietnam (67), 9.2% (22/240) of the *Campylobacter* spp. isolates derived from poultry between 2013 and 2021 exhibited resistance to both ciprofloxacin and erythromycin; however, all 22 isolates were still susceptible to gentamicin, making this antimicrobial a suitable treatment option. In contrast, 11.0% and 76.8% of *C. jejuni* and *C. coli* isolates from Vietnam, respectively, showed resistance to both macrolides and fluoroquinolones, with isolates collected from fresh chicken feces and chicken meat from retail between 2016 and 2018. Furthermore, 84.4% of the Vietnamese *C. coli* resistant to both substances were also resistant to gentamicin, leaving only few treatment options in case of severe outcomes of the disease.

Surveillance of the carbapenem ertapenem is mandatory in Europe for *C. spp* isolates from food and food animals since the Commission Implementing Decision (EU) 2020/1729 (170) came into force. Hence, the microdilution panel EUCAMP2 was updated to include ertapenem as substance for testing in the latest EUCAMP3 microtiter plate format. The current epidemiological cut-off value (ECOFF) of ertapenem for *Campylobacter* is 0.5 mg/L, defining isolates with MIC values above this value as resistant (171). However, as this antibiotic was introduced for monitoring thermotolerant *Campylobacter* spp. in 2021, there is only limited data on minimum inhibitory concentrations in the current EUCAST database (172). Therefore, adjustments of the ECOFF are likely. According to the current ECOFF, German *Campylobacter* isolates from humans and food obtained between 2018 and 2023 exhibited moderate resistance (6.8–40.0%), while Georgian human and chicken-derived isolates showed moderate to high-level resistance (37.0–82.0%) to the carbapenem antibiotic ertapenem (89, 173). Most recent results from zoonosis monitoring in chicken from retail in 2022 show even higher prevalences, with 6.4% of *C. jejuni* and 66.0% of *C. coli* being resistant to ertapenem (160). Carbapenems, like ertapenem and meropenem have already been used in humans for treating persistent

campylobacteriosis, resulting in the development of resistance to these antimicrobials in previously susceptible strains (174, 175). Vietnamese *Campylobacter* phenotype was only assessed with the EUCAMP2 plate, so that data on carbapenem resistance is still lacking and might be collected in the future.

Concerning species distribution of *C. spp.* isolated from chicken samples, it was intriguing to find that *C. coli* had a substantially higher prevalence of 74% compared to *C. jejuni* in Georgian backyard chickens and a prevalence of 90% in industrial chickens, which contrasts with other studies (176, 177). Meanwhile, the species distribution in human isolates from Georgia, with 82.0% being *C. jejuni* and 18.0% being *C. coli*, reflected a typical distribution, which is commonly observed among human isolates. A study from China showed a species shift from the previously predominant *C. jejuni* to more resistant *C. coli* over the course of seven years. The authors suggested that this species shift might have been likely induced by extended antimicrobial use, especially macrolides (178). Macrolide resistance associated with the point mutations in the 23S rRNA, is linked to a substantial fitness loss in *C. jejuni* (68), whereas *C. coli* does not seem to encounter the same fitness costs (69). However, in the Georgian isolates, none of the *Campylobacter* strains exhibited macrolide resistance, so the high prevalence of *C. coli* is unlikely to be attributed to this factor. In a study by Luangtongkum et al., conventional farms using antimicrobials were associated with a higher prevalence of *C. jejuni* and a lower prevalence of *C. coli* in broilers, but a higher prevalence of *C. coli* and a lower prevalence of *C. jejuni* in turkeys. Conversely, organic farms without antimicrobial use showed the opposite trends for both broilers and turkeys (179). Therefore, there appeared to be no causal relationship between antimicrobial use and the predominance of *C. coli*. Another study from Italy has also indicated that seasonality influences the prevalence of *C. spp.*, demonstrating a higher occurrence of *C. coli* during the spring and summer seasons (180). Furthermore, the usage of selective agars and enrichment broth seemed to favor the presence of *C. coli*

(181). Likewise, it was shown by Kramer et al. that *C. coli* was more likely to be isolated after enrichment if multispecies contamination was present (182).

### **7.2 In-depth analysis of antimicrobial resistance determinants resulted in identification of knowledge gaps in AMR prediction based on WGS**

In one of our studies, we analyzed nearly 500 *Campylobacter* isolates from Germany and Vietnam to identify and resolve discrepancies between short-read derived Whole-Genome Sequencing (WGS) and phenotypic data, and to evaluate the reliability of antimicrobial resistance prediction based on WGS. The findings showed regional differences in occurrence of resistance determinants, with substantially more resistance determinants being identified in *Campylobacter* isolates from Vietnam. Interestingly, distinct variants of certain genes, such as *tet(O)* and its mosaic variants, as well as *aadE*, were preferentially harbored by isolates from different geographical regions.

Although, several studies have already published whole-genome sequencing data alongside some phenotypic analysis of thermotolerant *Campylobacter* spp. (65, 66, 90, 104, 107, 108, 116), they did not provide in-depth analyses specifically focused on identifying and resolving discrepancies between whole-genome sequencing data and phenotypic resistance profiles. With our comprehensive and systematic approach, we were able to identify five principle discrepancies between pheno- and genotype in German and Vietnamese *Campylobacter* spp.. These five discrepancies were i) missing or falsely annotated AMR genes, ii) detection issues due to multiple copies or variant genes, iii) AMR gene inactivation by novel mutations and phase variability, iv) non-specific resistance indicated by MIC values just above the cut-off, and v) unknown resistance mechanisms in *Campylobacter* spp..

Certain antimicrobial resistance genes were either missing or inaccurately annotated

in databases. For example, the *aadE3* gene was absent from both the AMRFinderPlus and ResFinder databases. Yet, this gene had already been described prior (105). Additionally, the ribosomal L22 protein A103V mutation, falsely identified as conferring resistance in the AMRFinderPlus database, lacked correlation with erythromycin resistance. Suggestions that mutations in ribosomal proteins L4 and L22 play a role in macrolide resistance are largely drawn from observed resistances in other bacteria (86, 183), or based on the presence of specific mutations, although without establishing direct causation between a sole mutation and a particular phenotype (184). Several studies have shown that, at least for the proposed resistance mechanism of the point mutation A103V in ribosomal protein L22, there is no correlation with macrolide resistance (65, 67, 185). The mosaic tetracycline resistance gene *tet(O/32/O)* was not yet part of the AMRFinderPlus database (version 2023-08-08.2). Instead, it was identified as *tet(O)* with a reduced identity of approximately 93%. Gene alignments involving *tet(32)* and *tet(O)* demonstrated that the identified gene was indeed the mosaic resistance determinant *tet(O/32/O)*. Nevertheless, these issues can be easily addressed by curation of the AMRFinderPlus and ResFinder databases.

We encountered another inconsistency with antimicrobial resistance genes, which appeared either as multiple copies or variant genes. Short-read sequencing methods frequently struggled to precisely assign single reads to specific copies or variants of the genes, causing the assembler to struggle in creating complete sequences of resistance genes from the raw reads. This led to the generation of incomplete genes or lack of the resistant gene in the assemblies. Such assembly errors arising from repetitive regions are known and can be circumvented using long-read sequencing (186). We frequently observed lack of tetracycline resistance genes, especially in tetracycline resistant Vietnamese *Campylobacter* isolates, which harbored up to three copies or variant genes. The assembly process was further complicated due to the presence of the mosaic tetracycline resistance genes *tet(O/32/O)* and *tet(O/M/O)*, with

the latter exclusively present in Vietnamese *Campylobacter* spp.. Furthermore, among Vietnamese *Campylobacter* isolates, we detected tetracycline resistance genes exhibiting varying levels of *tet*(M) introgression into the *tet*(O) gene. It is known that ribosomal protection proteins (RPPs) like *tet*(O), *tet*(M), and *tet*(W) are likely to form chimeric structures where they can undergo diverse rearrangements (119). Whether these mosaic-like genes have advantageous traits in bacteria remains uncertain. From our phenotypic analysis we did not observe any differences in resistance to tetracycline arising from these genes. To address issues arising from multiple copies or variant genes, read mapping was performed using an artificial template consisting of three reference genes (*tet*(O), *tet*(O/M/O), and *tet*(O/32/O)), to which the trimmed short reads were aligned. True evidence was then achieved via long-read sequencing, which aligned well with results from read-mapping. Similarly, this phenomenon was noted with other resistance genes such as *aadE*, which existed in various variants, resulting in truncated genes being reported by AMRFinderPlus. The acquisition of multiple genes conferring the same resistance phenotype likely stems from the transfer of multidrug resistance islands, which harbor both the necessary resistance determinants and other resistance genes already present in the genome. Similarly, multiple genes conferring redundant phenotypes, including resistance to tetracycline and phenicol, were observed in isolates from food animals in China (187).

Another issue was the occurrence of point mutations. Mutations in the tetracycline resistance gene *tet*(W) apparently led to inactivation of *tet*(W), since the isolate was tetracycline sensitive, despite the presence of a full-length gene. Likewise, we observed point mutations in the gene *aad9*, which led to annotation of truncated resistance genes by AMRFinderPlus in over two-thirds of all isolates harboring this gene. Yet, phenotypic resistance was always detected in these isolates. From our sequencing data we identified mutations in a poly-C tract within the gene, which led to frameshifts and thus early termination of translation. Through application of selection

pressure with the antimicrobial spectinomycin, the gene reverted to a functional full-length *aad9* capable of inactivating the antibiotic substance. This led to the conclusion that *aad9* is a phase-variable gene able to undergo frameshifting but quickly reverting to a functional gene in presence of spectinomycin. Such phase variation might be important to reduce the cost of carrying AMR genes. Likewise, phase variation has been shown to be important in adaptation and in compensating for fitness costs in pathogenic bacteria (188, 189). In *Campylobacter* phase variation has been proposed e.g., a key mechanism regulating various genes crucial for host response (190-192).

Furthermore, we identified isolates with unknown resistance mechanisms. For instance, some isolates displayed resistance to ciprofloxacin without concurrent resistance to nalidixic acid, indicating unresolved aspects of (fluoro-)quinolone resistance in *Campylobacter*. Additionally, we identified a gentamicin resistant *C. coli* isolated in Germany in 2018 from turkey. Here, initial whole-genome sequencing and analysis approaches utilizing AMRFinderPlus and ResFinder led to absence of any known resistance determinant. Antimicrobial susceptibility testing using customized plate formats revealed that the isolate exhibited cross-resistance to the aminoglycosides apramycin, kanamycin, and tobramycin. Through a combined approach of natural transformation and whole-genome sequencing we were able to identify a novel point mutation within the 16S rRNA gene in the aminoacyl-tRNA binding (A-)site, where gentamicin is known to bind (193). This novel mutation in *Campylobacter* spp. has previously been described in other bacteria as the A1408G mutation (using *E. coli* numbering) (194-197). From our findings we could conclude that the resistance was transferrable via natural transformation in *Campylobacter* spp. but with a comparably low transformation rate. Additionally, Sanger sequencing revealed resistant transformants with different genotypes, which impacted the stability of the acquired resistance. Hence, the presence of the mutation A1387G in all three copies of the 16S rRNA gene was associated with stable resistance even after 45

passages, while presence of 1 or 2 copies led to rapid reversion to a sensitive phenotype after 15 passages. Likewise, in co-cultures of *Borrelia burgdorferi*, where resistant and sensitive strains were mixed, it was observed that the resistant strains were eventually outcompeted. This was evidenced by the absence of colonies from the cocultures on plates with the corresponding antibiotics after 100 generations (194). This study also observed no growth deficiencies in absence of selection pressure, which aligns with our findings. Hence, it can be concluded that, despite no growth deficiency is observable in absence of selection pressure, still the mutation leads to a fitness loss, and that a reversion to a sensitive phenotype was favored.

Additionally, we witnessed that transformants with a mixed population of 16S rRNA gene copies adapted to having only G at position 1387 at higher concentrations of tobramycin. This is probably due to a gradual transition from a sensitive to a fully resistant phenotype. Consequently, we concluded that colonies undergoing only partial transition still experience impeded growth due to the selective pressure at higher aminoglycoside concentrations, leading to a reduced growth rate. This also suggests that complete resistance is only apparent when all copies of 16S rRNA have undergone transition. Similar observations were made for isolates of *Nocardia farcinica* that were exposed to the aminoglycoside amikacin for an extended period (195).

To further elucidate the genetic basis of resistance, we employed Oxford Nanopore long-read sequencing technology. This approach enabled us to pinpoint the exact location of resistance genes within the genome. Our sequencing results revealed that resistance genes were predominantly located on the chromosome, particularly near transposase genes, while they were less frequently found on plasmids. Hence, we concluded that primary mobilization of resistance determinants in *Campylobacter* is likely through natural transformation and transposition and to a lesser extent through conjugation. For instance, the *Inu(C)* gene, found exclusively in Vietnamese *C. jejuni*

isolates, was located near a transposase gene. Similarly, literature from China reported this resistance gene in *C. coli* isolates from chickens, also positioned adjacent to a transposase gene, aligning with our findings (198). Additionally, *fexA* and *optrA* were found next to *IS1216* family transposase genes in *C. jejuni* and *C. coli* from poultry and swine in China, which corresponds with our long-read sequencing data (187). We observed the resistance cluster *aadE-sat4-aph(3')-IIIa* to be located either within the bacterial chromosome or on plasmids. When situated on the chromosome, it was found in close proximity to transposase genes, as reported by other researchers as well (199). Likewise, its presence on plasmids has also been described before (105, 200). Next to the *aadE-sat4-aph(3')-IIIa* gene cluster, both plasmids harboring resistance genes carried either the resistance gene *tet(O)* or *tet(O/32/O)*. Transmissible plasmids with tetracycline resistance genes are frequently encountered in *Campylobacter* spp., such as the plasmid pTet (201-203). We also observed genes encoding the Type IV Secretion system, which is crucial for conjugative DNA transfer in *Campylobacter* spp. (204).

Since macrolides are the preferred antimicrobials in treatment of severe and prolonged campylobacteriosis (27, 51, 53), high resistances and spread of associated resistance determinants should be viewed critically. It was intriguing to find that some *Campylobacter coli* isolates from Vietnam harbored the methyltransferase *erm(B)*, which also leads to high-level macrolide resistance. Our results from long-read sequencing showed that the gene was situated in a MDRI on the chromosome and that this MDRI was quite conserved throughout the investigated isolates. Likewise, other studies from China also found the gene to be part of different multidrug resistance genomic islands (76, 78). Additionally, a novel *erm* class methyltransferase, subsequently named *erm(N)*, was identified in five French and one Canadian clinical strain. Its consistent presence within the CRISPR-*cas9* operon suggested potential dissemination of this resistance determinant between France and Canada, aided by



travel (77, 205). Due to the transferability of the *erm*(B) gene via natural transformation (206), the rapid spread of macrolide resistance is particularly concerning. An indicator of its dissemination is that it has already been identified in many countries globally (72-78).

### **7.3 Novel pentaplex Real-Time PCR shows rapid and reliable detection of clinically important resistance determinants in *Campylobacter* spp.**

The findings from our genomic investigations into resistance determinants circulating globally in the zoonotic pathogen *Campylobacter* were used to develop a novel pentaplex real-time PCR system for routine detection. This novel Real-Time PCR system was specifically designed to target the most commonly encountered and clinically important resistance determinants relevant to the treatment of campylobacteriosis (121). Therefore, we decided to incorporate the detection of the GyrA\_T86I point mutation linked to fluoroquinolone resistance, the A2075G point mutation within the 23S rRNA gene, along with the *erm*(B) gene associated with macrolide resistance, and finally, the ribosomal protection protein *tet*(O) responsible for tetracycline resistance. Our aim was to identify resistance determinants without relying on time-consuming and labor-intensive phenotypic characterization, providing rapid tools for utilization in European monitoring surveys of circulating resistance determinants.

While there have been PCR detection systems described for identifying resistance determinants in *Campylobacter*, ours integrates a broader range of resistance determinants than those typically covered by existing systems. For instance, singleplex systems were previously utilized to assess the presence of point mutations, such as in the 23S rRNA gene using mismatch amplification mutation assay (MAMA) PCR (207). A real-time PCR assay, based on amplifying a fragment of the 23S rRNA gene was

designed to detect macrolide-associated mutations (64). Additionally, Hadiyan and colleagues used conventional endpoint PCR to detect the resistance genes *tet(O)*, *aph(3')*, *bla<sub>OXA</sub>* and *cmeB* in *Campylobacter* isolates from poultry meat samples (208). A duplex PCR system was also used to detect fluoroquinolone resistance associated with the T86I point mutation within the *gyrA* gene (209). The novel pentaplex PCR System for detection of four different resistance determinants (*tet(O)*, *erm(B)*, *gyrA\_T86I* and 23S rRNA\_A2075G) integrates the detection of the most frequent resistant determinants in *C. spp.* simultaneously.

Although MAMA PCR is commonly used for point mutation detection (210), the pentaplex PCR opted to use Locked Nucleotide Acid (LNA) oligonucleotides instead (211), due to their advantage in providing high specificity by thermally stabilizing the probes (212, 213). Furthermore, unlabeled LNA probes were added, containing the wild-type nucleotide sequence, to enhance the specificity of detecting *gyrA* and 23S rRNA point mutations. The developed multiplex assay does not detect the point mutations A2074G, A2074C, and A2074T (60-62), which may slightly constrain its range of application. Nevertheless, these mutations are rarely observed, while the A2075G mutation is the most frequent encountered mutation associated with macrolide resistance in *Campylobacter* (66, 67, 90, 124).

The comparison between phenotypic and genomic results obtained from EUCAMP3 and PCR, respectively, demonstrated 100% agreement, confirming the specificity of the system. Therefore, the pentaplex PCR system is well-suited as an alert tool for routine resistance monitoring. In future, there might be a need to develop an additional multiplex PCR system capable of detecting other circulating resistance determinants in *Campylobacter* worldwide. Notably, it might be crucial to include aminoglycoside and carbapenem resistance determinants in this expanded system for comprehensive monitoring.

### 7.4 Conclusion

Our studies have provided valuable insights into the occurrence of resistances in *Campylobacter* isolates from a global perspective. We successfully correlated phenotypic resistance with the presence of genomic determinants. In-depth analysis of whole-genome sequencing data revealed knowledge gaps in the prediction of AMR based on genomic information, emphasizing the need for ongoing refinement and validation of predictive tools. This includes improvements in database curation and sequencing methodologies. Furthermore, the development of a novel pentaplex Real-Time PCR system will enhance routine resistance monitoring in the near future. Our findings highlight the importance of comprehensive surveillance strategies to address ongoing dissemination and adaptation of AMR in thermotolerant *Campylobacter*, particularly in the context of global health.

## 8 References

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## 9 Appendix

### 9.1 List of Abbreviations

|        |  |
|--------|--|
| 2-DOS  | 2-deoxystreptamine   |
| AMR    | antimicrobial resistance                                     |
| ATP    | adenosine triphosphate                                       |
| BfR    | German Federal Institute for Risk Assessment                 |
| BVL    | German Federal Office of Consumer Protection and Food Safety |
| CDC    | Centers for Disease Control and Prevention                   |
| CRISPR | Clustered Regularly Interspaced Short Palindromic Repeats    |
| DALY   | Disability Adjusted Life Years                               |
| DART   | German Antimicrobial Resistance Strategy                     |
| ECDC   | European Centre for Disease Prevention and Control           |
| ECOFF  | Epidemiological Cut-Off                                      |
| EFSA   | European Food Safety Authority                               |
| EIA    | Enzymatic Immunoassay  |
| EUCAST | European Committee on Antimicrobial Susceptibility Testing   |
| FDA    | U.S. Food and Drug Administration                            |
| FERG   | Foodborne Disease Burden Epidemiology Reference Group        |
| LNA    | Locked Nucleic Acid  |
| MAMA   | Mismatch Amplification Mutation Assay                        |
| MDRI   | Multidrug Resistance Island                                  |
| MIC    | Minimum Inhibitory Concentration                             |
| NAP    | National Action Plan   |

|        |   |
|--------|---|
| NARMS  | National Antimicrobial Resistance Monitoring System |
| QRDR   | Quinolone Resistance Determining Region             |
| RPP    | Ribosome Protection Protein                         |
| rRNA   | ribosomal Ribonucleic Acid                          |
| RT-PCR | Real-Time Polymerase Chain Reaction                 |
| SAM    | S-adenosylmethionine                                |
| tRNA   | transfer Ribonucleic Acid                           |
| WGS    | Whole-Genome Sequencing                             |
| WHO    | World Health Organization                           |



## **9.2 Supplementary material of own publications**

### **9.2.1 Publication 1: Comparison of Antimicrobial Susceptibility Profiles of Thermotolerant *Campylobacter* spp. Isolated from Human and Poultry Samples in Georgia (Caucasus)**

# 9 Appendix

TabS1\_Complete\_sample list

data export 10.03.2022

| strain No.   | source         | original No. | isolation date | Species              | NGS_Species          | CHL | CIP    | ERY | ETP    | GEN    | TET   | Res profile   | Res type  |
|--------------|----------------|--------------|----------------|----------------------|----------------------|-----|--------|-----|--------|--------|-------|---------------|-----------|
| BIR-CA-19789 | broiler, feces | BY 5         | 12.02.2020     | Campylobacter coli   | Campylobacter coli   | 4   | 32     | <=1 | 0,5    | 0,5    | >64   | CIP, TET      | 2-fold    |
| BIR-CA-19797 | human stool    | H8           | 06.07.2020     | Campylobacter jejuni | Campylobacter jejuni | 4   | 16     | 2   | <=0,12 | 0,5    | <=0,5 | CIP           | 1-fold    |
| BIR-CA-19798 | human stool    | H5           | 08.07.2020     | Campylobacter jejuni | Campylobacter jejuni | <=2 | 8      | <=1 | <=0,12 | 0,5    | 64    | CIP, TET      | 2-fold    |
| BIR-CA-19799 | human stool    | H9           | 13.07.2020     | Campylobacter coli   | Campylobacter coli   | 4   | 16     | <=1 | 1      | 0,5    | >64   | CIP, ETP, TET | 3-fold    |
| BIR-CA-19802 | human stool    | H12          | 20.08.2020     | Campylobacter coli   | Campylobacter coli   | 4   | 32     | <=1 | 0,5    | 1      | >64   | CIP, TET      | 2-fold    |
| BIR-CA-19804 | human stool    | H18          | 29.09.2020     | Campylobacter coli   | Campylobacter coli   | <=2 | 8      | <=1 | 1      | 0,5    | >64   | CIP, ETP, TET | 3-fold    |
| BIR-CA-19805 | human stool    | H19          | 29.09.2020     | Campylobacter jejuni | Campylobacter jejuni | <=2 | 16     | <=1 | <=0,12 | 0,5    | <=0,5 | CIP           | 1-fold    |
| BIR-CA-19806 | human stool    | H20          | 13.10.2020     | Campylobacter jejuni | Campylobacter jejuni | <=2 | 16     | <=1 | <=0,12 | 0,5    | <=0,5 | CIP           | 1-fold    |
| BIR-CA-19807 | human stool    | H27          | 10.12.2020     | Campylobacter coli   | Campylobacter coli   | 4   | 32     | 2   | <=0,12 | 0,5    | >64   | CIP, TET      | 2-fold    |
| BIR-CA-19808 | human stool    | H28          | 15.12.2020     | Campylobacter coli   | Campylobacter coli   | 4   | 16     | 2   | 2      | 0,5    | <=0,5 | CIP, ETP      | 2-fold    |
| BIR-CA-19812 | human stool    | H34          | 05.03.2021     | Campylobacter jejuni | Campylobacter jejuni | <=2 | 16     | <=1 | <=0,12 | <=0,25 | >64   | CIP, TET      | 2-fold    |
| BIR-CA-19817 | human stool    | H39          | 13.05.2021     | Campylobacter jejuni | Campylobacter jejuni | <=2 | 16     | <=1 | <=0,12 | 0,5    | <=0,5 | CIP           | 1-fold    |
| BIR-CA-19820 | human stool    | H13          | 20.08.2020     | Campylobacter jejuni | Campylobacter jejuni | <=2 | <=0,12 | <=1 | <=0,12 | 0,5    | <=0,5 | sensitive     | sensitive |
| BIR-CA-19827 | broiler, feces | BY 15        | 03.07.2020     | Campylobacter coli   | Campylobacter coli   | <=2 | <=0,12 | <=1 | 0,5    | 0,5    | <=0,5 | sensitive     | sensitive |
| BIR-CA-19828 | broiler, feces | BY 18        | 03.07.2020     | Campylobacter jejuni | Campylobacter jejuni | <=2 | 16     | <=1 | <=0,12 | 0,5    | 64    | CIP, TET      | 2-fold    |
| BIR-CA-19831 | broiler, feces | BY 33        | 11.02.2021     | Campylobacter jejuni | Campylobacter jejuni | <=2 | 8      | <=1 | <=0,12 | <=0,25 | >64   | CIP, TET      | 2-fold    |
| BIR-CA-19833 | broiler, feces | BY 37        | 11.02.2021     | Campylobacter coli   | Campylobacter coli   | 4   | 16     | <=1 | 0,5    | 1      | >64   | CIP, TET      | 2-fold    |
| BIR-CA-19839 | broiler, feces | BY 45        | 23.02.2021     | Campylobacter jejuni | Campylobacter jejuni | <=2 | <=0,12 | <=1 | <=0,12 | 0,5    | <=0,5 | sensitive     | sensitive |
| BIR-CA-19845 | human stool    | H30          | 11.01.2021     | Campylobacter jejuni | Campylobacter jejuni | 4   | 32     | 2   | 0,25   | 0,5    | >64   | CIP, TET      | 2-fold    |
| BIR-CA-19846 | broiler, feces | BY 27        | 17.07.2020     | Campylobacter jejuni | Campylobacter jejuni | <=2 | 8      | <=1 | <=0,12 | 0,5    | 32    | CIP, TET      | 2-fold    |
| BIR-CA-19851 | human stool    | H41          | 08.06.2021     | Campylobacter jejuni | Campylobacter jejuni | 4   | 32     | <=1 | 0,25   | 0,5    | >64   | CIP, TET      | 2-fold    |
| BIR-CA-19853 | human stool    | H43          | 16.06.2021     | Campylobacter jejuni | Campylobacter jejuni | <=2 | <=0,12 | <=1 | <=0,12 | 0,5    | 64    | TET           | 1-fold    |
| BIR-CA-19854 | human stool    | H44          | 16.06.2021     | Campylobacter coli   | Campylobacter coli   | <=2 | 16     | <=1 | 2      | 1      | >64   | CIP, ETP, TET | 3-fold    |
| BIR-CA-19857 | human stool    | H47          | 01.07.2021     | Campylobacter coli   | Campylobacter coli   | <=2 | 32     | <=1 | 0,5    | 0,5    | >64   | CIP, TET      | 2-fold    |
| BIR-CA-19863 | human stool    | H53          | 30.07.2021     | Campylobacter jejuni | Campylobacter jejuni | <=2 | 16     | <=1 | <=0,12 | <=0,25 | <=0,5 | CIP           | 1-fold    |
| BIR-CA-19866 | human stool    | H56          | 23.09.2021     | Campylobacter jejuni | Campylobacter jejuni | <=2 | 16     | <=1 | <=0,12 | 0,5    | <=0,5 | CIP           | 1-fold    |
| BIR-CA-19874 | broiler, feces | BY 46        | 23.02.2021     | Campylobacter coli   | Campylobacter coli   | 4   | 16     | <=1 | 4      | 1      | <=0,5 | CIP, ETP      | 2-fold    |
| BIR-CA-19876 | broiler, feces | BY 48        | 26.02.2021     | Campylobacter jejuni | Campylobacter jejuni | 4   | 32     | <=1 | <=0,12 | 0,5    | >64   | CIP, TET      | 2-fold    |
| BIR-CA-19902 | human stool    | H29          | 25.12.2020     | Campylobacter coli   | Campylobacter coli   | <=2 | 8      | <=1 | 1      | 0,5    | 64    | CIP, ETP, TET | 3-fold    |
| BIR-CA-19906 | broiler, feces | BY 80        | 07.04.2021     | Campylobacter coli   | Campylobacter coli   | 4   | 8      | <=1 | 1      | 0,5    | <=0,5 | CIP, ETP      | 2-fold    |
| BIR-CA-19911 | broiler, feces | BY 66        | 01.04.2021     | Campylobacter jejuni | Campylobacter jejuni | <=2 | 8      | <=1 | <=0,12 | <=0,25 | >64   | CIP, TET      | 2-fold    |
| BIR-CA-19922 | broiler, feces | BY 86        | 07.04.2021     | Campylobacter coli   | Campylobacter coli   | <=2 | 32     | <=1 | 0,5    | 0,5    | >64   | CIP, TET      | 2-fold    |
| BIR-CA-19931 | broiler, feces | BY 95        | 07.04.2021     | Campylobacter jejuni | Campylobacter jejuni | <=2 | 4      | <=1 | <=0,12 | 0,5    | 16    | CIP, TET      | 2-fold    |
| BIR-CA-19951 | broiler, feces | BY 115       | 15.04.2021     | Campylobacter jejuni | Campylobacter jejuni | <=2 | 16     | <=1 | <=0,12 | 0,5    | >64   | CIP, TET      | 2-fold    |
| BIR-CA-19952 | broiler, feces | IndChi 1     | 03.06.2021     | Campylobacter coli   | Campylobacter coli   | <=2 | 16     | <=1 | 1      | 0,5    | >64   | CIP, ETP, TET | 3-fold    |
| BIR-CA-19961 | broiler, feces | IndChi 10    | 17.06.2021     | Campylobacter coli   | Campylobacter coli   | <=2 | 16     | <=1 | 1      | 0,5    | >64   | CIP, ETP, TET | 3-fold    |
| BIR-CA-19967 | broiler, feces | IndChi 16    | 17.06.2021     | Campylobacter jejuni | Campylobacter jejuni | 4   | 16     | <=1 | 0,25   | 0,5    | >64   | CIP, TET      | 2-fold    |
| BIR-CA-19989 | broiler, feces | IndChi 38    | 09.07.2021     | Campylobacter coli   | Campylobacter coli   | <=2 | 16     | <=1 | 0,5    | 0,5    | >64   | CIP, TET      | 2-fold    |
| BIR-CA-19995 | broiler, feces | IndChi 44    | 09.07.2021     | Campylobacter jejuni | Campylobacter jejuni | 4   | 16     | <=1 | <=0,12 | 0,5    | >64   | CIP, TET      | 2-fold    |
| BIR-CA-19996 | broiler, feces | IndChi 45    | 09.07.2021     | Campylobacter coli   | Campylobacter coli   | <=2 | 16     | <=1 | 1      | 0,5    | >64   | CIP, ETP, TET | 3-fold    |
| BIR-CA-19786 | broiler, feces | BY 1         | 28.10.2020     | Campylobacter coli   | n.a.                 | 4   | 32     | <=1 | 0,5    | 0,5    | >64   | CIP, TET      | 2-fold    |
| BIR-CA-19787 | broiler, feces | BY 3         | 12.02.2020     | Campylobacter coli   | n.a.                 | 4   | 32     | <=1 | 0,5    | 0,5    | >64   | CIP, TET      | 2-fold    |
| BIR-CA-19788 | broiler, feces | BY 4         | 12.02.2020     | Campylobacter coli   | n.a.                 | 4   | >32    | <=1 | 0,5    | 1      | >64   | CIP, TET      | 2-fold    |
| BIR-CA-19790 | broiler, feces | BY 6         | 18.06.2021     | Campylobacter coli   | n.a.                 | 8   | >32    | <=1 | 1      | 1      | >64   | CIP, ETP, TET | 3-fold    |
| BIR-CA-19791 | broiler, feces | BY 7         | 18.06.2021     | Campylobacter coli   | n.a.                 | 4   | >32    | <=1 | 1      | 1      | >64   | CIP, ETP, TET | 3-fold    |
| BIR-CA-19792 | broiler, feces | BY 9         | 21.02.2020     | Campylobacter coli   | n.a.                 | 4   | 32     | <=1 | 0,25   | 0,5    | >64   | CIP, TET      | 2-fold    |
| BIR-CA-19793 | human stool    | H2           | 21.06.2020     | Campylobacter jejuni | n.a.                 | 4   | 8      | <=1 | <=0,12 | 0,5    | >64   | CIP, TET      | 2-fold    |
| BIR-CA-19794 | human stool    | H3           | 21.06.2020     | Campylobacter coli   | n.a.                 | <=2 | 16     | 2   | 2      | 0,5    | >64   | CIP, ETP, TET | 3-fold    |
| BIR-CA-19795 | human stool    | H1           | 21.06.2020     | Campylobacter jejuni | n.a.                 | <=2 | 16     | <=1 | <=0,12 | <=0,25 | >64   | CIP, TET      | 2-fold    |
| BIR-CA-19796 | human stool    | H7           | 06.07.2020     | Campylobacter jejuni | n.a.                 | <=2 | 16     | <=1 | <=0,12 | 0,5    | >64   | CIP, TET      | 2-fold    |
| BIR-CA-19800 | human stool    | H10          | 15.07.2020     | Campylobacter jejuni | n.a.                 | <=2 | 16     | <=1 | <=0,12 | 0,5    | <=0,5 | CIP           | 1-fold    |
| BIR-CA-19801 | human stool    | H11          | 20.07.2020     | Campylobacter jejuni | n.a.                 | 4   | 16     | <=1 | 0,25   | 0,5    | >64   | CIP, TET      | 2-fold    |
| BIR-CA-19803 | human stool    | H14          | 04.09.2020     | Campylobacter jejuni | n.a.                 | <=2 | 16     | <=1 | <=0,12 | 0,5    | <=0,5 | CIP           | 1-fold    |
| BIR-CA-19809 | human stool    | H31          | 18.01.2021     | Campylobacter jejuni | n.a.                 | <=2 | 8      | <=1 | <=0,12 | 1      | >64   | CIP, TET      | 2-fold    |
| BIR-CA-19810 | human stool    | H32          | 11.02.2021     | Campylobacter jejuni | n.a.                 | <=2 | <=0,12 | <=1 | <=0,12 | 0,5    | <=0,5 | sensitive     | sensitive |
| BIR-CA-19811 | human stool    | H33          | 02.03.2021     | Campylobacter coli   | n.a.                 | <=2 | 8      | <=1 | <=0,12 | 0,5    | <=0,5 | CIP           | 1-fold    |
| BIR-CA-19813 | human stool    | H35          | 21.04.2021     | Campylobacter jejuni | n.a.                 | <=2 | <=0,12 | <=1 | <=0,12 | <=0,25 | <=0,5 | sensitive     | sensitive |
| BIR-CA-19814 | human stool    | H36          | 21.04.2021     | Campylobacter jejuni | n.a.                 | <=2 | <=0,12 | <=1 | <=0,12 | 1      | <=0,5 | sensitive     | sensitive |
| BIR-CA-19815 | human stool    | H37          | 26.04.2021     | Campylobacter jejuni | n.a.                 | <=2 | 16     | <=1 | <=0,12 | 1      | <=0,5 | CIP           | 1-fold    |
| BIR-CA-19816 | human stool    | H38          | 07.05.2021     | Campylobacter jejuni | n.a.                 | <=2 | 4      | <=1 | <=0,12 | 0,5    | 16    | CIP, TET      | 2-fold    |
| BIR-CA-19818 | human stool    | H4           | 06.07.2020     | Campylobacter coli   | n.a.                 | 4   | 32     | <=1 | <=0,12 | 0,5    | <=0,5 | CIP           | 1-fold    |
| BIR-CA-19819 | human stool    | H6           | 06.07.2020     | Campylobacter jejuni | n.a.                 | 4   | 16     | 2   | <=0,12 | 0,5    | <=0,5 | CIP           | 1-fold    |
| BIR-CA-19821 | human stool    | H15          | 11.09.2020     | Campylobacter jejuni | n.a.                 | <=2 | 8      | <=1 | <=0,12 | <=0,25 | <=0,5 | CIP           | 1-fold    |
| BIR-CA-19822 | human stool    | H16          | 11.09.2020     | Campylobacter jejuni | n.a.                 | <=2 | <=0,12 | <=1 | <=0,12 | 0,5    | <=0,5 | sensitive     | sensitive |
| BIR-CA-19823 | human stool    | H17          | 24.09.2020     | Campylobacter jejuni | n.a.                 | 4   | 16     | 4   | <=0,12 | 0,5    | <=0,5 | CIP           | 1-fold    |
| BIR-CA-19824 | broiler, feces | BY 2         | 28.10.2020     | Campylobacter coli   | n.a.                 | 4   | 32     | <=1 | 0,25   | 0,5    | >64   | CIP, TET      | 2-fold    |
| BIR-CA-19825 | broiler, feces | BY 8         | 18.06.2021     | Campylobacter coli   | n.a.                 | <=2 | 4      | <=1 | <=0,12 | 0,5    | 16    | CIP, TET      | 2-fold    |
| BIR-CA-19826 | broiler, feces | BY 14        | 03.07.2021     | Campylobacter coli   | n.a.                 | <=2 | 16     | <=1 | 1      | 1      | >64   | CIP, ETP, TET | 3-fold    |
| BIR-CA-19829 | broiler, feces | BY 31        | 11.02.2021     | Campylobacter coli   | n.a.                 | <=2 | 8      | <=1 | 0,5    | 0,5    | >64   | CIP, TET      | 2-fold    |
| BIR-CA-19830 | broiler, feces | BY 32        | 11.02.2021     | Campylobacter coli   | n.a.                 | <=2 | 8      | <=1 | 0,5    | 0,5    | >64   | CIP, TET      | 2-fold    |
| BIR-CA-19832 | broiler, feces | BY 35        | 11.02.2021     | Campylobacter coli   | n.a.                 | <=2 | 8      | <=1 | 0,25   | 0,5    | >64   | CIP, TET      | 2-fold    |
| BIR-CA-19834 | broiler, feces | BY 39        | 11.02.2021     | Campylobacter coli   | n.a.                 | 4   | 32     | <=1 | 0,25   | 0,5    | >64   | CIP, TET      | 2-fold    |
| BIR-CA-19835 | broiler, feces | BY 40        | 11.02.2021     | Campylobacter coli   | n.a.                 | 8   | 32     | <=1 | 0,5    | 0,5    | <=0,5 | CIP           | 1-fold    |
| BIR-CA-19836 | broiler, feces | BY 41        | 11.02.2021     | Campylobacter coli   | n.a.                 | <=2 | 16     | 2   | 0,5    | 0,5    | >64   | CIP, TET      | 2-fold    |
| BIR-CA-19837 | broiler, feces | BY 42        | 11.02.2021     | Campylobacter coli   | n.a.                 | 4   | 32     | 4   | 0,5    | 1      | <=0,5 | CIP           | 1-fold    |
| BIR-CA-19838 | broiler, feces | BY 43        | 23.02.2021     | Campylobacter coli   | n.a.                 | <=2 | 8      | <=1 | 1      | 0,5    | 64    | CIP, ETP, TET | 3-fold    |
| BIR-CA-19840 | human stool    | H22          | 13.10.2020     | Campylobacter jejuni | n.a.                 | <=2 | 16     | <=1 | <=0,12 | 0,5    | >64   | CIP, TET      | 2-fold    |
| BIR-CA-19841 | human stool    | H23          | 16.10.2020     | Campylobacter jejuni | n.a.                 | <=2 | 8      | <=1 | <=0,12 | <=0,25 | >64   | CIP, TET      | 2-fold    |
| BIR-CA-19842 | human stool    | H24          | 23.10.2020     | Campylobacter jejuni | n.a.                 | <=2 | 16     | <=1 | <=0,12 | 0,5    | <=0,5 | CIP           | 1-fold    |
| BIR-CA-19843 | human stool    | H25          | 20.11.2020     | Campylobacter jejuni | n.a.                 | <=2 | 16     | <=1 | 0,25   |        |       |               |           |





# 9 Appendix

TabS2\_WGS\_data\_overview

| strain No.   | source         | original No. | isolation date | Species              | NGS_Species          | coverage depth | number contigs | ST            | CC             | aspA | glnA | gltA | glyA | pgm | tkf | uncA | amr genecount | amr genecount acquired | amr genecount points | amr genes                   | mobile amr genecount                      | mobile amr genes                          | plasmids contigs                          | plasmids cumulativelength | plasmids circular | strain No.   | Res profile  |               |               |               |
|--------------|----------------|--------------|----------------|----------------------|----------------------|----------------|----------------|---------------|----------------|------|------|------|------|-----|-----|------|---------------|------------------------|----------------------|-----------------------------|---|---|---|---------------------------|-------------------|--------------|--------------|---------------|---------------|---------------|
| BIR-CA-19789 | broiler, feces | BY 5         | 12.02.2020     | Campylobacter coli   | Campylobacter coli   | 74.5           | 54             | 1058          | ST-828 complex | 33   | 39   | 30   | 82   | 104 | 35  | 17   | 3             | 2                      | 1                    | blaOXA-489;gyrA_T86I:tet(O) | 0   | None found                                | 1   | 26044                     | 0                 | BIR-CA-19789 | CIP, TET     |               |               |               |
| BIR-CA-19797 | human stool    | H8           | 06.07.2020     | Campylobacter jejuni | Campylobacter jejuni | 40             | 38             | ST-48 complex | 2              | 4    | 2    | 2    | 6    | 1   | 5   | 2    | 1             | 1                      | 1                    | blaOXA-193;gyrA_T86I        | 0   | None found                                | 1   | 29179                     | 0                 | BIR-CA-19797 | CIP          |               |               |               |
| BIR-CA-19798 | human stool    | H5           | 08.07.2020     | Campylobacter jejuni | Campylobacter jejuni | 76.0           | 45             | 11759         | ST-574         | 7    | 84   | 2    | 10   | 11  | 3   | 3    | 4             | 2                      | 2                    | 2                           | 50S_L22_A103V;blaOXA-193;gyrA_T86I:tet(O) | 0   | None found                                | 0                         | 0                 | 0            | BIR-CA-19798 | CIP, TET      |               |               |
| BIR-CA-19799 | human stool    | H9           | 13.07.2020     | Campylobacter coli   | Campylobacter coli   | 96.5           | 54             | 855           | ST-828 complex | 33   | 39   | 30   | 79   | 104 | 35  | 17   | 3             | 2                      | 1                    | 1                           | blaOXA-489;gyrA_T86I:tet(O)               | 0   | None found                                | 1                         | 26043             | 0            | BIR-CA-19799 | CIP, ETP, TET |               |               |
| BIR-CA-19802 | human stool    | H12          | 20.08.2020     | Campylobacter coli   | Campylobacter coli   | 93.8           | 56             | 11762         | ST-828 complex | 33   | 39   | 30   | 82   | 104 | 47  | 731  | 3             | 2                      | 1                    | 1                           | blaOXA-594;gyrA_T86I:tet(O)               | 0   | None found                                | 1                         | 40871             | 0            | BIR-CA-19802 | CIP, TET      |               |               |
| BIR-CA-19804 | human stool    | H18          | 29.09.2020     | Campylobacter coli   | Campylobacter coli   | 64.2           | 70             | 855           | ST-828 complex | 33   | 39   | 30   | 79   | 104 | 35  | 17   | 4             | 2                      | 2                    | 2                           | 50S_L22_A103V;blaOXA-193;gyrA_T86I:tet(O) | 0   | None found                                | 9                         | 43987             | 0            | BIR-CA-19804 | CIP, ETP, TET |               |               |
| BIR-CA-19805 | human stool    | H19          | 29.09.2020     | Campylobacter jejuni | Campylobacter jejuni | 80.1           | 31             | 356           | ST-353 complex | 14   | 17   | 5    | 2    | 11  | 3   | 6    | 1             | 0                      | 0                    | 1                           | gyrA_T86I                                 | 0   | None found                                | 0                         | 0                 | 0            | BIR-CA-19805 | CIP           |               |               |
| BIR-CA-19806 | human stool    | H20          | 13.10.2020     | Campylobacter coli   | Campylobacter coli   | 67.9           | 33             | 356           | ST-353 complex | 14   | 17   | 5    | 2    | 11  | 3   | 6    | 1             | 0                      | 0                    | 1                           | gyrA_T86I                                 | 0   | None found                                | 0                         | 0                 | 0            | BIR-CA-19806 | CIP           |               |               |
| BIR-CA-19807 | human stool    | H27          | 10.12.2020     | Campylobacter coli   | Campylobacter coli   | 94.6           | 29             | 854           | ST-828 complex | 33   | 38   | 30   | 82   | 104 | 43  | 17   | 3             | 2                      | 2                    | 1                           | 1   | aadE-Cc;gyrA_T86I:tet(O)                  | 0   | None found                | 1                 | 3397         | 0            | BIR-CA-19807  | CIP, TET      |               |
| BIR-CA-19808 | human stool    | H28          | 15.12.2020     | Campylobacter coli   | Campylobacter coli   | 53.9           | 38             | 8043          | none           | 33   | 66   | 30   | 79   | 113 | 35  | 17   | 2             | 1                      | 1                    | 1                           | blaOXA-193;gyrA_T86I                      | 0   | None found                                | 1                         | 44061             | 0            | BIR-CA-19808 | CIP, ETP      |               |               |
| BIR-CA-19812 | human stool    | H34          | 05.03.2021     | Campylobacter jejuni | Campylobacter jejuni | 67.2           | 135            | 531           | none           | 2    | 71   | 5    | 62   | 11  | 67  | 6    | 3             | 2                      | 1                    | 1                           | blaOXA-452;gyrA_T86I:tet(O)               | 0   | None found                                | 2                         | 5954              | 0            | BIR-CA-19812 | CIP, TET      |               |               |
| BIR-CA-19817 | human stool    | H39          | 13.05.2021     | Campylobacter jejuni | Campylobacter jejuni | 53.1           | 28             | 1947          | ST-22 complex  | 1    | 94   | 6    | 4    | 3   | 3   | 3    | 2             | 1                      | 1                    | 1                           | blaOXA-193;gyrA_T86I                      | 0   | None found                                | 0                         | 0                 | 0            | BIR-CA-19817 | CIP           |               |               |
| BIR-CA-19820 | human stool    | H13          | 20.08.2020     | Campylobacter jejuni | Campylobacter jejuni | 72.5           | 45             | 49            | ST-49 complex  | 3    | 1    | 5    | 17   | 11  | 11  | 6    | 1             | 1                      | 1                    | 0                           | blaOXA-461                                | 0   | None found                                | 2                         | 73430             | 0            | BIR-CA-19820 | sensitiv      |               |               |
| BIR-CA-19827 | broiler, feces | BY 15        | 03.07.2020     | Campylobacter coli   | Campylobacter coli   | 57.5           | 40             | 825           | ST-828 complex | 33   | 39   | 30   | 82   | 113 | 47  | 17   | 0             | 0                      | 0                    | 0                           | 0   | None found                                | 2   | 37212                     | 0                 | BIR-CA-19827 | sensitiv     |               |               |               |
| BIR-CA-19828 | broiler, feces | BY 18        | 03.07.2020     | Campylobacter jejuni | Campylobacter jejuni | 99.6           | 30             | 354           | ST-354 complex | 8    | 10   | 2    | 2    | 11  | 12  | 6    | 3             | 2                      | 2                    | 1                           | 1   | blaOXA-460;gyrA_T86I:tet(O)               | 0   | None found                | 0                 | 0            | 0            | BIR-CA-19828  | CIP, TET      |               |
| BIR-CA-19831 | broiler, feces | BY 33        | 11.02.2021     | Campylobacter jejuni | Campylobacter jejuni | 75.1           | 71             | 454           | ST-464 complex | 24   | 2    | 2    | 2    | 10  | 3   | 1    | 3             | 1                      | 1                    | 2                           | 50S_L22_A103V;gyrA_T86I:tet(O)            | 0   | None found                                | 0                         | 0                 | 0            | BIR-CA-19831 | CIP, TET      |               |               |
| BIR-CA-19833 | broiler, feces | BY 37        | 11.02.2021     | Campylobacter coli   | Campylobacter coli   | 70.3           | 35             | 828           | ST-828 complex | 33   | 39   | 30   | 82   | 104 | 43  | 17   | 4             | 3                      | 1                    | 1                           | 1   | aadE-Cc;blaOXA-489;gyrA_T86I:tet(O)       | 0   | None found                | 1                 | 28684        | 0            | BIR-CA-19833  | CIP, TET      |               |
| BIR-CA-19839 | broiler, feces | BY 45        | 23.02.2021     | Campylobacter jejuni | Campylobacter jejuni | 66.7           | 19             | 1723          | ST-354 complex | 8    | 17   | 2    | 2    | 11  | 12  | 6    | 1             | 1                      | 1                    | 0                           | 0   | None found                                | 0   | 0                         | 0                 | 0            | BIR-CA-19839 | sensitiv      |               |               |
| BIR-CA-19845 | human stool    | H30          | 11.01.2021     | Campylobacter jejuni | Campylobacter jejuni | 50.6           | 55             | 11759         | ST-574         | 7    | 84   | 2    | 10   | 11  | 3   | 3    | 4             | 2                      | 2                    | 2                           | 2   | 50S_L22_A103V;blaOXA-193;gyrA_T86I:tet(O) | 0   | None found                | 0                 | 0            | 0            | BIR-CA-19845  | CIP, TET      |               |
| BIR-CA-19846 | broiler, feces | BY 27        | 17.07.2020     | Campylobacter jejuni | Campylobacter jejuni | 80.6           | 56             | 305           | ST-574 complex | 9    | 53   | 2    | 10   | 11  | 3   | 3    | 4             | 2                      | 2                    | 2                           | 2   | 50S_L22_A103V;blaOXA-193;gyrA_T86I:tet(O) | 0   | None found                | 0                 | 0            | 0            | BIR-CA-19846  | CIP, TET      |               |
| BIR-CA-19851 | human stool    | H41          | 08.06.2021     | Campylobacter jejuni | Campylobacter jejuni | 58.6           | 56             | 607           | ST-607 complex | 8    | 2    | 5    | 53   | 11  | 3   | 1    | 3             | 2                      | 2                    | 1                           | 1   | blaOXA-193;gyrA_T86I:tet(O)               | 0   | None found                | 0                 | 0            | 0            | BIR-CA-19851  | CIP, TET      |               |
| BIR-CA-19853 | human stool    | H43          | 16.06.2021     | Campylobacter jejuni | Campylobacter jejuni | 102.9          | 33             | 658           | ST-658 complex | 2    | 4    | 2    | 4    | 19  | 3   | 6    | 2             | 2                      | 1                    | 1                           | 0   | blaOXA-193;tet(O)                         | 0   | None found                | 0                 | 0            | 0            | BIR-CA-19853  | TET           |               |
| BIR-CA-19854 | human stool    | H44          | 16.06.2021     | Campylobacter coli   | Campylobacter coli   | 93.3           | 49             | 902           | ST-828 complex | 33   | 39   | 30   | 79   | 104 | 43  | 17   | 3             | 2                      | 1                    | 1                           | 1   | blaOXA-489;gyrA_T86I:tet(O)               | 0   | None found                | 1                 | 26364        | 0            | BIR-CA-19854  | CIP, ETP, TET |               |
| BIR-CA-19857 | human stool    | H47          | 01.07.2021     | Campylobacter coli   | Campylobacter coli   | 97.6           | 52             | 902           | ST-828 complex | 33   | 39   | 30   | 79   | 104 | 43  | 17   | 4             | 3                      | 2                    | 1                           | 1   | 1   | aadE;blaOXA;gyrA_T86I:tet(O)              | 0                         | None found        | 0            | 0            | 0             | BIR-CA-19857  | CIP, TET      |
| BIR-CA-19863 | human stool    | H53          | 30.07.2021     | Campylobacter jejuni | Campylobacter jejuni | 51.0           | 50             | 356           | ST-353 complex | 14   | 17   | 5    | 2    | 11  | 3   | 6    | 1             | 0                      | 0                    | 1                           | gyrA_T86I                                 | 0   | None found                                | 0                         | 0                 | 0            | BIR-CA-19863 | CIP           |               |               |
| BIR-CA-19866 | human stool    | H66          | 23.09.2021     | Campylobacter jejuni | Campylobacter jejuni | 83.5           | 32             | 356           | ST-353 complex | 14   | 17   | 5    | 2    | 11  | 3   | 6    | 1             | 0                      | 0                    | 1                           | gyrA_T86I                                 | 0   | None found                                | 0                         | 0                 | 0            | BIR-CA-19866 | CIP           |               |               |
| BIR-CA-19874 | broiler, feces | BY 46        | 23.02.2021     | Campylobacter coli   | Campylobacter coli   | 102.3          | 38             | 902           | ST-828 complex | 33   | 39   | 30   | 79   | 104 | 43  | 17   | 2             | 0                      | 0                    | 0                           | 0   | None found                                | 0   | 0                         | 0                 | 0            | 0            | BIR-CA-19874  | CIP, ETP      |               |
| BIR-CA-19876 | broiler, feces | BY 48        | 26.02.2021     | Campylobacter jejuni | Campylobacter jejuni | 71.8           | 52             | 1723          | ST-354 complex | 8    | 17   | 2    | 2    | 11  | 12  | 6    | 3             | 2                      | 1                    | 1                           | 1   | blaOXA-460;gyrA_T86I:tet(O)               | 0   | None found                | 0                 | 0            | 0            | BIR-CA-19876  | CIP, TET      |               |
| BIR-CA-19902 | human stool    | H29          | 25.12.2020     | Campylobacter coli   | Campylobacter coli   | 99.9           | 41             | 3990          | ST-828 complex | 33   | 66   | 30   | 79   | 104 | 43  | 17   | 3             | 2                      | 2                    | 1                           | 1   | 1   | blaOXA-489;gyrA_T86I:tet(O)               | 0                         | None found        | 0            | 0            | 0             | BIR-CA-19902  | CIP, ETP, TET |
| BIR-CA-19906 | broiler, feces | BY 80        | 07.04.2021     | Campylobacter coli   | Campylobacter coli   | 68.4           | 55             | 828           | ST-828 complex | 33   | 39   | 30   | 82   | 104 | 43  | 17   | 2             | 1                      | 1                    | 1                           | 1   | blaOXA-193;gyrA_T86I                      | 0   | None found                | 0                 | 0            | 0            | BIR-CA-19906  | CIP, ETP      |               |
| BIR-CA-19911 | broiler, feces | BY 66        | 01.04.2021     | Campylobacter jejuni | Campylobacter jejuni | 37.3           | 50             | 2801          | ST-581 complex | 7    | 112  | 5    | 62   | 11  | 3   | 6    | 3             | 2                      | 1                    | 1                           | 1   | blaOXA;gyrA_T86I:tet(O)                   | 0   | None found                | 1                 | 1983         | 1            | BIR-CA-19911  | CIP, TET      |               |
| BIR-CA-19922 | broiler, feces | BY 86        | 07.04.2021     | Campylobacter coli   | Campylobacter coli   | 96.7           | 36             | 1058          | ST-828 complex | 33   | 39   | 30   | 82   | 104 | 35  | 17   | 3             | 2                      | 1                    | 1                           | 1   | 1   | blaOXA-489;gyrA_T86I:tet(O)               | 0                         | None found        | 1            | 26552        | 0             | BIR-CA-19922  | CIP, TET      |
| BIR-CA-19931 | broiler, feces | BY 95        | 07.04.2021     | Campylobacter jejuni | Campylobacter jejuni | 66.9           | 64             | 454           | ST-464 complex | 24   | 2    | 2    | 2    | 10  | 3   | 1    | 2             | 1                      | 1                    | 1                           | 1   | gyrA_T86I:tet(O)                          | 0   | None found                | 0                 | 0            | 0            | BIR-CA-19931  | CIP, TET      |               |
| BIR-CA-19951 | broiler, feces | BY 115       | 15.04.2021     | Campylobacter jejuni | Campylobacter jejuni | 97.0           | 33             | 45            | ST-45 complex  | 4    | 7    | 10   | 4    | 1   | 7   | 1    | 2             | 1                      | 1                    | 1                           | 1   | gyrA_T86I:tet(O)                          | 0   | None found                | 0                 | 0            | 0            | BIR-CA-19951  | CIP, TET      |               |
| BIR-CA-19952 | broiler, feces | IndChi 1     | 03.06.2021     | Campylobacter coli   | Campylobacter coli   | 98.5           | 46             | 855           | ST-828 complex | 33   | 39   | 30   | 79   | 104 | 35  | 17   | 4             | 2                      | 2                    | 2                           | 2   | 50S_L22_A103V;blaOXA-193;gyrA_T86I:tet(O) | 0   | None found                | 2                 | 29158        | 1            | BIR-CA-19952  | CIP, ETP, TET |               |
| BIR-CA-19961 | broiler, feces | IndChi 10    | 17.06.2021     | Campylobacter coli   | Campylobacter coli   | 62.4           | 39             | 855           | ST-828 complex | 33   | 39   | 30   | 79   | 104 | 35  | 17   | 4             | 2                      | 2                    | 2                           | 2   | 50S_L22_A103V;blaOXA-193;gyrA_T86I:tet(O) | 0   | None found                | 2                 | 29121        | 1            | BIR-CA-19961  | CIP, ETP, TET |               |
| BIR-CA-19967 | broiler, feces | IndChi 16    | 17.06.2021     | Campylobacter jejuni | Campylobacter jejuni | 51.0           | 27             | 3628          | ST-443 complex | 7    | 17   | 2    | 337  | 23  | 3   | 12   | 4             | 2                      | 2                    | 2                           | 2   | 50S_L22_A103V;blaOXA-193;gyrA_T86I:tet(O) | 0   | None found                | 0                 | 0            | 0            | BIR-CA-19967  | CIP, TET      |               |
| BIR-CA-19989 | broiler, feces | IndChi 38    | 09.07.2021     | Campylobacter coli   | Campylobacter coli   | 95.9           | 39             | 855           | ST-828 complex | 33   | 39   | 30   | 79   | 104 | 35  | 17   | 4             | 2                      | 2                    | 2                           | 2   | 2   | 50S_L22_A103V;blaOXA-193;gyrA_T86I:tet(O) | 0                         | None found        | 2            | 29500        | 0             | BIR-CA-19989  | CIP, TET      |
| BIR-CA-19995 | broiler, feces | IndChi 44    | 09.07.2021     | Campylobacter jejuni | Campylobacter jejuni | 101.1          | 37             | 3628          | ST-443 complex | 7    | 17   | 2    | 337  | 23  | 3   | 12   | 4             | 2                      | 2                    | 2                           | 2   | 2   | 50S_L22_A103V;blaOXA-193;gyrA_T86I:tet(O) | 0                         | None found        | 0            | 0            | 0             | BIR-CA-19995  | CIP, TET      |
| BIR-CA-19996 | broiler, feces | IndChi 45    | 09.07.2021     | Campylobacter coli   | Campylobacter coli   | 74.5           | 47             | 855           | ST-828 complex | 33   | 39   | 30   | 79   | 104 | 35  | 17   | 4             | 2                      | 2                    | 2                           | 2   | 2   | 50S_L22_A103V;blaOXA-193;gyrA_T86I:tet(O) | 0                         | None found        | 2            | 29454        | 1             | BIR-CA-19996  | CIP, ETP, TET |

## 9 Appendix

TabS3\_WGS\_data\_plasmids

| strain No.   | Contig      | Accession     | Identity | Query_length | Bitscore | Query_coverage | Details  |
|--------------|-------------|---------------|----------|--------------|----------|----------------|--|
| BfR-CA-19789 | contig00013 | NZ_CP017874.1 | 98.272   | 12671        | 22177    |                | 96 Campylobacter coli strain WA333 plasmid pCCDM33S, complete sequence               |
|              | contig00010 | NZ_CP017231.1 | 99.532   | 7482         | 13623    |                | 46 Campylobacter jejuni strain FORC_046 plasmid pFORC46.2, complete sequence         |
| BfR-CA-19797 |             |               |          |              |          |                |  |
| BfR-CA-19799 | contig00014 | NZ_CP023547.1 | 98.494   | 13013        | 22940    |                | 90 Campylobacter coli strain CFSAN032805 plasmid pCFSAN032805_2, complete sequence   |
| BfR-CA-19802 | contig00011 | NC_006134.1   | 98.882   | 20208        | 36056    |                | 49 Campylobacter coli plasmid pCC31, complete sequence                               |
| BfR-CA-19804 | contig00014 | NZ_CP017874.1 | 99.246   | 7031         | 12689    |                | 92 Campylobacter coli strain WA333 plasmid pCCDM33S, complete sequence               |
| BfR-CA-19804 | contig00015 | NZ_CP017870.1 | 99.949   | 9711         | 17906    |                | 97 Campylobacter coli strain MG1116 plasmid pCCDM116S, complete sequence             |
| BfR-CA-19804 | contig00017 | NZ_CP007187.1 | 94.148   | 1948         | 2896     |                | 72 Campylobacter coli RM1875 plasmid pRM1875_3.4kbp, complete sequence               |
| BfR-CA-19804 | contig00018 | NC_022656.1   | 98.359   | 4145         | 7273     |                | 100 Campylobacter coli 15-537360 plasmid pCC42yr, complete sequence                  |
| BfR-CA-19804 | contig00019 | NZ_CP017870.1 | 95.296   | 4124         | 6540     |                | 99 Campylobacter coli strain MG1116 plasmid pCCDM116S, complete sequence             |
| BfR-CA-19804 | contig00020 | NZ_CP017874.1 | 96.845   | 3708         | 6163     |                | 100 Campylobacter coli strain WA333 plasmid pCCDM33S, complete sequence              |
| BfR-CA-19804 | contig00021 | NZ_CP017870.1 | 98.886   | 3592         | 6405     |                | 99 Campylobacter coli strain MG1116 plasmid pCCDM116S, complete sequence             |
|              | contig00026 | NZ_CP028188.1 | 99.596   | 1732         | 3160     |                | 100 Campylobacter coli strain CFSAN054106 plasmid pGMI16-001, complete sequence      |
| BfR-CA-19804 | contig00027 | NZ_CP017870.1 | 99.934   | 1509         | 2782     |                | 100 Campylobacter coli strain MG1116 plasmid pCCDM116S, complete sequence            |
| BfR-CA-19807 | contig00013 | NZ_CP007187.1 | 94.793   | 3092         | 4754     |                | 89 Campylobacter coli RM1875 plasmid pRM1875_3.4kbp, complete sequence               |
|              | contig00011 | NZ_CP017872.1 | 98.265   | 13893        | 24271    |                | 31 Campylobacter coli strain BP3183 plasmid pCCDM183, complete sequence              |
| BfR-CA-19808 |             |               |          |              |          |                |  |
| BfR-CA-19812 | contig00018 | NZ_CP014345.1 | 99.898   | 3918         | 7212     |                | 100 Campylobacter jejuni strain RM3194 plasmid, complete sequence                    |
|              | contig00031 | NZ_CP017026.1 | 99.358   | 1713         | 3101     |                | 84 Campylobacter coli plasmid pCC14983A-1, complete sequence                         |
| BfR-CA-19812 |             |               |          |              |          |                |  |
| BfR-CA-19820 | contig00010 | NZ_CP010074.1 | 99.023   | 16076        | 28795    |                | 64 Campylobacter jejuni subsp. jejuni strain 01-1512 plasmid pCj2, complete sequence |
| BfR-CA-19820 | contig00011 | NZ_CP028186.1 | 97.59    | 19296        | 33024    |                | 53 Campylobacter jejuni strain CFSAN054107 plasmid pGMI16-002, complete sequence     |
| BfR-CA-19827 | contig00017 | NZ_CP007184.1 | 99.159   | 14393        | 25896    |                | 86 Campylobacter coli RM1875 plasmid pRM1875_35kb, complete sequence                 |
| BfR-CA-19827 | contig00018 | NZ_CP014746.1 | 95.905   | 6471         | 10414    |                | 70 Campylobacter jejuni strain OD267 plasmid pCJDM67 S, complete sequence            |
| BfR-CA-19833 | contig00011 | NC_022656.1   | 98.591   | 11571        | 20461    |                | 40 Campylobacter coli 15-537360 plasmid pCC42yr, complete sequence                   |
| BfR-CA-19854 | contig00013 | NC_022656.1   | 98.191   | 14155        | 24705    |                | 89 Campylobacter coli 15-537360 plasmid pCC42yr, complete sequence                   |
| BfR-CA-19952 | contig00011 | NZ_CP023547.1 | 98.511   | 11686        | 20593    |                | 66 Campylobacter coli strain CFSAN032805 plasmid pCFSAN032805_2, complete sequence   |
| BfR-CA-19952 | contig00014 | NZ_CP007187.1 | 94.583   | 3120         | 4774     |                | 90 Campylobacter coli RM1875 plasmid pRM1875_3.4kbp, complete sequence               |
| BfR-CA-19961 | contig00011 | NZ_CP017874.1 | 97.526   | 11721        | 19989    |                | 87 Campylobacter coli strain WA333 plasmid pCCDM33S, complete sequence               |
| BfR-CA-19961 | contig00013 | NZ_CP007187.1 | 94.558   | 3142         | 4802     |                | 91 Campylobacter coli RM1875 plasmid pRM1875_3.4kbp, complete sequence               |
| BfR-CA-19989 | contig00011 | NZ_CP023547.1 | 98.967   | 10939        | 19571    |                | 62 Campylobacter coli strain CFSAN032805 plasmid pCFSAN032805_2, complete sequence   |
| BfR-CA-19989 | contig00013 | NZ_CP007187.1 | 94.558   | 3142         | 4802     |                | 91 Campylobacter coli RM1875 plasmid pRM1875_3.4kbp, complete sequence               |
|              | contig00011 | NZ_CP017870.1 | 97.109   | 10825        | 18233    |                | 41 Campylobacter coli strain MG1116 plasmid pCCDM116S, complete sequence             |
| BfR-CA-19992 |             |               |          |              |          |                |  |
| BfR-CA-19996 | contig00011 | NZ_CP023547.1 | 98.967   | 10939        | 19571    |                | 63 Campylobacter coli strain CFSAN032805 plasmid pCFSAN032805_2, complete sequence   |
| BfR-CA-19996 | contig00013 | NZ_CP007187.1 | 94.583   | 3120         | 4774     |                | 90 Campylobacter coli RM1875 plasmid pRM1875_3.4kbp, complete sequence               |

**9.2.2 Publication 2: Multiplex Real-Time PCR for the Detection of Tetracycline, Ciprofloxacin, and Erythromycin Resistance Determinants from Human and Foodborne *Campylobacter jejuni* and *Campylobacter coli***

# 9 Appendix

| Sample overview   |                   |                    |                       |                 | Phenotypic resistance determined by broth microdilution |         |         |         |         |         | Multiresistance level |
|---|-------------------|--------------------|-----------------------|-----------------|---|---------|---------|---------|---------|---------|-----------------------|
| German isolates from food (FS <sub>1</sub> ) and human origin (HS <sub>1</sub> ). Vietnamese isolates from food (VE <sub>1</sub> ). |                   |                    |                       |                 | EUCAMP3   |         |         |         |         |         | 1                     |
|   |                   |                    |                       |                 | grey shading: resistant to antimicrobiol agent          |         |         |         |         |         | 2                     |
|   |                   |                    |                       |                 |   |         |         |         |         |         | 3                     |
|   |                   |                    |                       |                 |   |         |         |         |         |         | 4                     |
| Strain No. alias  | Country of origin | isolation source   | Campylobacter species | collection date | CHL MIC   | ERY MIC | GEN MIC | CIP MIC | TET MIC | FTP MIC | Multiresistance       |
| HS_1  | Germany           | human stool sample | <i>C. jejuni</i>      | 2019            | <=2   | <=1     | 0.5     | 4       | <=0.5   | <=0.12  | 1                     |
| HS_2  | Germany           | human stool sample | <i>C. jejuni</i>      | 2019            | 4   | <=1     | <=0.25  | 16      | 64      | <=0.12  | 2                     |
| HS_3  | Germany           | human stool sample | <i>C. jejuni</i>      | 2019            | <=2   | 4       | <=0.25  | <=0.12  | <=0.5   | <=0.12  | 0                     |
| HS_4  | Germany           | human stool sample | <i>C. jejuni</i>      | 2019            | <=2   | <=1     | <=0.25  | 8       | 64      | <=0.12  | 2                     |
| HS_5  | Germany           | human stool sample | <i>C. jejuni</i>      | 2019            | <=2   | <=1     | <=0.25  | 8       | >64     | <=0.12  | 2                     |
| HS_6  | Germany           | human stool sample | <i>C. jejuni</i>      | 2019            | <=2   | <=1     | <=0.25  | 8       | <=0.5   | <=0.12  | 1                     |
| HS_7  | Germany           | human stool sample | <i>C. jejuni</i>      | 2019            | <=2   | <=1     | 0.5     | <=0.12  | <=0.5   | <=0.12  | 0                     |
| HS_8  | Germany           | human stool sample | <i>C. jejuni</i>      | 2020            | <=2   | <=1     | <=0.25  | 8       | 32      | <=0.12  | 2                     |
| HS_9  | Germany           | human stool sample | <i>C. jejuni</i>      | 2020            | <=2   | <=1     | <=0.25  | <=0.12  | <=0.5   | <=0.12  | 0                     |
| HS_10   | Germany           | human stool sample | <i>C. coli</i>        | 2020            | 16  | 8       | 0.5     | >32     | >64     | 2       | 3                     |
| HS_11   | Germany           | human stool sample | <i>C. jejuni</i>      | 2020            | <=2   | <=1     | <=0.25  | 8       | <=0.5   | <=0.12  | 1                     |
| HS_12   | Germany           | human stool sample | <i>C. jejuni</i>      | 2020            | <=2   | <=1     | <=0.25  | 4       | 32      | <=0.12  | 2                     |
| HS_13   | Germany           | human stool sample | <i>C. jejuni</i>      | 2020            | <=2   | <=1     | <=0.25  | 8       | 64      | 0.5     | 2                     |
| HS_14   | Germany           | human stool sample | <i>C. jejuni</i>      | 2020            | <=2   | <=1     | <=0.25  | 8       | 64      | <=0.12  | 2                     |
| HS_15   | Germany           | human stool sample | <i>C. jejuni</i>      | 2020            | <=2   | <=1     | <=0.25  | <=0.12  | <=0.5   | <=0.12  | 0                     |
| HS_16   | Germany           | human stool sample | <i>C. jejuni</i>      | 2020            | <=2   | <=1     | <=0.25  | 16      | <=0.5   | <=0.12  | 1                     |
| HS_17   | Germany           | human stool sample | <i>C. jejuni</i>      | 2020            | <=2   | <=1     | <=0.25  | 16      | 64      | <=0.12  | 2                     |
| HS_18   | Germany           | human stool sample | <i>C. jejuni</i>      | 2020            | <=2   | <=1     | <=0.25  | 8       | 8       | <=0.12  | 2                     |
| HS_19   | Germany           | human stool sample | <i>C. coli</i>        | 2020            | <=2   | <=1     | 0.5     | 8       | 64      | 0.25    | 2                     |
| HS_20   | Germany           | human stool sample | <i>C. jejuni</i>      | 2020            | <=2   | <=1     | <=0.25  | <=0.12  | <=0.5   | <=0.12  | 0                     |
| HS_21   | Germany           | human stool sample | <i>C. coli</i>        | 2020            | <=2   | <=1     | 0.5     | 8       | 64      | 1       | 3                     |
| HS_22   | Germany           | human stool sample | <i>C. coli</i>        | 2020            | <=2   | 512     | 1       | 8       | <=0.5   | <=0.12  | 2                     |
| HS_23   | Germany           | human stool sample | <i>C. jejuni</i>      | 2020            | <=2   | <=1     | <=0.25  | 16      | >64     | >4      | 3                     |
| HS_24   | Germany           | human stool sample | <i>C. jejuni</i>      | 2020            | 4   | <=1     | <=0.25  | 16      | >64     | 1       | 3                     |
| HS_25   | Germany           | human stool sample | <i>C. jejuni</i>      | 2021            | <=2   | <=1     | 0.5     | 16      | 8       | <=0.12  | 2                     |
| HS_26   | Germany           | human stool sample | <i>C. jejuni</i>      | 2018            | <=2   | <=1     | <=0.25  | 8       | 64      | <=0.12  | 2                     |
| HS_27   | Germany           | human stool sample | <i>C. jejuni</i>      | 2018            | <=2   | <=1     | <=0.25  | 8       | <=0.5   | <=0.12  | 1                     |
| HS_28   | Germany           | human stool sample | <i>C. jejuni</i>      | 2019            | <=2   | <=1     | <=0.25  | 4       | 32      | <=0.12  | 2                     |
| HS_29   | Germany           | human stool sample | <i>C. jejuni</i>      | 2019            | <=2   | 2       | <=0.25  | 32      | >64     | <=0.12  | 2                     |
| HS_30   | Germany           | human stool sample | <i>C. jejuni</i>      | 2019            | <=2   | <=1     | <=0.25  | 8       | 4       | <=0.12  | 2                     |
| HS_31   | Germany           | human stool sample | <i>C. jejuni</i>      | 2019            | <=2   | <=1     | 0.5     | 8       | 64      | <=0.12  | 2                     |
| HS_32   | Germany           | human stool sample | <i>C. coli</i>        | 2019            | <=2   | <=1     | <=0.25  | 8       | 32      | 0.5     | 2                     |
| HS_33   | Germany           | human stool sample | <i>C. jejuni</i>      | 2019            | <=2   | <=1     | <=0.25  | 8       | 16      | <=0.12  | 2                     |
| HS_34   | Germany           | human stool sample | <i>C. jejuni</i>      | 2019            | <=2   | <=1     | <=0.25  | 8       | 2       | <=0.12  | 2                     |
| HS_35   | Germany           | human stool sample | <i>C. coli</i>        | 2019            | <=2   | <=1     | 0.5     | 16      | >64     | 1       | 3                     |
| HS_36   | Germany           | human stool sample | <i>C. jejuni</i>      | 2019            | <=2   | <=1     | <=0.25  | 8       | <=0.5   | 1       | 2                     |
| HS_37   | Germany           | human stool sample | <i>C. jejuni</i>      | 2019            | <=2   | <=1     | 0.5     | 8       | 64      | <=0.12  | 2                     |
| HS_38   | Germany           | human stool sample | <i>C. jejuni</i>      | 2019            | <=2   | <=1     | <=0.25  | 8       | 8       | <=0.12  | 2                     |
| HS_39   | Germany           | human stool sample | <i>C. jejuni</i>      | 2019            | <=2   | <=1     | <=0.25  | 16      | 64      | <=0.12  | 2                     |
| HS_40   | Germany           | human stool sample | <i>C. jejuni</i>      | 2019            | <=2   | <=1     | <=0.25  | 8       | 16      | <=0.12  | 2                     |
| HS_41   | Germany           | human stool sample | <i>C. jejuni</i>      | 2019            | <=2   | <=1     | <=0.25  | 8       | 16      | <=0.12  | 2                     |
| HS_42   | Germany           | human stool sample | <i>C. jejuni</i>      | 2019            | <=2   | <=1     | 0.5     | <=0.12  | <=0.5   | <=0.12  | 0                     |
| HS_43   | Germany           | human stool sample | <i>C. jejuni</i>      | 2019            | <=2   | <=1     | 0.5     | <=0.12  | <=0.5   | <=0.12  | 0                     |
| HS_44   | Germany           | human stool sample | <i>C. jejuni</i>      | 2019            | <=2   | <=1     | <=0.25  | 0.25    | <=0.5   | <=0.12  | 0                     |
| HS_45   | Germany           | human stool sample | <i>C. coli</i>        | 2019            | <=2   | 2       | 1       | 8       | <=0.5   | <=0.12  | 1                     |
| HS_46   | Germany           | human stool sample | <i>C. jejuni</i>      | 2019            | <=2   | <=1     | <=0.25  | 8       | 32      | <=0.12  | 2                     |
| HS_47   | Germany           | human stool sample | <i>C. jejuni</i>      | 2019            | <=2   | <=1     | <=0.25  | 8       | <=0.5   | <=0.12  | 1                     |
| HS_48   | Germany           | human stool sample | <i>C. jejuni</i>      | 2019            | <=2   | <=1     | <=0.25  | 8       | >64     | <=0.12  | 2                     |



# 9 Appendix

| Sample overview  |                   |                        |                       |                 | Phenotypic resistance determined by broth microdilution |         |         |         |         |         | Multiresistance level |
|--|-------------------|------------------------|-----------------------|-----------------|---|---------|---------|---------|---------|---------|-----------------------|
| German isolates from food (FS_) and human origin (HS_). Vietnamese isolates from food (VE_). |                   |                        |                       |                 | EUCAMP3   |         |         |         |         |         | 1                     |
|  |                   |                        |                       |                 | grey shading: resistant to antimicrobial agent          |         |         |         |         |         | 2                     |
|  |                   |                        |                       |                 |   |         |         |         |         |         | 3                     |
|  |                   |                        |                       |                 |   |         |         |         |         |         | 4                     |
| Strain No. alias   | Country of origin | Isolation source       | Campylobacter species | collection date | CHL MIC   | ERY MIC | GEN MIC | CIP MIC | TET MIC | FTP MIC | Multiresistance       |
| HS_49  | Germany           | human stool sample     | <i>C. jejuni</i>      | 2019            | <=2   | <=1     | <=0.25  | 8       | 16      | <=0.12  | 2                     |
| HS_50  | Germany           | human stool sample     | <i>C. jejuni</i>      | 2019            | <=2   | <=1     | <=0.25  | 8       | 32      | 0.25    | 2                     |
| FS_51  | Germany           | retail, chicken breast | <i>C. jejuni</i>      | 2021            | <=2   | <=1     | 0.5     | <=0.12  | <=0.5   | <=0.12  | 0                     |
| FS_52  | Germany           | retail, chicken breast | <i>C. jejuni</i>      | 2021            | <=2   | <=1     | <=0.25  | 8       | 32      | <=0.12  | 2                     |
| FS_53  | Germany           | retail, chicken breast | <i>C. jejuni</i>      | 2021            | <=2   | <=1     | <=0.25  | <=0.12  | <=0.5   | <=0.12  | 0                     |
| FS_54  | Germany           | retail, chicken breast | <i>C. jejuni</i>      | 2021            | <=2   | <=1     | <=0.25  | 8       | 64      | <=0.12  | 2                     |
| FS_55  | Germany           | retail, chicken breast | <i>C. coli</i>        | 2021            | <=2   | <=1     | 1       | 8       | <=0.5   | 0.25    | 1                     |
| FS_56  | Germany           | retail, chicken meat   | <i>C. jejuni</i>      | 2021            | <=2   | <=1     | <=0.25  | 8       | 64      | <=0.12  | 2                     |
| FS_57  | Germany           | retail, chicken leg    | <i>C. jejuni</i>      | 2021            | <=2   | <=1     | <=0.25  | <=0.12  | <=0.5   | <=0.12  | 0                     |
| FS_58  | Germany           | retail, chicken breast | <i>C. jejuni</i>      | 2021            | <=2   | <=1     | <=0.25  | 16      | >64     | <=0.12  | 2                     |
| FS_59  | Germany           | retail, chicken breast | <i>C. jejuni</i>      | 2021            | <=2   | <=1     | <=0.25  | 4       | <=0.5   | <=0.12  | 1                     |
| FS_60  | Germany           | retail, chicken breast | <i>C. jejuni</i>      | 2021            | <=2   | <=1     | 0.5     | <=0.12  | <=0.5   | <=0.12  | 0                     |
| FS_61  | Germany           | retail, chicken breast | <i>C. jejuni</i>      | 2021            | <=2   | <=1     | <=0.25  | <=0.12  | <=0.5   | <=0.12  | 0                     |
| FS_62  | Germany           | retail, chicken breast | <i>C. jejuni</i>      | 2021            | <=2   | <=1     | <=0.25  | 4       | 8       | <=0.12  | 2                     |
| FS_63  | Germany           | retail, chicken meat   | <i>C. jejuni</i>      | 2019            | <=2   | <=1     | <=0.25  | <=0.12  | <=0.5   | <=0.12  | 0                     |
| FS_64  | Germany           | retail, chicken meat   | <i>C. jejuni</i>      | 2019            | <=2   | <=1     | 0.5     | 16      | >64     | <=0.12  | 2                     |
| FS_65  | Germany           | retail, chicken breast | <i>C. jejuni</i>      | 2019            | <=2   | <=1     | <=0.25  | 16      | >64     | <=0.12  | 2                     |
| FS_66  | Germany           | retail, chicken breast | <i>C. coli</i>        | 2019            | <=2   | <=1     | 0.5     | 16      | >64     | 0.5     | 2                     |
| FS_67  | Germany           | retail, chicken breast | <i>C. coli</i>        | 2019            | <=2   | <=1     | 0.5     | <=0.12  | 32      | <=0.12  | 1                     |
| FS_68  | Germany           | retail, chicken breast | <i>C. jejuni</i>      | 2019            | <=2   | <=1     | <=0.25  | 8       | 64      | <=0.12  | 2                     |
| FS_69  | Germany           | retail, chicken breast | <i>C. jejuni</i>      | 2019            | <=2   | <=1     | <=0.25  | 16      | <=0.5   | <=0.12  | 1                     |
| FS_70  | Germany           | retail, chicken breast | <i>C. jejuni</i>      | 2019            | <=2   | <=1     | <=0.25  | <=0.12  | <=0.5   | <=0.12  | 0                     |
| FS_71  | Germany           | retail, chicken breast | <i>C. jejuni</i>      | 2019            | <=2   | <=1     | <=0.25  | 8       | 8       | <=0.12  | 2                     |
| FS_72  | Germany           | retail, chicken breast | <i>C. jejuni</i>      | 2019            | <=2   | <=1     | 0.5     | 8       | <=0.5   | 0.25    | 1                     |
| FS_73  | Germany           | retail, chicken breast | <i>C. jejuni</i>      | 2019            | <=2   | <=1     | <=0.25  | 8       | <=0.5   | <=0.12  | 1                     |
| FS_74  | Germany           | retail, chicken breast | <i>C. jejuni</i>      | 2019            | <=2   | <=1     | <=0.25  | 8       | >64     | <=0.12  | 2                     |
| FS_75  | Germany           | broiler, neck skin     | <i>C. jejuni</i>      | 2019            | <=2   | <=1     | <=0.25  | <=0.12  | <=0.5   | <=0.12  | 0                     |
| FS_76  | Germany           | retail, chicken breast | <i>C. coli</i>        | 2019            | <=2   | <=1     | 0.5     | <=0.12  | 64      | 1       | 2                     |
| FS_77  | Germany           | retail, chicken breast | <i>C. jejuni</i>      | 2019            | <=2   | <=1     | <=0.25  | 4       | <=0.5   | <=0.12  | 1                     |
| FS_78  | Germany           | retail, chicken breast | <i>C. jejuni</i>      | 2019            | <=2   | <=1     | <=0.25  | 4       | 16      | <=0.12  | 2                     |
| FS_79  | Germany           | retail, chicken breast | <i>C. coli</i>        | 2019            | <=2   | <=1     | 0.5     | 8       | <=0.5   | <=0.12  | 1                     |
| FS_80  | Germany           | retail, chicken leg    | <i>C. jejuni</i>      | 2019            | <=2   | <=1     | <=0.25  | <=0.12  | <=0.5   | <=0.12  | 0                     |
| FS_81  | Germany           | broiler, neck skin     | <i>C. jejuni</i>      | 2019            | <=2   | <=1     | 0.5     | 0.25    | 32      | <=0.12  | 1                     |
| FS_82  | Germany           | Chicken eggs           | <i>C. coli</i>        | 2020            | 4   | 4       | 0.5     | 0.25    | <=0.5   | 2       | 1                     |
| FS_83  | Germany           | broiler, neck skin     | <i>C. jejuni</i>      | 2019            | <=2   | <=1     | 0.5     | <=0.12  | <=0.5   | <=0.12  | 0                     |
| FS_84  | Germany           | broiler, neck skin     | <i>C. coli</i>        | 2019            | 4   | 2       | 0.5     | 16      | >64     | 0.5     | 2                     |
| FS_85  | Germany           | retail, chicken breast | <i>C. jejuni</i>      | 2019            | <=2   | <=1     | <=0.25  | 8       | <=0.5   | <=0.12  | 1                     |
| FS_86  | Germany           | retail, chicken breast | <i>C. jejuni</i>      | 2019            | <=2   | <=1     | <=0.25  | 8       | <=0.5   | <=0.12  | 1                     |
| FS_87  | Germany           | retail, chicken breast | <i>C. jejuni</i>      | 2019            | <=2   | <=1     | <=0.25  | 16      | 64      | <=0.12  | 2                     |
| FS_88  | Germany           | retail, chicken breast | <i>C. jejuni</i>      | 2019            | <=2   | <=1     | 0.5     | 16      | <=0.5   | <=0.12  | 1                     |
| FS_89  | Germany           | retail, chicken breast | <i>C. jejuni</i>      | 2020            | <=2   | <=1     | <=0.25  | 8       | 8       | <=0.12  | 2                     |
| FS_90  | Germany           | broiler, neck skin     | <i>C. coli</i>        | 2019            | <=2   | <=1     | 0.5     | <=0.12  | 64      | 0.5     | 1                     |
| FS_91  | Germany           | Unknown                | <i>C. jejuni</i>      | 2020            | 4   | <=1     | <=0.25  | 16      | >64     | 0.5     | 2                     |
| FS_92  | Germany           | turkey, neck skin      | <i>C. coli</i>        | 2020            | <=2   | <=1     | 1       | <=0.12  | >64     | 1       | 2                     |
| FS_93  | Germany           | turkey, neck skin      | <i>C. coli</i>        | 2020            | <=2   | <=1     | <=0.25  | <=0.12  | <=0.5   | 0.25    | 0                     |
| FS_94  | Germany           | broiler, neck skin     | <i>C. coli</i>        | 2020            | 4   | 2       | 0.5     | 0.25    | <=0.5   | 0.25    | 0                     |
| FS_95  | Germany           | broiler, neck skin     | <i>C. coli</i>        | 2020            | <=2   | <=1     | 0.5     | 8       | >64     | 4       | 3                     |

Table S1\_Sample overview

# 9 Appendix

| Sample overview  |              |                   |                                  |                       | Phenotypic resistance determined by broth microdilution |         |         |         |         |         | Multiresistance level |                 |
|--|--------------|-------------------|----------------------------------|-----------------------|---|---------|---------|---------|---------|---------|-----------------------|-----------------|
| German isolates from food (FS_) and human origin (HS_). Vietnamese isolates from food (VE_). |              |                   |                                  |                       | EUCAMP3   |         |         |         |         |         | 1                     |                 |
|  |              |                   |                                  |                       | grey shading: resistant to antimicrobial agent          |         |         |         |         |         | 2                     |                 |
|  |              |                   |                                  |                       |   |         |         |         |         |         | 3                     |                 |
|  |              |                   |                                  |                       |   |         |         |         |         |         | 2,4                   |                 |
| Strain No.   | alias        | Country of origin | isolation source                 | Campylobacter species | collection date   | CHL MIC | ERY MIC | GEN MIC | CIP MIC | TET MIC | FTP MIC               | Multiresistance |
| FS_96  |              | Germany           | broiler, neck skin               | <i>C. jejuni</i>      | 2020  | <=2     | <=1     | <=0.25  | <=0.12  | <=0.5   | 0.25                  | 0               |
| FS_97  |              | Germany           | broiler, neck skin               | <i>C. coli</i>        | 2020  | <=2     | <=1     | 0.5     | <=0.12  | <=0.5   | 0.5                   | 0               |
| FS_98  |              | Germany           | retail, chicken breast           | <i>C. coli</i>        | 2020  | <=2     | <=1     | 0.5     | 8       | 32      | 2                     | 3               |
| FS_99  |              | Germany           | retail, chicken breast           | <i>C. jejuni</i>      | 2020  | <=2     | <=1     | <=0.25  | 8       | 64      | <=0.12                | 2               |
| FS_100   |              | Germany           | Chicken eggs                     | <i>C. coli</i>        | 2020  | <=2     | <=1     | <=0.25  | 16      | <=0.5   | 4                     | 2               |
| HS_101   |              | Germany           | human stool sample, external lab | <i>C. coli</i>        | 2022  | <=2     | <=1     | 1       | 8       | 64      | 0.25                  | 2               |
| HS_102   |              | Germany           | human stool sample, external lab | <i>C. coli</i>        | 2022  | 4       | <=1     | 0.5     | 8       | 64      | 2                     | 3               |
| HS_103   |              | Germany           | human stool sample, external lab | <i>C. coli</i>        | 2022  | <=2     | 512     | 0.5     | 8       | 64      | 1                     | 4               |
| HS_104   |              | Germany           | human stool sample, external lab | <i>C. coli</i>        | 2022  | <=2     | <=1     | 0.5     | 8       | <=0.5   | 1                     | 2               |
| HS_105   |              | Germany           | human stool sample, external lab | <i>C. coli</i>        | 2022  | 4       | <=1     | 0.5     | 16      | 1       | 2                     | 2               |
| HS_106   |              | Germany           | human stool sample, external lab | <i>C. coli</i>        | 2022  | 4       | <=1     | <=0.25  | 0.25    | <=0.5   | 0.5                   | 0               |
| HS_107   |              | Germany           | human stool sample, external lab | <i>C. coli</i>        | 2022  | 4       | <=1     | 1       | 16      | 64      | 0.25                  | 2               |
| HS_108   |              | Germany           | human stool sample, external lab | <i>C. coli</i>        | 2022  | <=2     | <=1     | 0.5     | 8       | 64      | 0.5                   | 2               |
| HS_109   |              | Germany           | human stool sample, external lab | <i>C. coli</i>        | 2022  | <=2     | <=1     | 0.5     | 0.25    | <=0.5   | 0.5                   | 0               |
| HS_110   |              | Germany           | human stool sample, external lab | <i>C. coli</i>        | 2022  | <=2     | <=1     | 1       | 8       | 64      | 0.5                   | 2               |
| FS_111   | BRR-CA-19312 | Germany           | broiler, skin                    | <i>C. coli</i>        | 2020  | <=2     | <=1     | 0.5     | 8       | 64      | 0.5                   | 2               |
| FS_112   | BRR-CA-19320 | Germany           | retail, chicken breast           | <i>C. jejuni</i>      | 2020  | <=2     | <=1     | <=0.25  | 4       | <=0.5   | <=0.12                | 1               |
| FS_113   | BRR-CA-20317 | Germany           | retail, chicken breast           | <i>C. jejuni</i>      | 2021  | 8       | <=1     | <=0.25  | <=0.12  | 1       | 1                     | 1               |
| FS_114   | BRR-CA-20398 | Germany           | retail, chicken breast           | <i>C. jejuni</i>      | 2022  | <=2     | <=1     | <=0.25  | 16      | 64      | 2                     | 3               |
| FS_115   | BRR-CA-20788 | Germany           | retail, chicken meat             | <i>C. coli</i>        | 2022  | <=2     | <=1     | 0.5     | 16      | 64      | 2                     | 3               |
| FS_116   | BRR-CA-20832 | Germany           | broiler, ovum                    | <i>C. coli</i>        | 2022  | <=2     | <=1     | 0.5     | 0.25    | <=0.5   | 1                     | 1               |
| FS_117   | BRR-CA-20996 | Germany           | retail, chicken meat             | <i>C. jejuni</i>      | 2022  | <=2     | <=1     | <=0.25  | 8       | <=0.5   | <=0.12                | 1               |
| FS_118   | BRR-CA-21003 | Germany           | retail, chicken meat             | <i>C. coli</i>        | 2022  | 8       | 8       | 1       | <=0.12  | 64      | 0.5                   | 1               |
| FS_119   | BRR-CA-21076 | Germany           | retail, chicken breast           | <i>C. coli</i>        | 2022  | <=2     | 512     | 1       | 8       | <=0.5   | <=0.12                | 2               |
| FS_120   | BRR-CA-21077 | Germany           | retail, chicken breast           | <i>C. jejuni</i>      | 2022  | <=2     | <=1     | <=0.25  | 8       | <=0.5   | <=0.12                | 1               |
| HS_121   |              | Germany           | human stool sample, external lab | <i>C. coli</i>        | 2022  | 4       | <=1     | 1       | 16      | <=0.5   | 0.5                   | 1               |
| HS_122   |              | Germany           | human stool sample, external lab | <i>C. jejuni</i>      | 2022  | <=2     | <=1     | <=0.25  | 32      | 64      | <=0.12                | 2               |
| HS_123   |              | Germany           | human stool sample, external lab | <i>C. coli</i>        | 2023  | <=2     | <=1     | 0.5     | <=0.12  | 32      | 0.5                   | 1               |
| HS_124   |              | Germany           | human stool sample, external lab | <i>C. coli</i>        | 2023  | <=2     | <=1     | <=0.25  | 4       | 16      | 0.5                   | 2               |
| HS_125   |              | Germany           | human stool sample, external lab | <i>C. coli</i>        | 2023  | 4       | <=1     | 0.5     | 32      | 64      | 4                     | 3               |
| HS_126   |              | Germany           | human stool sample, external lab | <i>C. coli</i>        | 2023  | 4       | <=1     | 1       | <=0.12  | <=0.5   | 1                     | 1               |
| HS_127   |              | Germany           | human stool sample, external lab | <i>C. coli</i>        | 2023  | 4       | 2       | 0.5     | 16      | 64      | 0.25                  | 2               |
| HS_128   |              | Germany           | human stool sample, external lab | <i>C. coli</i>        | 2023  | <=2     | <=1     | <=0.25  | <=0.12  | <=0.5   | 0.5                   | 0               |
| FS_129   | BRR-CA-19086 | Germany           | broiler, meat                    | <i>C. jejuni</i>      | 2020  | <=2     | <=1     | 0.5     | 8       | 32      | 2                     | 3               |
| VE_01  | BRR-CA-15062 | Vietnam           | broiler, feces                   | <i>C. coli</i>        | 2017  | >64     | >512    | >16     | 32      | >64     | 1                     | 2,4             |
| VE_02  | BRR-CA-15077 | Vietnam           | broiler, feces                   | <i>C. coli</i>        | 2017  | 64      | >512    | >16     | 32      | >64     | 2                     | 2,4             |
| VE_03  | BRR-CA-15986 | Vietnam           | broiler, feces                   | <i>C. coli</i>        | 2016  | 4       | >512    | >16     | 32      | >64     | 4                     | 2,4             |
| VE_04  | BRR-CA-15987 | Vietnam           | broiler, feces                   | <i>C. coli</i>        | 2016  | 8       | >512    | 1       | 32      | >64     | 4                     | 4               |
| VE_05  | BRR-CA-15989 | Vietnam           | broiler, feces                   | <i>C. coli</i>        | 2016  | >64     | 512     | >16     | 32      | >64     | 2                     | 2,4             |
| VE_06  | BRR-CA-15990 | Vietnam           | broiler, feces                   | <i>C. coli</i>        | 2016  | >64     | >512    | >16     | >32     | >64     | >4                    | 2,4             |
| VE_07  | BRR-CA-15991 | Vietnam           | broiler, feces                   | <i>C. coli</i>        | 2016  | >64     | >512    | >16     | >32     | >64     | >4                    | 2,4             |
| VE_08  | BRR-CA-15994 | Vietnam           | broiler, feces                   | <i>C. coli</i>        | 2016  | 64      | 512     | >16     | >32     | >64     | 4                     | 2,4             |
| VE_09  | BRR-CA-16036 | Vietnam           | broiler, feces                   | <i>C. coli</i>        | 2017  | 4       | >512    | >16     | >32     | >64     | 1                     | 2,4             |
| VE_10  | BRR-CA-16046 | Vietnam           | broiler, feces                   | <i>C. coli</i>        | 2017  | 64      | >512    | >16     | 16      | >64     | >4                    | 2,4             |
| VE_11  | BRR-CA-16057 | Vietnam           | broiler, feces                   | <i>C. coli</i>        | 2017  | >64     | >512    | >16     | 16      | >64     | >4                    | 2,4             |
| VE_12  | BRR-CA-16059 | Vietnam           | broiler, feces                   | <i>C. coli</i>        | 2017  | >64     | >512    | >16     | 16      | >64     | >4                    | 2,4             |
| VE_13  | BRR-CA-16081 | Vietnam           | broiler, feces                   | <i>C. jejuni</i>      | 2017  | >64     | 2       | 0.5     | >32     | >64     | 4                     | 4               |
| VE_14  | BRR-CA-16092 | Vietnam           | broiler, feces                   | <i>C. jejuni</i>      | 2017  | >64     | >512    | >16     | >32     | >64     | 2                     | 2,4             |
| VE_15  | BRR-CA-16107 | Vietnam           | broiler, feces                   | <i>C. coli</i>        | 2017  | 16      | 512     | >16     | >32     | >64     | 2                     | 2,4             |
| VE_16  | BRR-CA-16198 | Vietnam           | broiler, feces                   | <i>C. coli</i>        | 2017  | >64     | >512    | >16     | 16      | >64     | 4                     | 2,4             |
| VE_17  | BRR-CA-16261 | Vietnam           | broiler, feces                   | <i>C. coli</i>        | 2017  | >64     | 256     | >16     | >32     | >64     | 4                     | 2,4             |
| VE_18  | BRR-CA-18689 | Vietnam           | broiler, feces                   | <i>C. jejuni</i>      | 2017  | >64     | <=1     | >16     | >32     | >64     | 0.5                   | 4               |
| VE_19  | BRR-CA-19033 | Vietnam           | broiler, meat                    | <i>C. coli</i>        | 2018  | 8       | 256     | >16     | >32     | >64     | 2                     | 2,4             |
| VE_20  | BRR-CA-19119 | Vietnam           | broiler, meat                    | <i>C. coli</i>        | 2018  | 8       | >512    | >16     | >32     | >64     | 1                     | 2,4             |
| VE_21  | BRR-CA-16298 | Vietnam           | broiler, feces                   | <i>C. coli</i>        | 2017  | 8       | 512     | 0.5     | 16      | >64     | 2                     | 4               |
| DSM 4688   |              |                   | reference strain                 | <i>C. jejuni</i>      |   | <=2     | <=1     | 0.5     | <=0.12  | <=0.5   | <=0.12                |                 |
| 2012-70-443-2  |              |                   | reference strain                 | <i>C. coli</i>        |   | <=2     | <=1     | 0.5     | <=0.12  | <=0.5   | <=0.12                |                 |

Table S1\_Sample overview

# 9 Appendix

## Primer and probe binding sites (based on Aquamis)

S: sensitivity; R: resistance

grey shading: phenotypically resistant isolate

(1\*): imperfect binding site with pm due to additional mutation A>G

Commonly, 2 binding sites for 2 primers (forward and reverse) and 1 binding site for the probe were identified

In some cases, more binding sites are observed for *tet* (O) and 23S *rRNA*, which indicate the presence of 2 or 3 copies of a gene: 4 or 6 binding sites for primers and 2 or 3 binding sites for the probe  
 degenerated nucleotide Y (C/T) in *gyrA\_Cc*: C for wt1 and pm1 ; T for wt2 and pm2

| strain No. | Campylobacter species | tetracycline |                |           | ciprofloxacin <i>C. coli</i> |         |             |             |             |             | ciprofloxacin <i>C. jejuni</i> |            |            | erythromycin |     |        |        |                |           |
|------------|-----------------------|--------------|----------------|-----------|------------------------------|---------|-------------|-------------|-------------|-------------|--------------------------------|------------|------------|--------------|-----|--------|--------|----------------|-----------|
|            |                       | TET R/S      | tet(O) primers | tet(O) wt | CIP R/S                      | gyrA_Cc | gyrA_Cc_wt1 | gyrA_Cc_wt2 | gyrA_Cc_pm1 | gyrA_Cc_pm2 | gyrA_Cj                        | gyrA_Cj_wt | gyrA_Cj_pm | ERY R/S      | 23S | 23S_wt | 23S_pm | erm(B) primers | erm(B) wt |
| HS_1       | <i>C. jejuni</i>      | 0            | 0              | 0         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 0            | 2   | 1      | 0      | 0              | 0         |
| HS_2       | <i>C. jejuni</i>      | 1            | 2              | 1         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 0            | 2   | 1      | 0      | 0              | 0         |
| HS_3       | <i>C. jejuni</i>      | 0            | 0              | 0         | 0                            | 0       | 0           | 0           | 0           | 0           | 2                              | 1          | 0          | 0            | 2   | 1      | 0      | 0              | 0         |
| HS_4       | <i>C. jejuni</i>      | 1            | 2              | 1         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 0            | 2   | 1      | 0      | 0              | 0         |
| HS_5       | <i>C. jejuni</i>      | 1            | 2              | 1         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 0            | 2   | 1      | 0      | 0              | 0         |
| HS_6       | <i>C. jejuni</i>      | 0            | 0              | 0         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 0            | 2   | 1      | 0      | 0              | 0         |
| HS_7       | <i>C. jejuni</i>      | 0            | 0              | 0         | 0                            | 0       | 0           | 0           | 0           | 0           | 2                              | 1          | 0          | 0            | 2   | 1      | 0      | 0              | 0         |
| HS_8       | <i>C. jejuni</i>      | 1            | 2              | 1         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 0            | 2   | 1      | 0      | 0              | 0         |
| HS_9       | <i>C. jejuni</i>      | 0            | 0              | 0         | 0                            | 0       | 0           | 0           | 0           | 0           | 2                              | 1          | 0          | 0            | 2   | 1      | 0      | 0              | 0         |
| HS_10      | <i>C. coli</i>        | 1            | 4              | 2         | 1                            | 2       | 0           | 0           | 1           | 0           | 0                              | 0          | 0          | 0            | 2   | 1      | 0      | 0              | 0         |
| HS_11      | <i>C. jejuni</i>      | 0            | 0              | 0         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 0            | 2   | 1      | 0      | 0              | 0         |
| HS_12      | <i>C. jejuni</i>      | 1            | 2              | 1         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 0            | 2   | 1      | 0      | 0              | 0         |
| HS_13      | <i>C. jejuni</i>      | 1            | 2              | 1         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 0            | 2   | 1      | 0      | 0              | 0         |
| HS_14      | <i>C. jejuni</i>      | 1            | 2              | 1         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 0            | 2   | 1      | 0      | 0              | 0         |
| HS_15      | <i>C. jejuni</i>      | 0            | 0              | 0         | 0                            | 0       | 0           | 0           | 0           | 0           | 2                              | 1          | 0          | 0            | 2   | 1      | 0      | 0              | 0         |
| HS_16      | <i>C. jejuni</i>      | 0            | 0              | 0         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 0            | 2   | 1      | 0      | 0              | 0         |
| HS_17      | <i>C. jejuni</i>      | 1            | 2              | 1         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 0            | 2   | 1      | 0      | 0              | 0         |
| HS_18      | <i>C. jejuni</i>      | 1            | 2              | 1         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 0            | 2   | 1      | 0      | 0              | 0         |
| HS_19      | <i>C. coli</i>        | 1            | 4              | 2         | 1                            | 2       | 0           | 0           | 1           | 0           | 0                              | 0          | 0          | 0            | 2   | 1      | 0      | 0              | 0         |
| HS_20      | <i>C. jejuni</i>      | 0            | 0              | 0         | 0                            | 0       | 0           | 0           | 0           | 0           | 2                              | 1          | 0          | 0            | 2   | 1      | 0      | 0              | 0         |
| HS_21      | <i>C. coli</i>        | 1            | 2              | 1         | 1                            | 2       | 0           | 0           | 1           | 0           | 0                              | 0          | 0          | 0            | 2   | 1      | 0      | 0              | 0         |
| HS_22      | <i>C. coli</i>        | 0            | 0              | 0         | 1                            | 2       | 0           | 0           | 1           | 0           | 0                              | 0          | 0          | 1            | 2   | 0      | 1      | 0              | 0         |
| HS_23      | <i>C. jejuni</i>      | 1            | 2              | 1         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 0            | 2   | 1      | 0      | 0              | 0         |
| HS_24      | <i>C. jejuni</i>      | 1            | 2              | 1         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 0            | 2   | 1      | 0      | 0              | 0         |
| HS_25      | <i>C. jejuni</i>      | 1            | 2              | 1         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 0            | 2   | 1      | 0      | 0              | 0         |
| HS_26      | <i>C. jejuni</i>      | 1            | 2              | 1         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 0            | 2   | 1      | 0      | 0              | 0         |
| HS_27      | <i>C. jejuni</i>      | 0            | 0              | 0         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 0            | 2   | 1      | 0      | 0              | 0         |
| HS_28      | <i>C. jejuni</i>      | 1            | 4              | 2         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 0            | 2   | 1      | 0      | 0              | 0         |
| HS_29      | <i>C. jejuni</i>      | 1            | 2              | 1         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 0            | 2   | 1      | 0      | 0              | 0         |
| HS_30      | <i>C. jejuni</i>      | 1            | 2              | 1         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 0            | 2   | 1      | 0      | 0              | 0         |
| HS_31      | <i>C. jejuni</i>      | 1            | 2              | 1         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 0            | 2   | 1      | 0      | 0              | 0         |
| HS_32      | <i>C. coli</i>        | 1            | 2              | 1         | 1                            | 2       | 0           | 0           | 1           | 0           | 0                              | 0          | 0          | 0            | 2   | 1      | 0      | 0              | 0         |
| HS_33      | <i>C. jejuni</i>      | 1            | 2              | 1         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 0            | 2   | 1      | 0      | 0              | 0         |
| HS_34      | <i>C. jejuni</i>      | 1            | 2              | 1         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 0            | 2   | 1      | 0      | 0              | 0         |
| HS_35      | <i>C. coli</i>        | 1            | 2              | 1         | 1                            | 2       | 0           | 0           | 1           | 0           | 0                              | 0          | 0          | 0            | 2   | 1      | 0      | 0              | 0         |
| HS_36      | <i>C. jejuni</i>      | 0            | 0              | 0         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 0            | 2   | 1      | 0      | 0              | 0         |
| HS_37      | <i>C. jejuni</i>      | 1            | 2              | 1         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 0            | 2   | 1      | 0      | 0              | 0         |
| HS_38      | <i>C. jejuni</i>      | 1            | 2              | 1         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 0            | 2   | 1      | 0      | 0              | 0         |
| HS_39      | <i>C. jejuni</i>      | 1            | 2              | 1         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 0            | 2   | 1      | 0      | 0              | 0         |
| HS_40      | <i>C. jejuni</i>      | 1            | 2              | 1         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 0            | 2   | 1      | 0      | 0              | 0         |
| HS_41      | <i>C. jejuni</i>      | 1            | 2              | 1         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 0            | 2   | 1      | 0      | 0              | 0         |

# 9 Appendix

## Primer and probe binding sites (based on Aquamis)

S: sensitivity; R: resistance

grey shading: phenotypically resistant isolate

(1\*): imperfect binding site with pm due to additional mutation A>G

Commonly, 2 binding sites for 2 primers (forward and reverse) and 1 binding site for the probe were identified

In some cases, more binding sites are observed for *tet* (O) and 23S *rRNA*, which indicate the presence of 2 or 3 copies of a gene: 4 or 6 binding sites for primers and 2 or 3 binding sites for the probe  
 degenerated nucleotide Y (C/T) in *gyrA\_Cc*: C for wt1 and pm1; T for wt2 and pm2

| strain No. | Campylobacter species | tetracycline |                |           | ciprofloxacin <i>C. coli</i> |         |             |             |             |             | ciprofloxacin <i>C. jejuni</i> |            |            | erythromycin |     |        |        |                |           |
|------------|-----------------------|--------------|----------------|-----------|------------------------------|---------|-------------|-------------|-------------|-------------|--------------------------------|------------|------------|--------------|-----|--------|--------|----------------|-----------|
|            |                       | TET R/S      | tet(O) primers | tet(O) wt | CIP R/S                      | gyrA_Cc | gyrA_Cc_wt1 | gyrA_Cc_wt2 | gyrA_Cc_pm1 | gyrA_Cc_pm2 | gyrA_Cj                        | gyrA_Cj_wt | gyrA_Cj_pm | ERY R/S      | 23S | 23S_wt | 23S_pm | erm(B) primers | erm(B) wt |
| HS_42      | <i>C. jejuni</i>      | 0            | 0              | 0         | 0                            | 0       | 0           | 0           | 0           | 0           | 2                              | 1          | 0          | 0            | 2   | 1      | 0      | 0              | 0         |
| HS_43      | <i>C. jejuni</i>      | 0            | 0              | 0         | 0                            | 0       | 0           | 0           | 0           | 0           | 2                              | 1          | 0          | 0            | 2   | 1      | 0      | 0              | 0         |
| HS_44      | <i>C. jejuni</i>      | 0            | 0              | 0         | 0                            | 0       | 0           | 0           | 0           | 0           | 2                              | 1          | 0          | 0            | 2   | 1      | 0      | 0              | 0         |
| HS_45      | <i>C. coli</i>        | 0            | 0              | 0         | 1                            | 2       | 0           | 0           | 0           | 1           | 0                              | 0          | 0          | 0            | 2   | 1      | 0      | 0              | 0         |
| HS_46      | <i>C. jejuni</i>      | 1            | 2              | 1         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 0            | 2   | 1      | 0      | 0              | 0         |
| HS_47      | <i>C. jejuni</i>      | 0            | 0              | 0         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 0            | 2   | 1      | 0      | 0              | 0         |
| HS_48      | <i>C. jejuni</i>      | 1            | 2              | 1         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 0            | 2   | 1      | 0      | 0              | 0         |
| HS_49      | <i>C. jejuni</i>      | 1            | 2              | 1         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 0            | 2   | 1      | 0      | 0              | 0         |
| HS_50      | <i>C. jejuni</i>      | 1            | 2              | 1         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 0            | 2   | 1      | 0      | 0              | 0         |
| FS_51      | <i>C. jejuni</i>      | 0            | 0              | 0         | 0                            | 0       | 0           | 0           | 0           | 0           | 2                              | 1          | 0          | 0            | 2   | 1      | 0      | 0              | 0         |
| FS_52      | <i>C. jejuni</i>      | 1            | 4              | 2         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 0            | 2   | 1      | 0      | 0              | 0         |
| FS_53      | <i>C. jejuni</i>      | 0            | 0              | 0         | 0                            | 0       | 0           | 0           | 0           | 0           | 2                              | 1          | 0          | 0            | 2   | 1      | 0      | 0              | 0         |
| FS_54      | <i>C. jejuni</i>      | 1            | 2              | 1         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 0            | 2   | 1      | 0      | 0              | 0         |
| FS_55      | <i>C. coli</i>        | 0            | 0              | 0         | 1                            | 2       | 0           | 0           | 1           | 0           | 0                              | 0          | 0          | 0            | 4   | 2      | 0      | 0              | 0         |
| FS_56      | <i>C. jejuni</i>      | 1            | 4              | 2         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 0            | 2   | 1      | 0      | 0              | 0         |
| FS_57      | <i>C. jejuni</i>      | 0            | 0              | 0         | 0                            | 0       | 0           | 0           | 0           | 0           | 2                              | 1          | 0          | 0            | 2   | 1      | 0      | 0              | 0         |
| FS_58      | <i>C. jejuni</i>      | 1            | 2              | 1         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 0            | 2   | 1      | 0      | 0              | 0         |
| FS_59      | <i>C. jejuni</i>      | 0            | 0              | 0         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 0            | 2   | 1      | 0      | 0              | 0         |
| FS_60      | <i>C. jejuni</i>      | 0            | 0              | 0         | 0                            | 0       | 0           | 0           | 0           | 0           | 2                              | 1          | 0          | 0            | 2   | 1      | 0      | 0              | 0         |
| FS_61      | <i>C. jejuni</i>      | 0            | 0              | 0         | 0                            | 0       | 0           | 0           | 0           | 0           | 2                              | 1          | 0          | 0            | 2   | 1      | 0      | 0              | 0         |
| FS_62      | <i>C. jejuni</i>      | 1            | 2              | 1         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 0            | 2   | 1      | 0      | 0              | 0         |
| FS_63      | <i>C. jejuni</i>      | 0            | 0              | 0         | 0                            | 0       | 0           | 0           | 0           | 0           | 2                              | 1          | 0          | 0            | 2   | 1      | 0      | 0              | 0         |
| FS_64      | <i>C. jejuni</i>      | 1            | 2              | 1         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 0            | 2   | 1      | 0      | 0              | 0         |
| FS_65      | <i>C. jejuni</i>      | 1            | 2              | 1         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 0            | 2   | 1      | 0      | 0              | 0         |
| FS_66      | <i>C. coli</i>        | 1            | 2              | 1         | 1                            | 2       | 0           | 0           | 1           | 0           | 0                              | 0          | 0          | 0            | 2   | 1      | 0      | 0              | 0         |
| FS_67      | <i>C. coli</i>        | 1            | 2              | 1         | 0                            | 2       | 1           | 0           | 0           | 0           | 0                              | 0          | 0          | 0            | 2   | 1      | 0      | 0              | 0         |
| FS_68      | <i>C. jejuni</i>      | 1            | 2              | 1         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 0            | 2   | 1      | 0      | 0              | 0         |
| FS_69      | <i>C. jejuni</i>      | 0            | 0              | 0         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 0            | 6   | 3      | 0      | 0              | 0         |
| FS_70      | <i>C. jejuni</i>      | 0            | 0              | 0         | 0                            | 0       | 0           | 0           | 0           | 0           | 2                              | 1          | 0          | 0            | 2   | 1      | 0      | 0              | 0         |
| FS_71      | <i>C. jejuni</i>      | 1            | 2              | 1         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 0            | 2   | 1      | 0      | 0              | 0         |
| FS_72      | <i>C. jejuni</i>      | 0            | 0              | 0         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 0            | 6   | 3      | 0      | 0              | 0         |
| FS_73      | <i>C. jejuni</i>      | 0            | 0              | 0         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 0            | 2   | 1      | 0      | 0              | 0         |
| FS_74      | <i>C. jejuni</i>      | 1            | 2              | 1         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 0            | 2   | 1      | 0      | 0              | 0         |
| FS_75      | <i>C. jejuni</i>      | 0            | 0              | 0         | 0                            | 0       | 0           | 0           | 0           | 0           | 2                              | 1          | 0          | 0            | 2   | 1      | 0      | 0              | 0         |
| FS_76      | <i>C. coli</i>        | 1            | 2              | 1         | 0                            | 2       | 1           | 0           | 0           | 0           | 0                              | 0          | 0          | 0            | 2   | 1      | 0      | 0              | 0         |
| FS_77      | <i>C. jejuni</i>      | 0            | 0              | 0         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 0            | 2   | 1      | 0      | 0              | 0         |
| FS_78      | <i>C. jejuni</i>      | 1            | 2              | 1         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 0            | 2   | 1      | 0      | 0              | 0         |
| FS_79      | <i>C. coli</i>        | 0            | 0              | 0         | 1                            | 2       | 0           | 0           | 1           | 0           | 0                              | 0          | 0          | 0            | 2   | 1      | 0      | 0              | 0         |
| FS_80      | <i>C. jejuni</i>      | 0            | 0              | 0         | 0                            | 0       | 0           | 0           | 0           | 0           | 2                              | 1          | 0          | 0            | 2   | 1      | 0      | 0              | 0         |
| FS_81      | <i>C. jejuni</i>      | 1            | 2              | 1         | 0                            | 0       | 0           | 0           | 0           | 0           | 2                              | 1          | 0          | 0            | 2   | 1      | 0      | 0              | 0         |

# 9 Appendix

## Primer and probe binding sites (based on Aquamis)

S: sensitivity; R: resistance

grey shading: phenotypically resistant isolate

(1\*): imperfect binding site with pm due to additional mutation A>G

Commonly, 2 binding sites for 2 primers (forward and reverse) and 1 binding site for the probe were identified

In some cases, more binding sites are observed for *tet* (O) and 23S *rRNA*, which indicate the presence of 2 or 3 copies of a gene: 4 or 6 binding sites for primers and 2 or 3 binding sites for the probe  
degenerated nucleotide Y (C/T) in *gyrA\_Cc*: C for wt1 and pm1; T for wt2 and pm2

| strain No. | Campylobacter species | tetracycline |                |           | ciprofloxacin <i>C. coli</i> |         |             |             |             |             | ciprofloxacin <i>C. jejuni</i> |            |            | erythromycin |     |        |        |                |           |
|------------|-----------------------|--------------|----------------|-----------|------------------------------|---------|-------------|-------------|-------------|-------------|--------------------------------|------------|------------|--------------|-----|--------|--------|----------------|-----------|
|            |                       | TET R/S      | tet(O) primers | tet(O) wt | CIP R/S                      | gyrA_Cc | gyrA_Cc_wt1 | gyrA_Cc_wt2 | gyrA_Cc_pm1 | gyrA_Cc_pm2 | gyrA_Cj                        | gyrA_Cj_wt | gyrA_Cj_pm | ERY R/S      | 23S | 23S_wt | 23S_pm | erm(B) primers | erm(B) wt |
| FS_82      | <i>C. coli</i>        | 0            | 0              | 0         | 0                            | 2       | 1           | 0           | 0           | 0           | 0                              | 0          | 0          | 0            | 2   | 1      | 0      | 0              | 0         |
| FS_83      | <i>C. jejuni</i>      | 0            | 0              | 0         | 0                            | 0       | 0           | 0           | 0           | 0           | 2                              | 1          | 0          | 0            | 2   | 1      | 0      | 0              | 0         |
| FS_84      | <i>C. coli</i>        | 1            | 2              | 1         | 1                            | 2       | 0           | 0           | 1           | 0           | 0                              | 0          | 0          | 0            | 2   | 1      | 0      | 0              | 0         |
| FS_85      | <i>C. jejuni</i>      | 0            | 0              | 0         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 0            | 2   | 1      | 0      | 0              | 0         |
| FS_86      | <i>C. jejuni</i>      | 0            | 0              | 0         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 0            | 2   | 1      | 0      | 0              | 0         |
| FS_87      | <i>C. jejuni</i>      | 1            | 2              | 1         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 0            | 2   | 1      | 0      | 0              | 0         |
| FS_88      | <i>C. jejuni</i>      | 0            | 0              | 0         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 0            | 2   | 1      | 0      | 0              | 0         |
| FS_89      | <i>C. jejuni</i>      | 1            | 2              | 1         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 0            | 2   | 1      | 0      | 0              | 0         |
| FS_90      | <i>C. coli</i>        | 1            | 2              | 1         | 0                            | 2       | 1           | 0           | 0           | 0           | 0                              | 0          | 0          | 0            | 2   | 1      | 0      | 0              | 0         |
| FS_91      | <i>C. jejuni</i>      | 1            | 2              | 1         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 0            | 2   | 1      | 0      | 0              | 0         |
| FS_92      | <i>C. coli</i>        | 1            | 2              | 1         | 0                            | 2       | 1           | 0           | 0           | 0           | 0                              | 0          | 0          | 0            | 2   | 1      | 0      | 0              | 0         |
| FS_93      | <i>C. coli</i>        | 0            | 0              | 0         | 0                            | 2       | 1           | 0           | 0           | 0           | 0                              | 0          | 0          | 0            | 2   | 1      | 0      | 0              | 0         |
| FS_94      | <i>C. coli</i>        | 0            | 0              | 0         | 0                            | 2       | 1           | 0           | 0           | 0           | 0                              | 0          | 0          | 0            | 2   | 1      | 0      | 0              | 0         |
| FS_95      | <i>C. coli</i>        | 1            | 2              | 1         | 1                            | 2       | 0           | 0           | 1           | 0           | 0                              | 0          | 0          | 0            | 2   | 1      | 0      | 0              | 0         |
| FS_96      | <i>C. jejuni</i>      | 0            | 0              | 0         | 0                            | 0       | 0           | 0           | 0           | 0           | 2                              | 1          | 0          | 0            | 2   | 1      | 0      | 0              | 0         |
| FS_97      | <i>C. coli</i>        | 0            | 0              | 0         | 0                            | 2       | 1           | 0           | 0           | 0           | 0                              | 0          | 0          | 0            | 2   | 1      | 0      | 0              | 0         |
| FS_98      | <i>C. coli</i>        | 1            | 2              | 1         | 1                            | 2       | 0           | 0           | 1           | 0           | 0                              | 0          | 0          | 0            | 2   | 1      | 0      | 0              | 0         |
| FS_99      | <i>C. jejuni</i>      | 1            | 2              | 1         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 0            | 2   | 1      | 0      | 0              | 0         |
| FS_100     | <i>C. coli</i>        | 0            | 0              | 0         | 1                            | 2       | 0           | 0           | 1           | 0           | 0                              | 0          | 0          | 0            | 2   | 1      | 0      | 0              | 0         |
| HS_101     | <i>C. coli</i>        | 1            | 2              | 1         | 1                            | 2       | 0           | 0           | 1           | 0           | 0                              | 0          | 0          | 0            | 2   | 1      | 0      | 0              | 0         |
| HS_102     | <i>C. coli</i>        | 1            | 4              | 2         | 1                            | 2       | 0           | 0           | 1           | 0           | 0                              | 0          | 0          | 0            | 2   | 1      | 0      | 0              | 0         |
| HS_103     | <i>C. coli</i>        | 1            | 2              | 1         | 1                            | 2       | 0           | 0           | 1           | 0           | 0                              | 0          | 0          | 1            | 2   | 0      | 1      | 0              | 0         |
| HS_104     | <i>C. coli</i>        | 0            | 0              | 0         | 1                            | 2       | 0           | 0           | 1           | 0           | 0                              | 0          | 0          | 0            | 2   | 1      | 0      | 0              | 0         |
| HS_105     | <i>C. coli</i>        | 0            | 0              | 0         | 1                            | 2       | 0           | 0           | 1           | 0           | 0                              | 0          | 0          | 0            | 2   | 1      | 0      | 0              | 0         |
| HS_106     | <i>C. coli</i>        | 0            | 0              | 0         | 0                            | 2       | 1           | 0           | 0           | 0           | 0                              | 0          | 0          | 0            | 2   | 1      | 0      | 0              | 0         |
| HS_107     | <i>C. coli</i>        | 1            | 2              | 1         | 1                            | 2       | 0           | 0           | 1           | 0           | 0                              | 0          | 0          | 0            | 2   | 1      | 0      | 0              | 0         |
| HS_108     | <i>C. coli</i>        | 1            | 2              | 1         | 1                            | 2       | 0           | 0           | 1           | 0           | 0                              | 0          | 0          | 0            | 2   | 1      | 0      | 0              | 0         |
| HS_109     | <i>C. coli</i>        | 0            | 0              | 0         | 0                            | 2       | 1           | 0           | 0           | 0           | 0                              | 0          | 0          | 0            | 2   | 1      | 0      | 0              | 0         |
| HS_110     | <i>C. coli</i>        | 1            | 2              | 1         | 1                            | 2       | 0           | 0           | 1           | 0           | 0                              | 0          | 0          | 0            | 4   | 2      | 0      | 0              | 0         |
| FS_111     | <i>C. coli</i>        | 1            | 2              | 1         | 1                            | 2       | 0           | 0           | 1           | 0           | 0                              | 0          | 0          | 0            | 2   | 1      | 0      | 0              | 0         |
| FS_112     | <i>C. jejuni</i>      | 0            | 0              | 0         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 0            | 4   | 2      | 0      | 0              | 0         |
| FS_113     | <i>C. jejuni</i>      | 0            | 0              | 0         | 0                            | 0       | 0           | 0           | 0           | 0           | 2                              | 1          | 0          | 0            | 2   | 1      | 0      | 0              | 0         |
| FS_114     | <i>C. jejuni</i>      | 1            | 2              | 1         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 0            | 2   | 1      | 0      | 0              | 0         |
| FS_115     | <i>C. coli</i>        | 1            | 2              | 1         | 1                            | 2       | 0           | 0           | 1           | 0           | 0                              | 0          | 0          | 0            | 2   | 1      | 0      | 0              | 0         |
| FS_116     | <i>C. coli</i>        | 0            | 0              | 0         | 0                            | 2       | 1           | 0           | 0           | 0           | 0                              | 0          | 0          | 0            | 2   | 1      | 0      | 0              | 0         |
| FS_117     | <i>C. jejuni</i>      | 0            | 0              | 0         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 0            | 4   | 2      | 0      | 0              | 0         |
| FS_118     | <i>C. coli</i>        | 1            | 2              | 1         | 0                            | 2       | 1           | 0           | 0           | 0           | 0                              | 0          | 0          | 0            | 2   | 1      | 0      | 0              | 0         |
| FS_119     | <i>C. coli</i>        | 0            | 0              | 0         | 1                            | 2       | 0           | 0           | 1           | 0           | 0                              | 0          | 0          | 1            | 2   | 0      | 1      | 0              | 0         |
| FS_120     | <i>C. jejuni</i>      | 0            | 0              | 0         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 0            | 2   | 1      | 0      | 0              | 0         |
| HS_121     | <i>C. coli</i>        | 0            | 0              | 0         | 1                            | 2       | 0           | 0           | 1           | 0           | 0                              | 0          | 0          | 0            | 4   | 2      | 0      | 0              | 0         |
| HS_122     | <i>C. jejuni</i>      | 1            | 2              | 1         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 0            | 2   | 1      | 0      | 0              | 0         |
| HS_123     | <i>C. coli</i>        | 1            | 2              | 1         | 0                            | 2       | 1           | 0           | 0           | 0           | 0                              | 0          | 0          | 0            | 2   | 1      | 0      | 0              | 0         |
| HS_124     | <i>C. coli</i>        | 1            | 2              | 1         | 1                            | 2       | 0           | 0           | 1           | 0           | 0                              | 0          | 0          | 0            | 2   | 1      | 0      | 0              | 0         |
| HS_125     | <i>C. coli</i>        | 1            | 2              | 1         | 1                            | 2       | 0           | 0           | 1           | 0           | 0                              | 0          | 0          | 0            | 2   | 1      | 0      | 0              | 0         |

## 9 Appendix

### Primer and probe binding sites (based on Aquamis)

S: sensitivity; R: resistance

grey shading: phenotypically resistant isolate

(1\*): imperfect binding site with pm due to additional mutation A>G

Commonly, 2 binding sites for 2 primers (forward and reverse) and 1 binding site for the probe were identified

In some cases, more binding sites are observed for *tet* (O) and *23S rRNA*, which indicate the presence of 2 or 3 copies of a gene: 4 or 6 binding sites for primers and 2 or 3 binding sites for the probe  
degenerated nucleotide Y (C/T) in *gyrA\_Cc*: C for wt1 and pm1 ; T for wt2 and pm2

| strain No. | <i>Campylobacter</i> species | tetracycline |                |           | ciprofloxacin <i>C. coli</i> |                |                    |                    |                    | ciprofloxacin <i>C. jejuni</i> |                |                   | erythromycin      |         |     |        |        |                |           |
|------------|------------------------------|--------------|----------------|-----------|------------------------------|----------------|--------------------|--------------------|--------------------|--------------------------------|----------------|-------------------|-------------------|---------|-----|--------|--------|----------------|-----------|
|            |                              | TET R/S      | tet(O) primers | tet(O) wt | CIP R/S                      | <i>gyrA_Cc</i> | <i>gyrA_Cc_wt1</i> | <i>gyrA_Cc_wt2</i> | <i>gyrA_Cc_pm1</i> | <i>gyrA_Cc_pm2</i>             | <i>gyrA_Cj</i> | <i>gyrA_Cj_wt</i> | <i>gyrA_Cj_pm</i> | ERY R/S | 23S | 23S_wt | 23S_pm | erm(B) primers | erm(B) wt |
| HS_126     | <i>C. coli</i>               | 0            | 0              | 0         | 0                            | 2              | 1                  | 0                  | 0                  | 0                              | 0              | 0                 | 0                 | 0       | 2   | 1      | 0      | 0              | 0         |
| HS_127     | <i>C. coli</i>               | 1            | 2              | 1         | 1                            | 2              | 0                  | 0                  | 1                  | 0                              | 0              | 0                 | 0                 | 0       | 2   | 1      | 0      | 0              | 0         |
| HS_128     | <i>C. coli</i>               | 0            | 0              | 0         | 0                            | 2              | 1                  | 0                  | 0                  | 0                              | 0              | 0                 | 0                 | 0       | 2   | 1      | 0      | 0              | 0         |
| FS_129     | <i>C. jejuni</i>             | 1            | 2              | 1         | 1                            | 0              | 0                  | 0                  | 0                  | 0                              | 2              | 0                 | (1*)+A>G          | 0       | 2   | 1      | 0      | 0              | 0         |

# 9 Appendix

## Primer and probe binding sites (based on Aquamis)

S: sensitivity; R: resistance

grey shading: phenotypically resistant isolate

(1\*): imperfect binding site with pm due to additional mutation A>G

Commonly, 2 binding sites for 2 primers (forward and reverse) and 1 binding site for the probe were identified

In some cases, more binding sites are observed for *tet* (O) and 23S *rRNA*, which indicate the presence of 2 or 3 copies of a gene: 4 or 6 binding sites for primers and 2 or 3 binding sites for the probe  
 degenerated nucleotide Y (C/T) in *gyrA\_Cc*: C for wt1 and pm1 ; T for wt2 and pm2

| strain No.    | Campylobacter species | tetracycline |                |           | ciprofloxacin <i>C. coli</i> |         |             |             |             |             | ciprofloxacin <i>C. jejuni</i> |            |            | erythromycin |     |        |        |                |           |
|---------------|-----------------------|--------------|----------------|-----------|------------------------------|---------|-------------|-------------|-------------|-------------|--------------------------------|------------|------------|--------------|-----|--------|--------|----------------|-----------|
|               |                       | TET R/S      | tet(O) primers | tet(O) wt | CIP R/S                      | gyrA_Cc | gyrA_Cc_wt1 | gyrA_Cc_wt2 | gyrA_Cc_pm1 | gyrA_Cc_pm2 | gyrA_Cj                        | gyrA_Cj_wt | gyrA_Cj_pm | ERY R/S      | 23S | 23S_wt | 23S_pm | erm(B) primers | erm(B) wt |
| VE_01         | <i>C. coli</i>        | 1            | 2              | 1         | 1                            | 2       | 0           | 0           | 1           | 0           | 0                              | 0          | 0          | 1            | 2   | 1      | 0      | 2              | 1         |
| VE_02         | <i>C. coli</i>        | 1            | 2              | 1         | 1                            | 2       | 0           | 0           | 1           | 0           | 0                              | 0          | 0          | 1            | 2   | 0      | 1      | 0              | 0         |
| VE_03         | <i>C. coli</i>        | 1            | 3              | 1         | 1                            | 2       | 0           | 0           | 1           | 0           | 0                              | 0          | 0          | 1            | 2   | 0      | 1      | 0              | 0         |
| VE_04         | <i>C. coli</i>        | 1            | 2              | 1         | 1                            | 2       | 0           | 0           | 1           | 0           | 0                              | 0          | 0          | 1            | 2   | 1      | 0      | 2              | 1         |
| VE_05         | <i>C. coli</i>        | 1            | 2              | 1         | 1                            | 2       | 0           | 0           | 1           | 0           | 0                              | 0          | 0          | 1            | 2   | 1      | 0      | 2              | 1         |
| VE_06         | <i>C. coli</i>        | 1            | 4              | 1         | 1                            | 2       | 0           | 0           | 1           | 0           | 0                              | 0          | 0          | 1            | 2   | 0      | 1      | 0              | 0         |
| VE_07         | <i>C. coli</i>        | 1            | 3              | 1         | 1                            | 2       | 0           | 0           | 1           | 0           | 0                              | 0          | 0          | 1            | 2   | 0      | 1      | 0              | 0         |
| VE_08         | <i>C. coli</i>        | 1            | 2              | 1         | 1                            | 2       | 0           | 0           | 1           | 0           | 0                              | 0          | 0          | 1            | 2   | 1      | 0      | 2              | 1         |
| VE_09         | <i>C. coli</i>        | 1            | 4              | 1         | 1                            | 2       | 0           | 0           | 1           | 0           | 0                              | 0          | 0          | 1            | 2   | 0      | 1      | 0              | 0         |
| VE_10         | <i>C. coli</i>        | 1            | 2              | 1         | 1                            | 2       | 0           | 0           | 1           | 0           | 0                              | 0          | 0          | 1            | 2   | 1      | 0      | 2              | 1         |
| VE_11         | <i>C. coli</i>        | 1            | 2              | 1         | 1                            | 2       | 0           | 0           | 1           | 0           | 0                              | 0          | 0          | 1            | 2   | 0      | 1      | 0              | 0         |
| VE_12         | <i>C. coli</i>        | 1            | 2              | 1         | 1                            | 2       | 0           | 0           | 1           | 0           | 0                              | 0          | 0          | 1            | 2   | 0      | 1      | 0              | 0         |
| VE_13         | <i>C. jejuni</i>      | 1            | 2              | 1         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 0            | 2   | 1      | 0      | 0              | 0         |
| VE_14         | <i>C. jejuni</i>      | 1            | 2              | 1         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 1            | 2   | 0      | 1      | 0              | 0         |
| VE_15         | <i>C. coli</i>        | 1            | 3              | 1         | 1                            | 2       | 0           | 0           | 1           | 0           | 0                              | 0          | 0          | 1            | 2   | 1      | 0      | 2              | 1         |
| VE_16         | <i>C. coli</i>        | 1            | 2              | 1         | 1                            | 2       | 0           | 0           | 1           | 0           | 0                              | 0          | 0          | 1            | 2   | 0      | 1      | 0              | 0         |
| VE_17         | <i>C. coli</i>        | 1            | 3              | 1         | 1                            | 2       | 0           | 0           | 1           | 0           | 0                              | 0          | 0          | 1            | 2   | 1      | 0      | 2              | 1         |
| VE_18         | <i>C. jejuni</i>      | 1            | 2              | 1         | 1                            | 0       | 0           | 0           | 0           | 0           | 2                              | 0          | 1          | 0            | 2   | 1      | 0      | 0              | 0         |
| VE_19         | <i>C. coli</i>        | 1            | 3              | 1         | 1                            | 2       | 0           | 0           | 1           | 0           | 0                              | 0          | 0          | 1            | 2   | 1      | 0      | 2              | 1         |
| VE_20         | <i>C. coli</i>        | 1            | 3              | 1         | 1                            | 2       | 0           | 0           | 1           | 0           | 0                              | 0          | 0          | 1            | 2   | 1      | 0      | 2              | 1         |
| VE_21         | <i>C. coli</i>        | 1            | 2              | 1         | 1                            | 2       | 0           | 0           | (1*)+A>G    | 0           | 0                              | 0          | 0          | 1            | 2   | 1      | 0      | 2              | 1         |
| DSM 4688      | <i>C. jejuni</i>      | 0            | 0              | 0         | 0                            | 0       | 0           | 0           | 0           | 0           | 2                              | 1          | 0          | 0            | 2   | 1      | 0      | 0              | 0         |
| 2012-70-443-2 | <i>C. coli</i>        | 0            | 0              | 0         | 0                            | 2       | 1           | 0           | 0           | 0           | 0                              | 0          | 0          | 0            | 2   | 1      | 0      | 0              | 0         |

**Pentaplex real-time PCR assay:  
Screening of *tet* (O), *GyrA*\_T86I, *erm* (B), 23S *rRNA* \_A2075G, IAC**

|                          |                             |
|--------------------------|-----------------------------|
| real-time PCR-instrument | AriaMx                      |
| detection system         | FAM, ROX, HEX, Cy5, ATTO425 |
| mastermix                | QuantiNova, Qiagen          |

| Components                           | Concentration of working solutions | Final concentration in PCR assay | Volume in $\mu$ L for 1 sample |
|--------------------------------------|------------------------------------|----------------------------------|--------------------------------|
| <b>QuantiNova Multiplex</b>          | 4x                                 | 1x                               | 6,250                          |
| PCR-grade water                      |                                    | -                                | 2,750                          |
| tet(O)-fw                            | 20 $\mu$ M                         | 150 nM                           | 0,188                          |
| tet(O)-re                            | 20 $\mu$ M                         | 150 nM                           | 0,188                          |
| tet(O)-probe (FAM)                   | 10 $\mu$ M                         | 100 nM                           | 0,250                          |
| <i>gyrA</i> _Cj_fw                   | 20 $\mu$ M                         | 400 nM                           | 0,500                          |
| <i>gyrA</i> _Cj_re                   | 20 $\mu$ M                         | 400 nM                           | 0,500                          |
| <i>gyrA</i> _Cj_wt                   | 20 $\mu$ M                         | 600 nM                           | 0,750                          |
| <i>gyrA</i> _Cj_pm (ROX)             | 10 $\mu$ M                         | 200 nM                           | 0,500                          |
| <i>gyrA</i> _Cc_fw                   | 20 $\mu$ M                         | 400 nM                           | 0,500                          |
| <i>gyrA</i> _Cc_re                   | 20 $\mu$ M                         | 400 nM                           | 0,500                          |
| <i>gyrA</i> _Cc_wt                   | 20 $\mu$ M                         | 600 nM                           | 0,750                          |
| <i>gyrA</i> _Cc_pm (ROX)             | 10 $\mu$ M                         | 200 nM                           | 0,500                          |
| <i>erm</i> (B)_fw                    | 20 $\mu$ M                         | 400 nM                           | 0,500                          |
| <i>erm</i> (B)_re                    | 20 $\mu$ M                         | 400 nM                           | 0,500                          |
| <i>erm</i> (B)-probe (Cy5)           | 10 $\mu$ M                         | 200 nM                           | 0,500                          |
| 23S_A2075G_fw                        | 20 $\mu$ M                         | 400 nM                           | 0,500                          |
| 23S_A2075G_re                        | 20 $\mu$ M                         | 400 nM                           | 0,500                          |
| 23S_A2075G_wt                        | 20 $\mu$ M                         | 600 nM                           | 0,750                          |
| 23S_A2075G_pm (HEX)                  | 10 $\mu$ M                         | 200 nM                           | 0,500                          |
| IPC-ntb2-fw                          | 20 $\mu$ M                         | 300 nM                           | 0,375                          |
| IPC-ntb2-re                          | 20 $\mu$ M                         | 300 nM                           | 0,375                          |
| IPC-ntb2-probe (ATTO425)             | 10 $\mu$ M                         | 150 nM                           | 0,375                          |
| IPC-ntb2 plasmid, 50 copies/ $\mu$ L | 50 copies/ $\mu$ L                 | 50 copies                        | 1,000                          |
| <b>Total volume of mastermix</b>     |                                    | -                                | <b>20,000</b>                  |
| Sample DNA, Extraction control, NTC  |                                    | -                                | 5                              |
| <b>Total volume of one reaction</b>  |                                    | -                                | <b>25</b>                      |

**Thermal profile**

|                      | Phase                       | Time   | Temperature | Cycles |
|----------------------|-----------------------------|--------|-------------|--------|
|                      | <b>Initial denaturation</b> | 2 min  | 95 °C       | 1 ×    |
| <b>Amplification</b> | denaturation                | 10 sec | 95 °C       | 40 ×   |
|                      | annealing                   | 20 sec | 60 °C       |        |
|                      | elongation                  | 20 sec | 72 °C       |        |



**Pentaplex real-time PCR assay:  
Screening of *tet* (O), *GyrA*\_T86I, *erm* (B), 23S *rRNA* \_A2075G, IAC**

real-time PCR-instrument      Quantstudio5, CFX96 Touch System  
detection system                FAM, ROX, HEX, Cy5, Cy5.5  
mastermix                         QuantiNova, Qiagen

| Components                           | Concentration of working solutions | Final concentration in PCR assay | Volume in $\mu$ L for 1 sample |
|--------------------------------------|------------------------------------|----------------------------------|--------------------------------|
| QuantiNova Multiplex                 | 4x                                 | 1x                               | 6,250                          |
| PCR-grade water                      |                                    | -                                | 2,750                          |
| tet(O)-fw                            | 20 $\mu$ M                         | 150 nM                           | 0,188                          |
| tet(O)-re                            | 20 $\mu$ M                         | 150 nM                           | 0,188                          |
| tet(O)-probe (FAM)                   | 10 $\mu$ M                         | 100 nM                           | 0,250                          |
| <i>gyrA</i> _Cj_fw                   | 20 $\mu$ M                         | 400 nM                           | 0,500                          |
| <i>gyrA</i> _Cj_re                   | 20 $\mu$ M                         | 400 nM                           | 0,500                          |
| <i>gyrA</i> _Cj_wt                   | 20 $\mu$ M                         | 600 nM                           | 0,750                          |
| <i>gyrA</i> _Cj_pm (ROX)             | 10 $\mu$ M                         | 200 nM                           | 0,500                          |
| <i>gyrA</i> _Cc_fw                   | 20 $\mu$ M                         | 400 nM                           | 0,500                          |
| <i>gyrA</i> _Cc_re                   | 20 $\mu$ M                         | 400 nM                           | 0,500                          |
| <i>gyrA</i> _Cc_wt                   | 20 $\mu$ M                         | 600 nM                           | 0,750                          |
| <i>gyrA</i> _Cc_pm (ROX)             | 10 $\mu$ M                         | 200 nM                           | 0,500                          |
| <i>erm</i> (B)_fw                    | 20 $\mu$ M                         | 400 nM                           | 0,500                          |
| <i>erm</i> (B)_re                    | 20 $\mu$ M                         | 400 nM                           | 0,500                          |
| <i>erm</i> (B)-probe (Cy5)           | 10 $\mu$ M                         | 200 nM                           | 0,500                          |
| 23S_A2075G_fw                        | 20 $\mu$ M                         | 400 nM                           | 0,500                          |
| 23S_A2075G_re                        | 20 $\mu$ M                         | 400 nM                           | 0,500                          |
| 23S_A2075G_wt                        | 20 $\mu$ M                         | 600 nM                           | 0,750                          |
| 23S_A2075G_pm (HEX)                  | 10 $\mu$ M                         | 200 nM                           | 0,500                          |
| IPC-ntb2-fw                          | 20 $\mu$ M                         | 300 nM                           | 0,375                          |
| IPC-ntb2-re                          | 20 $\mu$ M                         | 300 nM                           | 0,375                          |
| IPC-ntb2-probe (Cy5.5)               | 10 $\mu$ M                         | 150 nM                           | 0,375                          |
| IPC-ntb2 plasmid, 50 copies/ $\mu$ L | 50 copies/ $\mu$ L                 | 50 copies                        | 1,000                          |
| <b>Total volume of mastermix</b>     |                                    | -                                | <b>20,000</b>                  |
| Sample DNA, Extraction control, NTC  |                                    | -                                | 5                              |
| <b>Total volume of one reaction</b>  |                                    | -                                | <b>25</b>                      |

**Thermal profile**

|                      | Phase                       | Time   | Temperature | Cycles |
|----------------------|-----------------------------|--------|-------------|--------|
|                      | <b>Initial denaturation</b> | 2 min  | 95 °C       | 1 ×    |
| <b>Amplification</b> | denaturation                | 10 sec | 95 °C       | 40 ×   |
|                      | annealing                   | 20 sec | 60 °C       |        |
|                      | elongation                  | 20 sec | 72 °C       |        |

**Triplex real-time PCR assay:  
Screening of *tet* (O), GyrA\_T86I, IAC**

real-time PCR-instrument      AriaMx, Quantstudio5, CFX96 Touch System  
detection system                FAM, ROX, HEX  
mastermix                         QuantiNova, Qiagen

| Components                                 | Concentration of working solutions | Final concentration in PCR assay | Volume in $\mu\text{L}$ for 1 sample |
|--|------------------------------------|----------------------------------|--------------------------------------|
| <b>QuantiNova Multiplex</b>                | 4x                                 | 1x                               | 6,250                                |
| PCR-grade water                            |                                    | -                                | 6,500                                |
| tet(O)-fw                                  | 20 $\mu\text{M}$                   | 150 nM                           | 0,188                                |
| tet(O)-re                                  | 20 $\mu\text{M}$                   | 150 nM                           | 0,188                                |
| tet(O)-probe ( <b>FAM</b> )                | 10 $\mu\text{M}$                   | 100 nM                           | 0,250                                |
| gyrA_Cj_fw                                 | 20 $\mu\text{M}$                   | 400 nM                           | 0,500                                |
| gyrA_Cj_re                                 | 20 $\mu\text{M}$                   | 400 nM                           | 0,500                                |
| gyrA_Cj_wt                                 | 20 $\mu\text{M}$                   | 600 nM                           | 0,750                                |
| gyrA_Cj_pm ( <b>ROX</b> )                  | 10 $\mu\text{M}$                   | 200 nM                           | 0,500                                |
| gyrA_Cc_fw                                 | 20 $\mu\text{M}$                   | 400 nM                           | 0,500                                |
| gyrA_Cc_re                                 | 20 $\mu\text{M}$                   | 400 nM                           | 0,500                                |
| gyrA_Cc_wt                                 | 20 $\mu\text{M}$                   | 600 nM                           | 0,750                                |
| gyrA_Cc_pm ( <b>ROX</b> )                  | 10 $\mu\text{M}$                   | 200 nM                           | 0,500                                |
| IPC-ntb2-fw                                | 20 $\mu\text{M}$                   | 300 nM                           | 0,375                                |
| IPC-ntb2-re                                | 20 $\mu\text{M}$                   | 300 nM                           | 0,375                                |
| IPC-ntb2-probe ( <b>HEX</b> )              | 10 $\mu\text{M}$                   | 150 nM                           | 0,375                                |
| IPC-ntb2 plasmid, 50 copies/ $\mu\text{L}$ | 50 copies/ $\mu\text{L}$           | 50 copies                        | 1,000                                |
| <b>Total volume of mastermix</b>           |                                    | -                                | <b>20,000</b>                        |
| Sample DNA, Extraction control, NTC        |                                    | -                                | 5                                    |
| <b>Total volume of one reaction</b>        |                                    | -                                | <b>25</b>                            |

**Thermal profile**

|                      | Phase                       | Time   | Temperature | Cycles |
|----------------------|-----------------------------|--------|-------------|--------|
|                      | <b>Initial denaturation</b> | 2 min  | 95 °C       | 1 ×    |
| <b>Amplification</b> | denaturation                | 10 sec | 95 °C       | 40 ×   |
|                      | annealing                   | 20 sec | 60 °C       |        |
|                      | elongation                  | 20 sec | 72 °C       |        |

**Duplex real-time PCR assay:  
Screening of *erm* (B), 23S rRNA\_A2075G**

|                          |  |
|--------------------------|--|
| real-time PCR-instrument | AriaMx, Quantstudio5, CFX96 Touch System |
| detection system         | FAM, HEX                                 |
| mastermix                | QuantiNova, Qiagen                       |

| Components                          | Concentration of working solutions | Final concentration in PCR assay | Volume in $\mu\text{L}$ for 1 sample |
|-------------------------------------|------------------------------------|----------------------------------|--------------------------------------|
| QuantiNova Multiplex                | 4x                                 | 1x                               | 6,250                                |
| PCR-grade water                     |                                    | -                                | 9,000                                |
| <i>erm</i> (B)_fw                   | 20 $\mu\text{M}$                   | 400 nM                           | 0,500                                |
| <i>erm</i> (B)_re                   | 20 $\mu\text{M}$                   | 400 nM                           | 0,500                                |
| <i>erm</i> (B)-probe (FAM)          | 10 $\mu\text{M}$                   | 200 nM                           | 0,500                                |
| 23S_A2075G_fw                       | 20 $\mu\text{M}$                   | 400 nM                           | 0,500                                |
| 23S_A2075G_re                       | 20 $\mu\text{M}$                   | 400 nM                           | 0,500                                |
| 23S_A2075G_wt                       | 20 $\mu\text{M}$                   | 600 nM                           | 0,750                                |
| 23S_A2075G_pm (HEX)                 | 10 $\mu\text{M}$                   | 200 nM                           | 0,500                                |
| <b>Total volume of mastermix</b>    |                                    | -                                | <b>20,000</b>                        |
| Sample DNA, Extraction control, NTC |                                    | -                                | 5                                    |
| <b>Total volume of one reaction</b> |                                    | -                                | <b>25</b>                            |

**Thermal profile**

|                      | Phase                       | Time   | Temperature | Cycles |
|----------------------|-----------------------------|--------|-------------|--------|
|                      | <b>Initial denaturation</b> | 2 min  | 95 °C       | 1 ×    |
| <b>Amplification</b> | denaturation                | 10 sec | 95 °C       | 40 ×   |
|                      | annealing                   | 20 sec | 60 °C       |        |
|                      | elongation                  | 20 sec | 72 °C       |        |

## 9 Appendix

| <u>QuantiNova and AriaMx</u>             |                   |            |              |                  | <u>HiDi polymerase and AriaMx</u>             |                   |            |              |                  |
|--|-------------------|------------|--------------|------------------|---|-------------------|------------|--------------|------------------|
| <b>VE_01, C. coli</b>                    |                   |            |              |                  | <b>VE_01, C. coli</b>                         |                   |            |              |                  |
| <u>Target</u>                            | <u>Efficiency</u> | <u>RSq</u> | <u>Slope</u> | <u>Intercept</u> | <u>Target</u>                                 | <u>Efficiency</u> | <u>RSq</u> | <u>Slope</u> | <u>Intercept</u> |
| <i>tet</i> (O)                           | 103.06            | 0.997      | -3.25        | 35.8             | <i>tet</i> (O)                                | 87.33             | 0.988      | -3.67        | 38.0             |
| GyrA_T86I                                | 98.40             | 0.994      | -3.36        | 36.1             | GyrA_T86I                                     | 95.00             | 0.982      | -3.45        | 37.3             |
| <i>erm</i> (B)                           | 109.70            | 0.991      | -3.11        | 36.7             | <i>erm</i> (B)                                | 104.27            | 0.987      | -3.22        | 36.6             |
| <b>VE-14 C. jejuni</b>                   |                   |            |              |                  | <b>VE-14 C. jejuni</b>                        |                   |            |              |                  |
| <u>Target</u>                            | <u>Efficiency</u> | <u>RSq</u> | <u>Slope</u> | <u>Intercept</u> | <u>Target</u>                                 | <u>Efficiency</u> | <u>RSq</u> | <u>Slope</u> | <u>Intercept</u> |
| <i>tet</i> (O)                           | 105.30            | 0.999      | -3.20        | 34.5             | <i>tet</i> (O)                                | 89.72             | 0.993      | -3.60        | 36.2             |
| GyrA_T86I                                | 91.22             | 0.997      | -3.55        | 36.9             | <i>gyrA</i> _T86I                             | 81.29             | 0.986      | -3.87        | 37.3             |
| 23S rRNA_A2075G                          | 97.95             | 0.999      | -3.37        | 35.2             | 23S rRNA_A2075G                               | 93.56             | 0.991      | -3.49        | 37.0             |
| <u>QuantiNova and Quantstudio5</u>       |                   |            |              |                  | <u>HiDi polymerase and Quantstudio5</u>       |                   |            |              |                  |
| <b>VE_01, C. coli</b>                    |                   |            |              |                  | <b>VE_01, C. coli</b>                         |                   |            |              |                  |
| <u>Target</u>                            | <u>Efficiency</u> | <u>RSq</u> | <u>Slope</u> | <u>Intercept</u> | <u>Target</u>                                 | <u>Efficiency</u> | <u>RSq</u> | <u>Slope</u> | <u>Intercept</u> |
| <i>tet</i> (O)                           | 96.77             | 0.998      | -3.40        | 35.2             | <i>tet</i> (O)                                | 91.40             | 0.992      | -3.55        | 35.4             |
| GyrA_T86I                                | 93.66             | 0.996      | -3.48        | 36.4             | GyrA_T86I                                     | 88.35             | 0.996      | -3.64        | 37.4             |
| <i>erm</i> (B)                           | 99.66             | 0.998      | -3.33        | 36.8             | <i>erm</i> (B)                                | 89.70             | 0.997      | -3.60        | 35.7             |
| <b>VE-14 C. jejuni</b>                   |                   |            |              |                  | <b>VE-14 C. jejuni</b>                        |                   |            |              |                  |
| <u>Target</u>                            | <u>Efficiency</u> | <u>RSq</u> | <u>Slope</u> | <u>Intercept</u> | <u>Target</u>                                 | <u>Efficiency</u> | <u>RSq</u> | <u>Slope</u> | <u>Intercept</u> |
| <i>tet</i> (O)                           | 105.11            | 0.994      | -3.21        | 33.7             | <i>tet</i> (O)                                | 93.24             | 0.988      | -3.50        | 33.8             |
| GyrA_T86I                                | 93.76             | 0.997      | -3.48        | 36.5             | GyrA_T86I                                     | 81.10             | 0.996      | -3.88        | 37.8             |
| 23S rRNA_A2075G                          | 99.20             | 0.997      | -3.34        | 35.1             | 23S rRNA_A2075G                               | 92.42             | 0.995      | -3.52        | 34.0             |
| <u>QuantiNova and CFX96 Touch System</u> |                   |            |              |                  | <u>HiDi polymerase and CFX96 Touch System</u> |                   |            |              |                  |
| <b>VE_01, C. coli</b>                    |                   |            |              |                  | <b>VE_01, C. coli</b>                         |                   |            |              |                  |
| <u>Target</u>                            | <u>Efficiency</u> | <u>RSq</u> | <u>Slope</u> | <u>Intercept</u> | <u>Target</u>                                 | <u>Efficiency</u> | <u>RSq</u> | <u>Slope</u> | <u>Intercept</u> |
| <i>tet</i> (O)                           | 95.14             | 0.994      | -3.44        | 37.3             | <i>tet</i> (O)                                | 86.00             | 0.988      | -3.71        | 36.3             |
| GyrA_T86I                                | 94.62             | 0.994      | -3.46        | 37.7             | GyrA_T86I                                     | 89.43             | 0.992      | -3.6         | 36.2             |
| <i>erm</i> (B)                           | 101.32            | 0.994      | -3.29        | 37.5             | <i>erm</i> (B)                                | 93.42             | 0.995      | -3.49        | 36.2             |
| <b>VE-14 C. jejuni</b>                   |                   |            |              |                  | <b>VE-14 C. jejuni</b>                        |                   |            |              |                  |
| <u>Target</u>                            | <u>Efficiency</u> | <u>RSq</u> | <u>Slope</u> | <u>Intercept</u> | <u>Target</u>                                 | <u>Efficiency</u> | <u>RSq</u> | <u>Slope</u> | <u>Intercept</u> |
| <i>tet</i> (O)                           | 100.07            | 0.997      | -3.32        | 36.1             | <i>tet</i> (O)                                | 87.15             | 0.987      | -3.67        | 34.8             |
| GyrA_T86I                                | 89.78             | 0.994      | -3.59        | 37.2             | GyrA_T86I                                     | 82.98             | 0.982      | -3.81        | 38.7             |
| 23S rRNA_A2075G                          | 94.93             | 0.996      | -3.45        | 36.9             | 23S rRNA_A2075G                               | 94.03             | 0.998      | -3.47        | 34.5             |

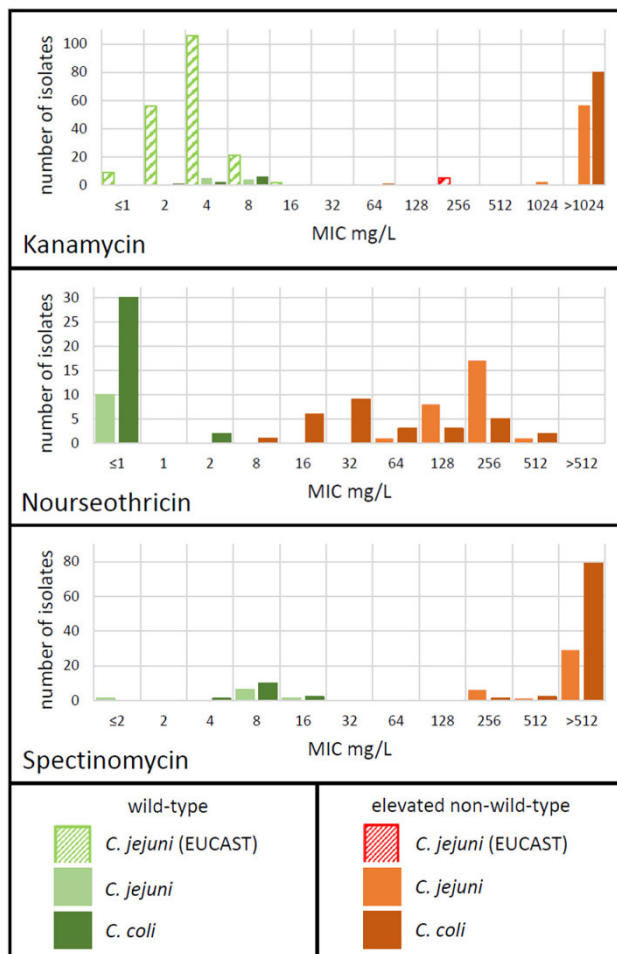


**9.2.3 Publication 3: Identification of knowledge gaps in whole-genome sequence analysis of multi-resistant thermotolerant *Campylobacter* spp.**

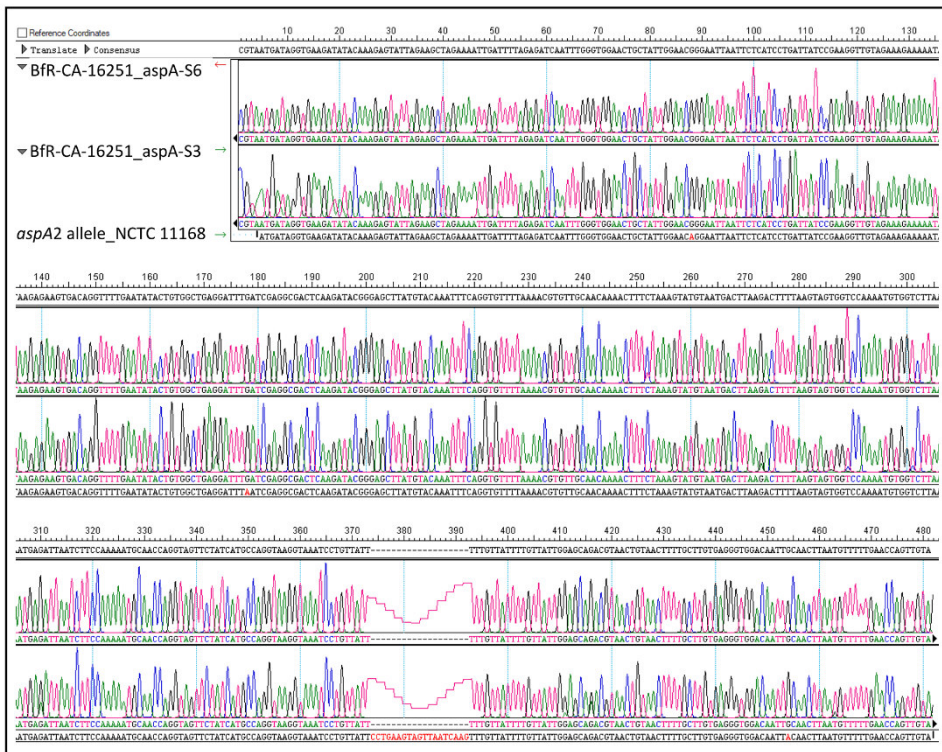
**Supplementary Figures**

Identification of knowledge gaps in whole-genome sequence analysis of multi-resistant thermotolerant *Campylobacter* spp.

Michael Zarske, Huong Quynh Luu, Carlus Deneke, Marie-Theres Knüver, Maja Thieck, Ha Thi Thu Hoang, Nancy Bretschneider, Ngoc Thi Pham, Ingrid Huber, Kerstin Stingl

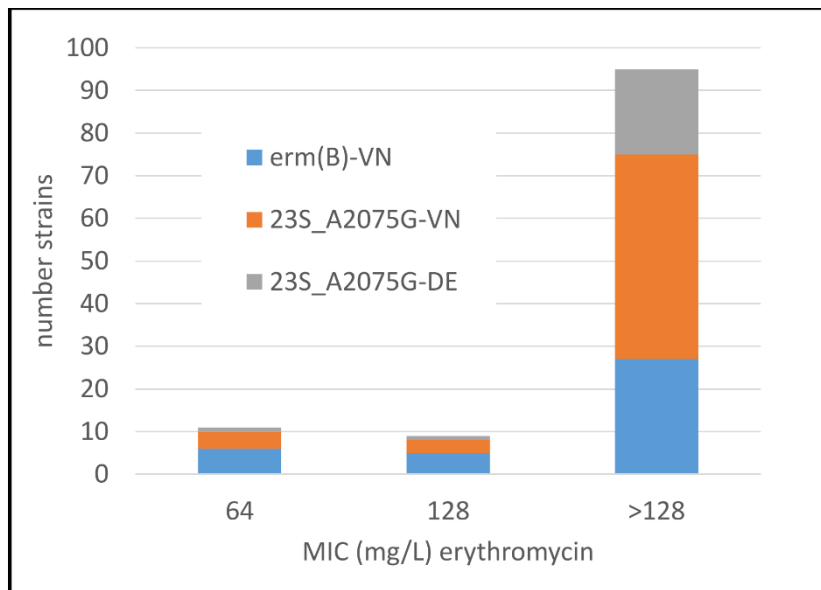


**Figure S1.** Distributions of MIC values of *Campylobacter* spp. isolates for antimicrobials lacking an epidemiological cutoff (ECOFF) value. Isolates were categorized into wild-type (green) and elevated non-wild-type (red) based on their MICs. Hatched bars, data from EUCAST (last accession on 11/16/2023); filled bars, results of this study.

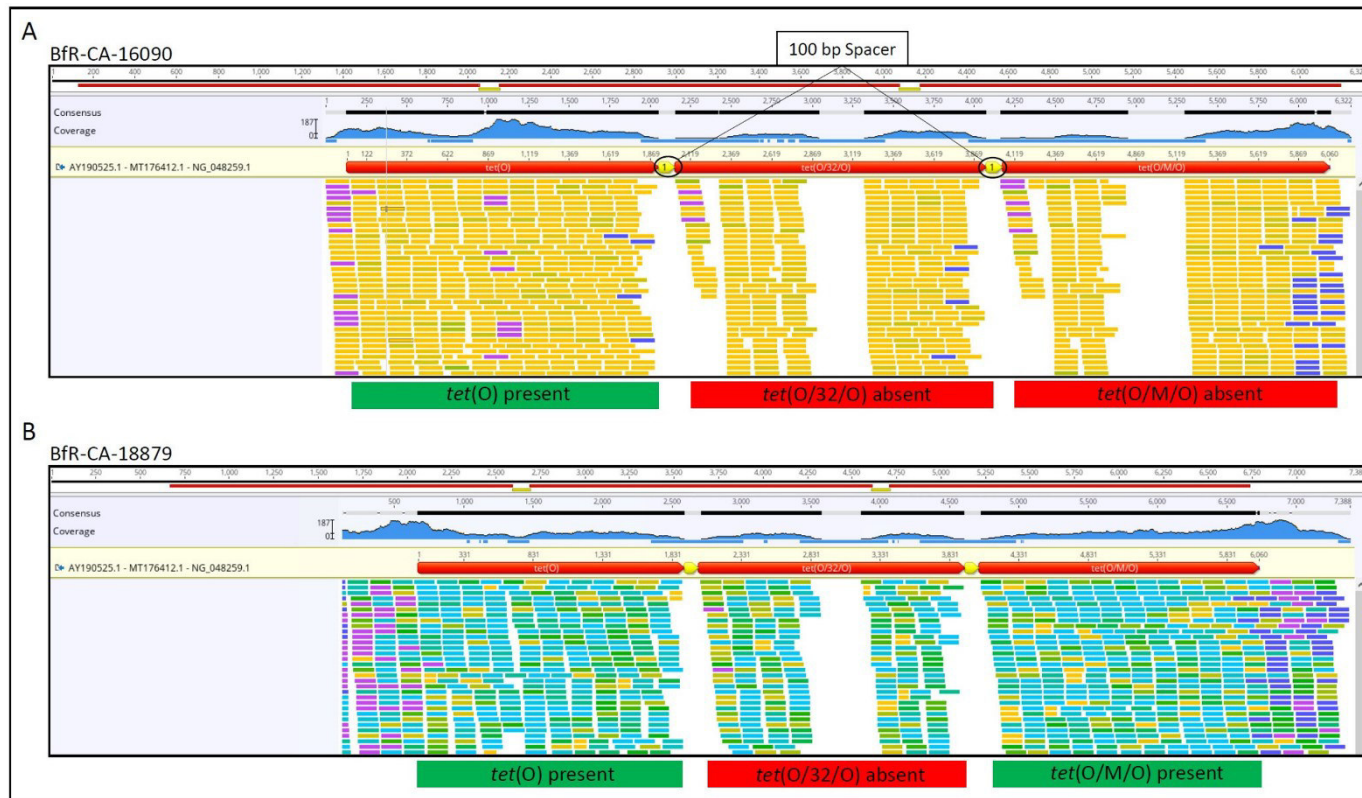


**Figure S2.** Sanger sequencing traces confirmed a deletion of 19 bp in *aspA* of BfR-CA-16251. A PCR fragment of *aspA*, amplified with primers *aspA*-A9/-A10 from BfR-CA-16251, was subjected to Sanger sequencing using primers *aspA*-S3 and *aspA*-S6. The obtained sequences were aligned to the MLST allele 2 of *aspA*, as present in *C. jejuni* NCTC 11168 (NC\_002163.1), utilizing Lasergene SeqMan Pro (LaserGene 17, DNASTAR Inc., Madison, WI, USA). The deletion of 19 bases (highlighted in red) within *aspA* in BfR-CA-16251, also observed upon assembly of short-read NGS data, was confirmed. The ruler annotates the base positions in the *aspA* fragment, defined as an allele for MLST at PubMLST (45).

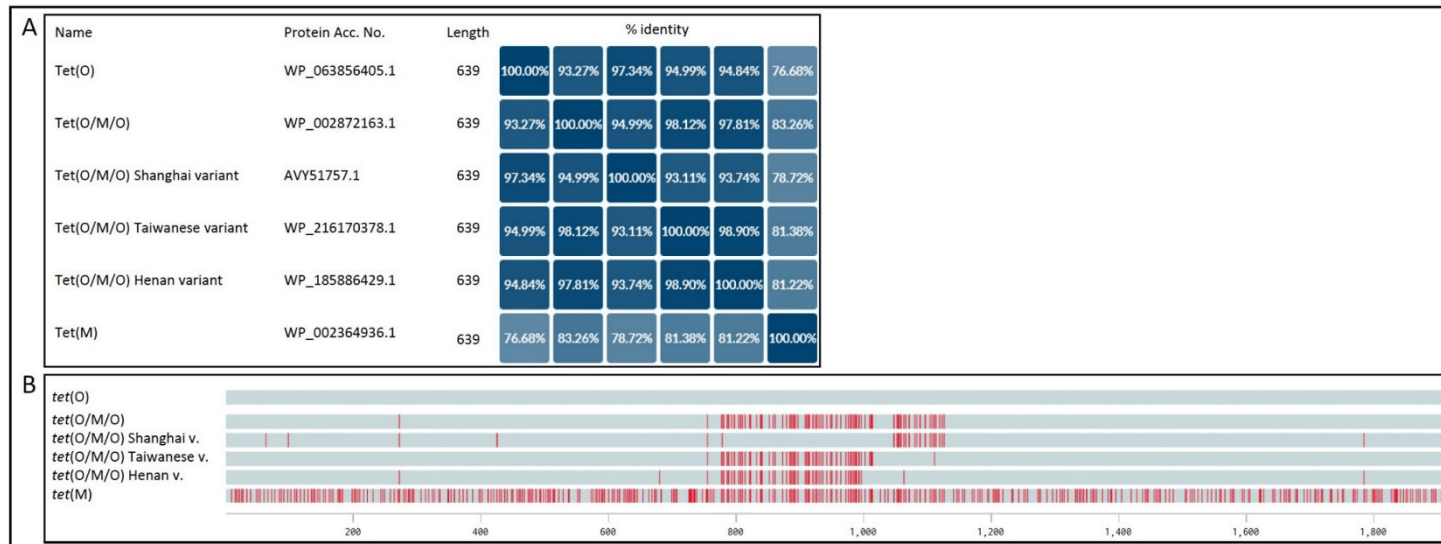




**Figure S3.** Minimum inhibitory concentration of erythromycin is similarly distributed among *erm(B)* and 23S\_A2075G carrying *Campylobacter* isolates. The obtained results demonstrate that MIC values of erythromycin cannot differentiate between the presence of *erm(B)* and 23S\_A2075G.



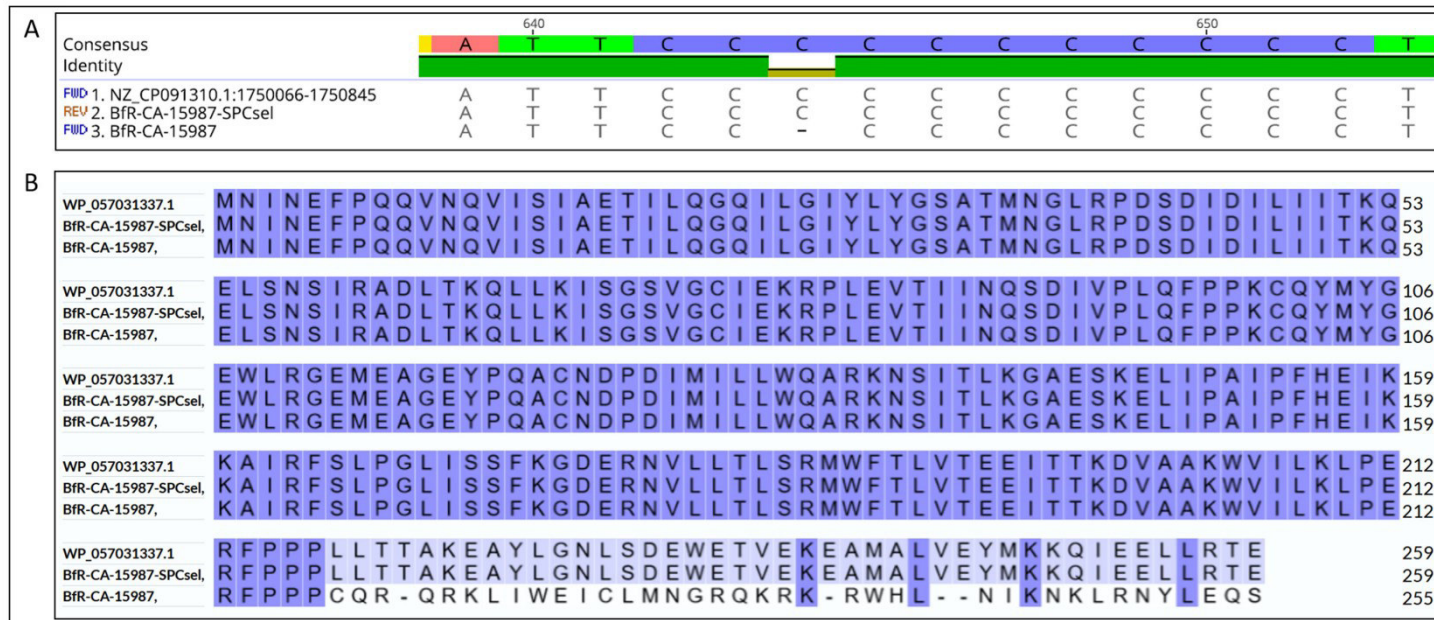
**Figure S4.** Identification of multiple copies of *tet(O)* variant genes using Geneious Prime. An artificial template of three reference genes, *tet(O)* (Acc. AY190525.1), *tet(O/32/O)* (Acc. MT176412.1) and *tet(O/M/O)* (Acc. NG\_048259.1), separated by 100 bp spacers (highlighted in yellow) was created for mapping of trimmed raw reads from isolates showing tetracycline resistance but displaying absence or only partial *tet(O)* genes according to AMRFinderPlus. Two examples of strains carrying multiple *tet(O)* variants are shown. **A.** Read mapping of BfR-CA-16090 to the template indicated full-length presence of *tet(O)*. **B.** Mapping reads of BfR-CA-18879 revealed presence of both *tet(O)* and *tet(O/M/O)* genes.



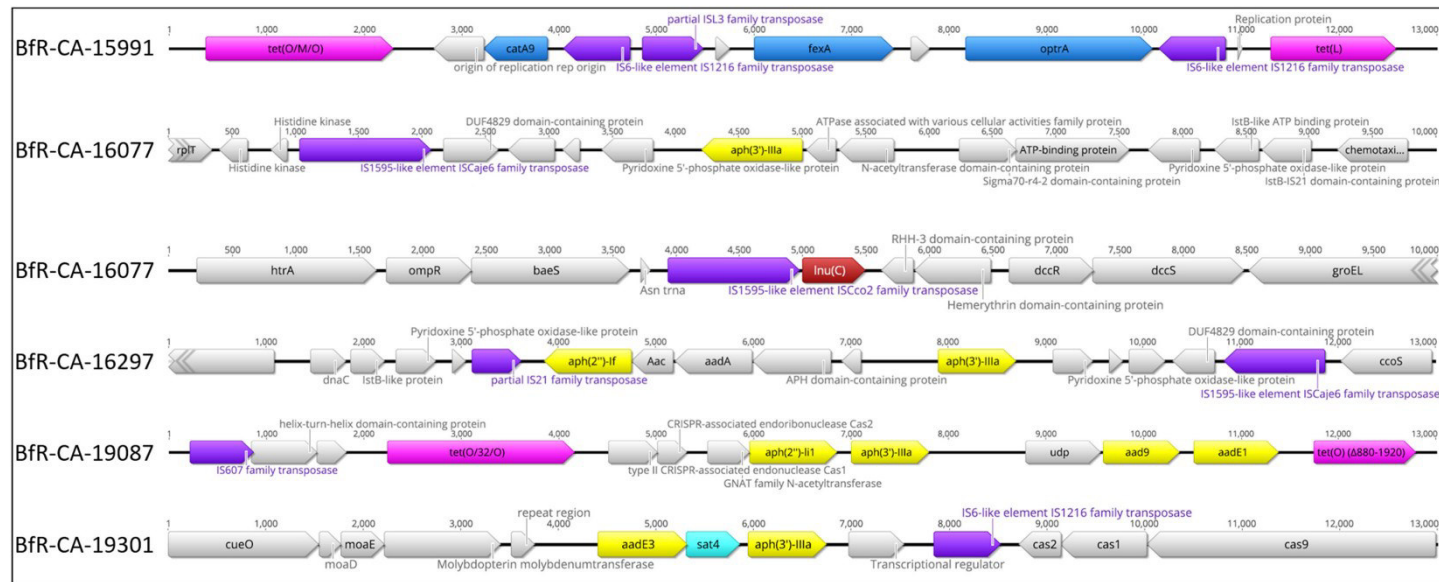
**Figure S5.** Visualization of different *tet(O/M/O)* variants found in Vietnamese *Campylobacter* spp.. **A** Percent of amino acid identity shared among the reference sequences. NCBI Protein Acc. No. and respective lengths are illustrated. Percent identity matrix generated with UniProt Align tool (56). **B** Nucleotide Alignment highlighting the areas of *tet(M)* introgression into *tet(O)* among the different *tet(O/M/O)* variants found in Vietnamese isolates. NCBI Nucleotide Accession of genes shown are presented in Table S3. Alignment generated with Benchling's DNA sequence alignment tool (110).

|                |   |     |
|----------------|---|-----|
| WP_000691721.1 | MKIIINIGILAHVDAGKTTLTESLLYASGAISEPGSVEKGTTRTDTMFLERQRGITIQAAVTSFQWH         | 66  |
| BfR-CA-15267   | MKIIINIGILAHVDAGKTTLTESLLYASGAISEPGSVEKGTTRTDTMFLERQRGITIQAAVTSFQWH         | 66  |
| BfR-CA-16942   | MKIIINIGILAHVDAGKTTLTESLLYASGAISEPGSVEKGTTRTDTMFLERQRGITIQAAVTSFQWH         | 66  |
| BfR-CA-18353   | MKIIINIGILAHVDAGKTTLTESLLYASGAISEPGSVEKGTTRTDTMFLERQRGITIQAAVTSFQWH         | 66  |
| WP_000691721.1 | RCKVNIVDTPGHMDFLAEVYRSLAVLDGAILVISA KDG VQAQTRILFHALRKMNIPTVIFINKIDQ        | 132 |
| BfR-CA-15267   | RCKVNIVDTPGHMDFLAEVYRSLAVLDGAILVISA KDG VQAQTRILFHALRKMNIPTVIFINKIDQ        | 132 |
| BfR-CA-16942   | RCKVNIVDTPGHMDFLAEVYRSLAVLDGAILVISA KDG VQAQTRILFHALRKMNIPTVIFINKIDQ        | 132 |
| BfR-CA-18353   | RCKVNIVDTPGHMDFLAEVYRSLAVLDGAILVISA KDG VQAQTRILFHALRKMNIPTVIFINKIDQ        | 132 |
| WP_000691721.1 | AGVDLQSVVQSVRDKLSADIIIKQTVSLSPEIVLEENTDIEAWDAVIENNDKLLKEYIAGEPISRE          | 198 |
| BfR-CA-15267   | AGVDLQSVVQSVRDKLSADIIIKQTVSLSPEIVLEENTDIEAWDAVIENNDKLLKEYIAGEPISRE          | 198 |
| BfR-CA-16942   | AGVDLQSVVQSVRDKLSADIIIKQTVSLSPEIVLEENTNIEAWDAVIENNDKLLKEYIAGEPISRE          | 198 |
| BfR-CA-18353   | AGVDLQSVVQSVRDKLSADIIIKQTVSLSPEIVLEENTNIEAWDAVIENNDKLLKEYIAGEPISRE          | 198 |
| WP_000691721.1 | KLVREEEQRRVQDASLFPVYYGSAKKGLGIQPLMDAVTGLFQPIGEQGSAAALCGSVFKVEYTD CGQR       | 264 |
| BfR-CA-15267   | KLVREEEQRRVQDASLFPVYYGSAKKGLGIQPLMDAVTGLFQPIGEQGSAAALCGSVFKVEYTD CGQR       | 264 |
| BfR-CA-16942   | KLVREEEQRRVQDASLFPVYYGSAKKGLGIQPLMDAVTGLFQPIGEQGSAAALCGSVFKVEYTD CGQR       | 264 |
| BfR-CA-18353   | KLVREEEQRRVQDASLFPVYYGSAKKGLGIQPLMDAVTGLFQPIGEQGSAAALCGSVFKVEYTD CGQR       | 264 |
| WP_000691721.1 | RVYLRRLYSGLT LRLRDTVALAGREK LKITEMRIPSKGEIVRTDTAYPGEIVILP SDSVRLNDV L G D P | 330 |
| BfR-CA-15267   | RVYLRRLYSGLT LRLRDTVALAGREK LKITEMRIPSKGEIVRTDTAYPGEIVILP SDSVRLNDV L G D P | 330 |
| BfR-CA-16942   | RVYLRRLYSGLT LRLRDTVALAGREK LKITEMRIPSKGEIVRTDTAYPGEIVILP SDSVRLNDV L G D P | 330 |
| BfR-CA-18353   | RVYLRRLYSGLT LRLRDTVALAGREK LKITEMRIPSKGEIVRTDTAYPGEIVILP SDSVRLNDV L G D P | 330 |
| WP_000691721.1 | TRLPRKRWR EDPLPMLR TS IAPK TAAQRERLLDAL TQLADTD PLLRCEVDSITHEIILSFLGRVQL    | 396 |
| BfR-CA-15267   | TRLPRKRWR EDPLPMLR TS IAPK TAAQRERLLDAL TQLADTD PLLRCEVDSITHEIILSFLGRVQL    | 396 |
| BfR-CA-16942   | TRLPRKRWR EDPLPMLR TS IAPK TAAQRERLLDAL TQLADTD PLLRCEVDSITHEIILSFLGRVQL    | 396 |
| BfR-CA-18353   | TRLPRKRWR EDPLPMLR TS IAPK TAAQRERLLDAL TQLADTD PLLRCEVDSITHEIILSFLGRVQL    | 396 |
| WP_000691721.1 | EVV SALLSEKYKLETVVKEPTVIYMERPLKAASHTIHIEVPPNPFWASIGLSVTPLPLGSGVQYES         | 462 |
| BfR-CA-15267   | EVV SALLSEKYKLETVVKEPTVIYMERPLKAASHTIHIEVPPNPFWASIGLSVTPLPLGSGVQYES         | 462 |
| BfR-CA-16942   | EVV SALLSEKYKLETVVKEPTVIYMERPLKAASHTIHIEVPPNPFWASIGLSVTPLPLGSGVQYES         | 462 |
| BfR-CA-18353   | EVV SALLSEKYKLETVVKEPTVIYMERPLKAASHTIHIEVPPNPFWASIGLSVTPLPLGSGVQYES         | 462 |
| WP_000691721.1 | RVSLG YLNQSFQNAVRDGI RYGLEQGLFGWNVTDCKICFEYGLYYS PVSTPADFRSLAPIVLEQAL       | 528 |
| BfR-CA-15267   | RVSLG YLNQSFQNAVRDGI RYGLEQGLFGWNVTDCKICFEYGLYYS PVSTPADFRSLAPIVLEQAL       | 528 |
| BfR-CA-16942   | RVSLG YLNQSFQNAVRDGI RYGLEQGLFGWNVTDCKICFEYGLYYS PVSTPADFRSLAPIVLEQAL       | 528 |
| BfR-CA-18353   | RVSLG YLNQSFQNAVRDGI RYGLEQGLFGWNVTDCKICFEYGLYYS PVSTPADFRSLAPIVLEQAL       | 528 |
| WP_000691721.1 | KESGTQLLEPYLSFTLYAPREYLSRAYHDAPKYCATIETVQVKKDEVVFTGEIPARCIQAYRTDLA          | 594 |
| BfR-CA-15267   | KESGTQLLEPYLSFTLYAPREYLSRAYHDAPKYCATIETVQVKKDEVVFTGEIPARCIQAYRTDLA          | 594 |
| BfR-CA-16942   | KESGTQLLEPYLSFTLYAPREYLSRAYHDAPKYCATIETVQVKKDEVVFTDEIPARCIQAYRTDLA          | 594 |
| BfR-CA-18353   | KESGTQLLEPYLSFTLYAPREYLSRAYHDAPKYCATIETVQVKKDEVVFTDEIPARCIQAYRTDLA          | 594 |
| WP_000691721.1 | FYTNGQSVCLTELKGYQAAV G K P V I Q P R R P N S R L D K V R Y M F Q K I M      | 639 |
| BfR-CA-15267   | FYTNGQSVCLTELKGYQAAV G K P V I Q P R R P N S R L D K V R Y M F Q K I M      | 639 |
| BfR-CA-16942   | FYTNGQSVCLTELKGYQAAV G K P V I Q P R R P N S R L D K V R Y M F Q K I M      | 639 |
| BfR-CA-18353   | FYTNGQSVCLTELKGYQAAV G K P V I Q P R R P N S R L D K V R Y M F Q K I M      | 639 |

**Figure S6.** Protein Alignment of reference Tet(W) (WP\_000691721.1) and translated gene assemblies of the three isolates harboring *tet(W)*. The tetracycline sensitive isolates, BfR-CA-16942 and BfR-CA-18353, showed two amino acid substitutions (D171N and G579D) in Tet(W), while Tet(W) of the tetracycline resistant BfR-CA-15267 was 100 % identical to the reference protein. Protein alignment was created with Uniprot Align tool (56).



**Figure S7.** *aad9* is a phase-variable gene frequently inactivated by frame-shifting. **A**, Gene alignment of the poly-C tract in reference gene *aad9* (NZ\_CP091310.1) with the corresponding region in BfR-CA-15987 and the spectinomycin re-selected BfR-CA-15987-SPCsel; **B**, Protein alignment of reference Aad9 (WP\_057031337.1) with translated *aad9* gene assemblies obtained from BfR-CA-15987 before and after re-selection (BfR-CA-15987-SPCsel) on spectinomycin. Re-selection induced insertion of 1 cytosine into the poly-C tract (leading to 11 cytosines) (**A**), resulting in the restoration of full-length protein (**B**). Nucleotide and protein alignments were created with Geneious Prime software (**A**) and the Uniprot Align tool (56) (**B**), respectively.



**Figure S8.** AMR genes in proximity to transposase genes not depicted in Figure 5 but part of Table 4. Transposase genes are marked in purple. AMR genes from different antimicrobial classes are depicted in different colors; blue, phenicol resistance genes (*catA*, *fexA*, *optrA*), red, *Inu(C)* genes; light purple, *tet* genes; yellow, aminoglycoside resistance genes; Grey arrows, non-AMR related genes.

## Supplementary Tables

**Identification of knowledge gaps in whole-genome sequence analysis of multi-resistant thermotolerant *Campylobacter* spp.**

Michael Zarske, Huong Quynh Luu, Carlus Deneke, Marie-Theres Knüver, Maja Thieck, Ha Thi Thu Hoang, Nancy Bretschneider, Ngoc Thi Pham, Ingrid Huber, Kerstin Sting

## Legends:

|                        |  |
|------------------------|--|
| <b>Table S1</b>        | <b>Sample overview, including geno- and phenotype of AMR and WGS data overview;</b> All samples were sequenced by Illumina short-read technology |
| blue shading           | Samples additionally sequenced by Oxford Nanopore Technology   |
| green shading          | phenotypical sensitive   |
| red shading            | phenotypical resistant   |
| red                    | Genes not found  |
| <del>crossed-out</del> | Genes that were falsely annotated  |
| <b>bold</b>            | Genes only partially found, but manually confirmed as full-length  |
| <b>bold + blue</b>     | aad9 falsely annotated as truncated due to frame-shifting in the poly-C tract  |
| tet(O) <sub>x</sub>    | Additional full-length or partial allelic variants of tet(O) genes identified through read mapping using Geneious Prime Software                 |
| yellow shading         | new allele variants and ST-Types   |

|                 |  |
|-----------------|--|
| <b>Table S2</b> | <b>Proof of principle comparison of AMR gene detection based on SKESA or Shovill genome assemblies</b> |
|                 | The coverage of the gene is expressed as a percentage of the full-length translated protein sequence   |
| red             | Genes not found  |
| <b>bold</b>     | Genes only partially found, but manually confirmed as full-length                                      |
| red shading     | differences in AMR detection based on SKESA vs. Shovill  |

|                 |  |
|-----------------|--|
| <b>Table S3</b> | <b>Reference sequences, showing the closest related NCBI RefSeq entries for the antimicrobial resistance genes found within this study</b> |
|-----------------|--|

|                 |   |
|-----------------|---|
| <b>Table S4</b> | <b>Statistical analysis of phenotypic resistance among <i>C. jejuni</i> and <i>C. coli</i> in Vietnam and Germany</b> |
|                 | Odds ratio (OR) with 95 % confidence interval (CI) were calculated  |
| <b>bold</b>     | Odds ratios with p-values of less than 0.05   |

|                 |  |
|-----------------|--|
| <b>Table S5</b> | <b>Ridom SeqSphere+ distance matrix, highlighting the phylogenetic diversity based on cgMLST</b> |
| green shading   | isolate pairs with ≤ 10 allele distance  |
| orange shading  | isolate pairs with 11-100 allele distance  |
| no shading      | isolates with >100 allele distance   |

9 Appendix

| Sample overview   |               |              |                   |                  |       |                 |                      | phenotypic resistance determined by broth microdilution with EUCAMP2 and custom plate formats |     |      |       |        |     |      |       |           |     |     |     |     |                         |                    |
|---|---------------|--------------|-------------------|------------------|-------|-----------------|----------------------|---|-----|------|-------|--------|-----|------|-------|-----------|-----|-----|-----|-----|-------------------------|--------------------|
| Samples highlighted in blue were additionally sequenced with Oxford Nanopore Technology |               |              |                   |                  |       |                 |                      | green shading, sensitive; red shading, resistant  |     |      |       |        |     |      |       |           |     |     |     |     |                         |                    |
| BioProject No.  | BioSample No. | isolate No.  | Country of origin | isolation source | Alias | collection date | Species              | CIP   | NAL | ERY  | TET   | GEN    | STR | AMP  | KAN   | NTC (STC) | CHL | FLO | SPC | LCM | Res profile [EUCAMP2]   | Res type [EUCAMP2] |
| PRJNA872862   | SAMN34728690  | BFR-CA-11843 | Germany           | broiler, meat    |       | 04.12.2013      | Campylobacter coli   | >4  | 64  | 4    | 32    | 0,5    | 2   |      |       |           |     |     |     |     | CIP, NAL, TET           | 2-fold             |
| PRJNA872862   | SAMN34728691  | BFR-CA-11858 | Germany           | broiler, meat    |       | 14.01.2014      | Campylobacter coli   | 8   | 64  | 128  | 32    | 0,5    | 0,5 |      |       |           |     |     |     |     | CIP, ERY, NAL, TET      | 3-fold             |
| PRJNA872862   | SAMN34728692  | BFR-CA-11892 | Germany           | broiler, meat    |       | 14.01.2014      | Campylobacter coli   | 16  | 64  | <=1  | 32    | 0,5    | 0,5 |      |       |           |     |     |     |     | CIP, NAL, TET           | 2-fold             |
| PRJNA872862   | SAMN34728693  | BFR-CA-11893 | Germany           | broiler, cecum   |       | 03.02.2014      | Campylobacter coli   | 0,25  | 4   | 2    | <=0,5 | 0,5    | 1   | 8    | 8     | 2         | 4   |     |     |     | sensitive               | sensitive          |
| PRJNA872862   | SAMN34728694  | BFR-CA-11930 | Germany           | broiler, meat    |       | 04.03.2014      | Campylobacter coli   | <=0,12  | 8   | <=1  | >64   | 1      | 2   |      |       |           |     |     |     |     | TET                     | 1-fold             |
| PRJNA872862   | SAMN34728695  | BFR-CA-12208 | Germany           | broiler, meat    |       | 13.06.2014      | Campylobacter coli   | 16  | 64  | <=1  | 64    | 0,25   | 2   | 512  | >1024 | 256       |     |     |     |     | CIP, NAL, TET           | 2-fold             |
| PRJNA872862   | SAMN34728696  | BFR-CA-12658 | Germany           | broiler, meat    |       | 03.07.2014      | Campylobacter coli   | >16   | 32  | >128 | 32    | <=0,12 | 1   |      |       |           |     |     |     |     | CIP, ERY, NAL, TET      | 3-fold             |
| PRJNA872862   | SAMN34728697  | BFR-CA-12887 | Germany           | broiler, cecum   |       | 09.10.2014      | Campylobacter coli   | 16  | 64  | <=1  | <=0,5 | 0,5    | 1   |      |       |           |     |     |     |     | CIP, NAL                | 1-fold             |
| PRJNA872862   | SAMN34728698  | BFR-CA-12897 | Germany           | broiler, liver   |       | 16.10.2014      | Campylobacter coli   | 8   | 64  | <=1  | 64    | 0,5    | 2   |      |       |           |     |     |     |     | CIP, NAL, TET           | 2-fold             |
| PRJNA872862   | SAMN34728699  | BFR-CA-12906 | Germany           | broiler, liver   |       | 06.10.2014      | Campylobacter coli   | >16   | 64  | <=1  | >64   | 0,5    | 1   |      |       |           |     |     |     |     | CIP, NAL, TET           | 2-fold             |
| PRJNA872862   | SAMN34728700  | BFR-CA-12973 | Germany           | broiler, feces   |       | 20.10.2014      | Campylobacter coli   | <=0,12  | 4   | <=1  | 64    | 0,5    | 2   |      |       |           |     |     |     |     | TET                     | 1-fold             |
| PRJNA872862   | SAMN34728701  | BFR-CA-13310 | Germany           | broiler, meat    |       | 17.06.2015      | Campylobacter coli   | 16  | >64 | <=1  | <=0,5 | 0,5    | 2   |      |       |           |     |     |     |     | CIP, NAL                | 1-fold             |
| PRJNA872862   | SAMN34728702  | BFR-CA-13470 | Germany           | broiler, meat    |       | 16.09.2015      | Campylobacter coli   | 8   | 64  | 2    | >64   | 0,5    | 1   |      |       |           |     |     |     |     | CIP, NAL, TET           | 2-fold             |
| PRJNA872862   | SAMN34728703  | BFR-CA-13526 | Germany           | broiler, meat    |       | 16.09.2015      | Campylobacter coli   | 16  | 64  | >128 | >64   | 0,5    | >16 |      |       |           |     |     |     |     | CIP, ERY, NAL, STR, TET | 4-fold             |
| PRJNA872862   | SAMN34728704  | BFR-CA-13528 | Germany           | broiler, meat    |       | 24.09.2015      | Campylobacter coli   | >16   | 64  | <=1  | >64   | 0,5    | 1   |      |       |           |     |     |     |     | CIP, NAL, TET           | 2-fold             |
| PRJNA872862   | SAMN34728705  | BFR-CA-13537 | Germany           | broiler, heart   |       | 06.10.2015      | Campylobacter coli   | 16  | 64  | <=1  | >64   | 0,5    | 2   |      |       |           |     |     |     |     | CIP, NAL, TET           | 2-fold             |
| PRJNA595957   | SAMN13577889  | BFR-CA-13895 | Germany           | broiler, egg     |       | 24.02.2016      | Campylobacter coli   | 0,5   | 4   | 2    | <=0,5 | 0,25   | 1   |      |       |           |     |     |     |     | sensitive               | sensitive          |
| PRJNA648048   | SAMN15617887  | BFR-CA-13918 | Germany           | broiler, meat    |       | 17.02.2016      | Campylobacter jejuni | <=0,12  | 8   | <=1  | <=0,5 | 0,5    | 1   |      |       |           |     |     |     |     | sensitive               | sensitive          |
| PRJNA595957   | SAMN13577890  | BFR-CA-13919 | Germany           | turkey, cecum    |       | 07.03.2016      | Campylobacter coli   | >16   | 32  | <=1  | <=0,5 | 0,25   | 1   |      |       |           |     |     |     |     | CIP, NAL                | 1-fold             |
| PRJNA648048   | SAMN15617888  | BFR-CA-13937 | Germany           | broiler, meat    |       | 07.03.2016      | Campylobacter jejuni | >16   | >64 | 2    | <=0,5 | 0,5    | 1   |      |       |           |     |     |     |     | CIP, NAL                | 1-fold             |
| PRJNA872862   | SAMN34728706  | BFR-CA-13938 | Germany           | turkey, cecum    |       | 14.03.2016      | Campylobacter coli   | 8   | 32  | <=1  | <=0,5 | 0,5    | 1   |      | >1024 | <=1       |     |     | 8   |     | CIP, NAL                | 1-fold             |
| PRJNA648048   | SAMN15617889  | BFR-CA-13939 | Germany           | broiler, meat    |       | 14.03.2016      | Campylobacter jejuni | 16  | 32  | <=1  | 16    | 0,25   | 0,5 |      |       |           |     |     |     |     | CIP, NAL, TET           | 2-fold             |
| PRJNA595957   | SAMN13577891  | BFR-CA-13953 | Germany           | turkey, cecum    |       | 07.03.2016      | Campylobacter coli   | >16   | >64 | <=1  | >64   | 0,5    | 1   | >512 | >1024 | 32        |     |     |     |     | CIP, NAL, TET           | 2-fold             |
| PRJNA872862   | SAMN34728707  | BFR-CA-13971 | Germany           | turkey, cecum    |       | 21.03.2016      | Campylobacter coli   | 8   | 16  | >128 | 32    | 0,25   | 1   |      |       |           |     |     |     |     | CIP, ERY, TET           | 3-fold             |
| PRJNA872862   | SAMN34728708  | BFR-CA-13985 | Germany           | turkey, cecum    |       | 21.03.2016      | Campylobacter jejuni | 16  | 64  | <=1  | 64    | 0,5    | >16 | >512 | >1024 | 128       |     |     |     |     | CIP, NAL, STR, TET      | 3-fold             |
| PRJNA648048   | SAMN15617890  | BFR-CA-14088 | Germany           | broiler, meat    |       | 27.04.2016      | Campylobacter jejuni | 16  | 32  | <=1  | <=0,5 | 0,5    | 4   |      |       |           |     |     |     |     | CIP, NAL                | 1-fold             |
| PRJNA648048   | SAMN15617891  | BFR-CA-14109 | Germany           | broiler, meat    |       | 03.02.2016      | Campylobacter jejuni | 0,25  | 4   | <=1  | <=0,5 | 0,5    | 2   |      |       |           |     |     |     |     | sensitive               | sensitive          |
| PRJNA648048   | SAMN15617892  | BFR-CA-14180 | Germany           | broiler, meat    |       | 31.05.2016      | Campylobacter jejuni | 16  | 64  | <=1  | 32    | 0,5    | 1   |      |       |           |     |     |     |     | CIP, NAL, TET           | 2-fold             |
| PRJNA648048   | SAMN15617893  | BFR-CA-14181 | Germany           | broiler, meat    |       | 31.05.2016      | Campylobacter jejuni | 16  | 64  | <=1  | <=0,5 | 0,5    | 2   |      |       |           |     |     |     |     | CIP, NAL                | 1-fold             |
| PRJNA872862   | SAMN34728709  | BFR-CA-14216 | Germany           | turkey, cecum    |       | 07.06.2016      | Campylobacter coli   | >16   | 32  | 4    | 32    | 0,5    | 2   |      |       |           |     |     |     |     | CIP, NAL, TET           | 2-fold             |
| PRJNA595957   | SAMN13577892  | BFR-CA-14226 | Germany           | turkey, cecum    |       | 14.06.2016      | Campylobacter coli   | >16   | 32  | 2    | 64    | 1      | 8   |      |       |           |     |     |     |     | CIP, NAL, STR, TET      | 3-fold             |
| PRJNA872862   | SAMN34728710  | BFR-CA-14373 | Germany           | broiler, cecum   |       | 26.07.2016      | Campylobacter jejuni | 8   | >64 | <=1  | >64   | 0,5    | 0,5 |      |       |           |     |     |     |     | CIP, NAL, TET           | 2-fold             |
| PRJNA562653   | SAMN12647218  | BFR-CA-14430 | Germany           | broiler, meat    |       | 10.08.2016      | Campylobacter jejuni | >16   | >64 | <=1  | >64   | 0,5    | 1   |      |       |           |     |     |     |     | CIP, NAL, TET           | 2-fold             |
| PRJNA595957   | SAMN13577893  | BFR-CA-14582 | Germany           | broiler, meat    |       | 05.09.2016      | Campylobacter coli   | >16   | 64  | <=1  | <=0,5 | 0,5    | 2   |      |       |           |     |     |     |     | CIP, NAL                | 1-fold             |
| PRJNA872862   | SAMN34728711  | BFR-CA-14583 | Germany           | broiler, meat    |       | 14.09.2016      | Campylobacter coli   | 16  | 32  | <=1  | 32    | 0,25   | 0,5 |      |       |           |     |     |     |     | CIP, NAL, TET           | 2-fold             |
| PRJNA595957   | SAMN13577894  | BFR-CA-14610 | Germany           | turkey, cecum    |       | 25.04.2016      | Campylobacter coli   | 4   | 32  | <=1  | <=0,5 | 0,5    | 1   |      |       |           |     |     |     |     | CIP, NAL                | 1-fold             |
| PRJNA595957   | SAMN13577895  | BFR-CA-14731 | Germany           | turkey, meat     |       | 06.10.2016      | Campylobacter coli   | 0,5   | 4   | <=1  | <=0,5 | 0,5    | 1   |      |       |           |     |     |     |     | sensitive               | sensitive          |
| PRJNA872862   | SAMN34728712  | BFR-CA-14781 | Germany           | broiler, cecum   |       | 24.10.2016      | Campylobacter jejuni | 16  | 64  | <=1  | 64    | 0,5    | 1   |      |       |           |     |     |     |     | CIP, NAL, TET           | 2-fold             |
| PRJNA595957   | SAMN13577896  | BFR-CA-14810 | Germany           | broiler, egg     |       | 25.10.2016      | Campylobacter coli   | 0,25  | 4   | <=1  | <=0,5 | 0,5    | 16  |      | >1024 | 512       |     |     | 4   |     | STR                     | 1-fold             |
| PRJNA872862   | SAMN34728713  | BFR-CA-14815 | Germany           | broiler, cecum   |       | 24.10.2016      | Campylobacter coli   | 16  | 64  | <=1  | 64    | 1      | 2   |      |       |           |     |     |     |     | CIP, NAL, TET           | 2-fold             |
| PRJNA595957   | SAMN13577897  | BFR-CA-14825 | Germany           | turkey, cecum    |       | 07.11.2016      | Campylobacter coli   | 8   | 32  | <=1  | <=0,5 | <=0,12 | 0,5 |      |       |           |     |     |     |     | CIP, NAL                | 1-fold             |
| PRJNA595957   | SAMN13577898  | BFR-CA-14833 | Germany           | broiler, egg     |       | 08.11.2016      | Campylobacter coli   | 0,5   | 8   | 2    | <=0,5 | 0,25   | 1   |      |       |           |     |     |     |     | sensitive               | sensitive          |
| PRJNA872862   | SAMN34728714  | BFR-CA-14857 | Germany           | broiler, skin    |       | 07.11.2016      | Campylobacter jejuni | >16   | <=1 | <=1  | 64    | 0,5    | 1   | >512 | 8     | <=1       | <=2 | 2   | 8   | 4   | CIP, TET                | 2-fold             |
| PRJNA872862   | SAMN34728715  | BFR-CA-14872 | Germany           | broiler, cecum   |       | 31.10.2016      | Campylobacter jejuni | >16   | <=1 | <=1  | <=0,5 | 0,25   | 1   |      |       |           |     |     |     |     | CIP                     | 1-fold             |





9 Appendix

|             |              |              |         |                |       |            |                      |     |     |      |     |      |     |      |       |     |     |     |      |                              |                              |                              |        |
|-------------|--------------|--------------|---------|----------------|-------|------------|----------------------|-----|-----|------|-----|------|-----|------|-------|-----|-----|-----|------|------------------------------|------------------------------|------------------------------|--------|
| PRJNA872862 | SAMN34728755 | BFR-CA-16006 | Vietnam | broiler, feces | CG75  | 25.12.2016 | Campylobacter coli   | 16  | 64  | >128 | >64 | >16  | >16 | 512  | >1024 |     | 64  |     | >512 | CIP, ERY, GEN, NAL, STR, TET | 4-fold                       |                              |        |
| PRJNA872862 | SAMN34728756 | BFR-CA-16007 | Vietnam | broiler, feces | CG76  | 25.12.2016 | Campylobacter jejuni | >16 | >64 | <=1  | >64 | >16  | 1   | 512  | >1024 |     | 64  |     | >512 | CIP, GEN, NAL, TET           | 3-fold                       |                              |        |
| PRJNA872862 | SAMN34728757 | BFR-CA-16008 | Vietnam | broiler, feces | CG79  | 25.12.2016 | Campylobacter jejuni | >16 | >64 | <=1  | >64 | 0,5  | 1   |      |       |     |     |     |      | CIP, NAL, TET                | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34728758 | BFR-CA-16010 | Vietnam | broiler, feces | CG81  | 25.12.2016 | Campylobacter coli   | >16 | >64 | 4    | >64 | 0,5  | 16  |      |       | <=1 |     |     | >512 | CIP, NAL, STR, TET           | 3-fold                       |                              |        |
| PRJNA872862 | SAMN34728759 | BFR-CA-16011 | Vietnam | broiler, feces | CG82  | 25.12.2016 | Campylobacter jejuni | 0,5 | 16  | <=1  | >64 | 0,5  | 1   |      |       |     |     |     |      | TET                          | 1-fold                       |                              |        |
| PRJNA872862 | SAMN34728760 | BFR-CA-16012 | Vietnam | broiler, feces | CG83  | 25.12.2016 | Campylobacter jejuni | >16 | >64 | <=1  | >64 | 0,5  | 1   |      |       |     |     |     |      | CIP, NAL, TET                | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34728761 | BFR-CA-16013 | Vietnam | broiler, feces | CG84  | 25.12.2016 | Campylobacter jejuni | >16 | >64 | 2    | >64 | >16  | >16 |      |       |     | 128 |     | >512 | CIP, GEN, NAL, STR, TET      | 3-fold                       |                              |        |
| PRJNA872862 | SAMN34728762 | BFR-CA-16014 | Vietnam | broiler, feces | CG88  | 25.12.2016 | Campylobacter jejuni | >16 | >64 | <=1  | >64 | 0,5  | 1   |      |       |     |     |     |      | CIP, NAL, TET                | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34728763 | BFR-CA-16015 | Vietnam | broiler, feces | CG89  | 25.12.2016 | Campylobacter jejuni | >16 | >64 | <=1  | >64 | 0,25 | 1   |      |       |     |     |     |      | CIP, NAL, TET                | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34728764 | BFR-CA-16016 | Vietnam | broiler, feces | CG90  | 25.12.2016 | Campylobacter coli   | >16 | 64  | 128  | >64 | >16  | >16 | 512  | >1024 |     | 128 |     | >512 | CIP, ERY, GEN, NAL, STR, TET | 4-fold                       |                              |        |
| PRJNA872862 | SAMN34728765 | BFR-CA-16021 | Vietnam | broiler, feces | CG95  | 25.12.2016 | Campylobacter jejuni | >16 | >64 | <=1  | >64 | >16  | 1   | >512 | >1024 |     | 128 |     | >512 | CIP, GEN, NAL, TET           | 3-fold                       |                              |        |
| PRJNA872862 | SAMN34728766 | BFR-CA-16022 | Vietnam | broiler, feces | CG99  | 12.01.2017 | Campylobacter jejuni | 8   | 64  | <=1  | >64 | 0,25 | 1   |      |       |     |     |     |      | CIP, NAL, TET                | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34728767 | BFR-CA-16023 | Vietnam | broiler, feces | CG101 | 12.01.2017 | Campylobacter coli   | 16  | 64  | 4    | >64 | >16  | >16 | 256  | >1024 |     | 16  |     | >512 | CIP, GEN, NAL, STR, TET      | 3-fold                       |                              |        |
| PRJNA872862 | SAMN34728768 | BFR-CA-16024 | Vietnam | broiler, feces | CG102 | 12.01.2017 | Campylobacter jejuni | >16 | >64 | <=1  | >64 | 0,5  | 2   |      | >1024 |     | <=1 |     |      | CIP, NAL, TET                | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34728769 | BFR-CA-16026 | Vietnam | broiler, feces | CG107 | 12.01.2017 | Campylobacter jejuni | 8   | 64  | 2    | 64  | 0,5  | 1   |      |       |     |     |     |      | CIP, NAL, TET                | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34728770 | BFR-CA-16027 | Vietnam | broiler, feces | CG108 | 12.01.2017 | Campylobacter coli   | 16  | 64  | >128 | >64 | >16  | >16 | >512 | >1024 |     | 128 |     | >512 | CIP, ERY, GEN, NAL, STR, TET | 4-fold                       |                              |        |
| PRJNA872862 | SAMN34728771 | BFR-CA-16028 | Vietnam | broiler, feces | CG109 | 12.01.2017 | Campylobacter coli   | >16 | 64  | 2    | >64 | >16  | >16 |      | >1024 |     | 4   |     | >512 | CIP, GEN, NAL, STR, TET      | 3-fold                       |                              |        |
| PRJNA872862 | SAMN34728772 | BFR-CA-16029 | Vietnam | broiler, feces | CG111 | 12.01.2017 | Campylobacter jejuni | >16 | >64 | <=1  | >64 | >16  | 2   | 512  | >1024 |     | 128 |     | >512 | CIP, GEN, NAL, TET           | 3-fold                       |                              |        |
| PRJNA872862 | SAMN34728773 | BFR-CA-16030 | Vietnam | broiler, feces | CG112 | 12.01.2017 | Campylobacter jejuni | >16 | >64 | <=1  | >64 | >16  | 1   | >512 | >1024 |     | 64  |     | >512 | CIP, GEN, NAL, TET           | 3-fold                       |                              |        |
| PRJNA872862 | SAMN34728774 | BFR-CA-16031 | Vietnam | broiler, feces | CG113 | 12.01.2017 | Campylobacter jejuni | >16 | >64 | <=1  | >64 | 0,25 | 1   |      |       |     |     |     |      | CIP, NAL, TET                | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34728775 | BFR-CA-16032 | Vietnam | broiler, feces | CG114 | 12.01.2017 | Campylobacter jejuni | >16 | >64 | <=1  | >64 | 0,5  | 1   |      |       |     |     |     |      | CIP, NAL, TET                | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34728776 | BFR-CA-16033 | Vietnam | broiler, feces | CG115 | 12.01.2017 | Campylobacter jejuni | >16 | >64 | <=1  | >64 | >16  | 2   |      |       |     | 128 |     | >512 | CIP, GEN, NAL, TET           | 3-fold                       |                              |        |
| PRJNA872862 | SAMN34728777 | BFR-CA-16034 | Vietnam | broiler, feces | CG116 | 12.01.2017 | Campylobacter jejuni | >16 | >64 | <=1  | >64 | >16  | 1   |      |       |     | 128 |     | >512 | CIP, GEN, NAL, TET           | 3-fold                       |                              |        |
| PRJNA872862 | SAMN34728778 | BFR-CA-16035 | Vietnam | broiler, feces | CG117 | 12.01.2017 | Campylobacter jejuni | >16 | >64 | <=1  | >64 | 0,5  | 1   |      |       |     |     |     |      | CIP, NAL, TET                | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34728779 | BFR-CA-16036 | Vietnam | broiler, feces | CG119 | 12.01.2017 | Campylobacter coli   | >16 | 64  | >128 | >64 | >16  | >16 | 32   | >1024 |     | <=1 | 4   | 2    | 8                            | >128                         | CIP, ERY, GEN, NAL, STR, TET | 4-fold |
| PRJNA872862 | SAMN34728780 | BFR-CA-16039 | Vietnam | broiler, feces | CG122 | 13.02.2017 | Campylobacter jejuni | 16  | >64 | <=1  | >64 | 0,5  | 2   |      |       |     |     |     |      | CIP, NAL, TET                | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34728781 | BFR-CA-16040 | Vietnam | broiler, feces | CG123 | 13.02.2017 | Campylobacter coli   | >16 | >64 | 128  | >64 | >16  | >16 |      |       |     | 64  |     | >512 | >128                         | CIP, ERY, GEN, NAL, STR, TET | 4-fold                       |        |
| PRJNA872862 | SAMN34728782 | BFR-CA-16041 | Vietnam | broiler, feces | CG124 | 13.02.2017 | Campylobacter jejuni | >16 | >64 | <=1  | 1   | 0,5  | 2   | 256  | 8     |     | <=1 | <=2 | 8    | 8                            | 16                           | CIP, NAL                     | 1-fold |
| PRJNA872862 | SAMN34728783 | BFR-CA-16042 | Vietnam | broiler, feces | CG125 | 13.02.2017 | Campylobacter jejuni | 8   | >64 | <=1  | >64 | 0,5  | 2   |      |       |     |     |     |      |                              | CIP, NAL, TET                | 2-fold                       |        |
| PRJNA872862 | SAMN34728784 | BFR-CA-16043 | Vietnam | broiler, feces | CG126 | 13.02.2017 | Campylobacter jejuni | >16 | >64 | <=1  | >64 | 0,25 | 1   |      |       |     |     |     |      |                              | CIP, NAL, TET                | 2-fold                       |        |
| PRJNA872862 | SAMN34728785 | BFR-CA-16044 | Vietnam | broiler, feces | CG127 | 13.02.2017 | Campylobacter jejuni | >16 | 64  | >128 | >64 | >16  | 1   | 256  | >1024 |     | 8   |     | <=2  | >128                         | CIP, ERY, GEN, NAL, TET      | 4-fold                       |        |
| PRJNA872862 | SAMN34728786 | BFR-CA-16045 | Vietnam | broiler, feces | CG128 | 13.02.2017 | Campylobacter jejuni | >16 | >64 | <=1  | >64 | 0,25 | >16 |      |       |     |     |     |      |                              | CIP, NAL, STR, TET           | 3-fold                       |        |
| PRJNA872862 | SAMN34728787 | BFR-CA-16046 | Vietnam | broiler, feces | CG129 | 13.02.2017 | Campylobacter coli   | 16  | >64 | >128 | 32  | >16  | >16 | >512 | >1024 |     | <=1 | 64  | 4    | >512                         | >128                         | CIP, ERY, GEN, NAL, STR, TET | 4-fold |
| PRJNA872862 | SAMN34728788 | BFR-CA-16048 | Vietnam | broiler, feces | CG131 | 13.02.2017 | Campylobacter coli   | 16  | 64  | <=1  | 64  | >16  | >16 |      |       |     |     |     |      |                              | CIP, GEN, NAL, STR, TET      | 3-fold                       |        |
| PRJNA872862 | SAMN34728789 | BFR-CA-16052 | Vietnam | broiler, feces | CG137 | 13.02.2017 | Campylobacter coli   | >16 | 64  | >128 | 64  | >16  | >16 | >512 | >1024 |     | 128 |     | >512 |                              | CIP, ERY, GEN, NAL, STR, TET | 4-fold                       |        |
| PRJNA872862 | SAMN34728790 | BFR-CA-16053 | Vietnam | broiler, feces | CG141 | 13.02.2017 | Campylobacter coli   | 16  | 64  | >128 | 64  | >16  | >16 |      |       |     | 128 |     | >512 |                              | CIP, ERY, GEN, NAL, STR, TET | 4-fold                       |        |
| PRJNA872862 | SAMN34728791 | BFR-CA-16056 | Vietnam | broiler, feces | CG145 | 10.03.2017 | Campylobacter jejuni | >16 | >64 | 2    | >64 | 0,5  | 1   | >512 | >1024 |     | <=2 |     |      |                              | CIP, NAL, TET                | 2-fold                       |        |
| PRJNA872862 | SAMN34728792 | BFR-CA-16057 | Vietnam | broiler, feces | CG147 | 10.03.2017 | Campylobacter coli   | >16 | 64  | >128 | 64  | >16  | >16 | >512 | >1024 |     | <=1 | 128 | >16  | >512                         | >128                         | CIP, ERY, GEN, NAL, STR, TET | 4-fold |
| PRJNA872862 | SAMN34728793 | BFR-CA-16058 | Vietnam | broiler, feces | CG148 | 10.03.2017 | Campylobacter jejuni | >16 | >64 | <=1  | >64 | 0,5  | 2   |      |       |     |     |     |      |                              | CIP, NAL, TET                | 2-fold                       |        |
| PRJNA872862 | SAMN34728794 | BFR-CA-16059 | Vietnam | broiler, feces | CG149 | 10.03.2017 | Campylobacter coli   | >16 | 64  | >128 | >64 | >16  | >16 | >512 | >1024 |     | <=1 | 128 | >16  | >512                         | >128                         | CIP, ERY, GEN, NAL, STR, TET | 4-fold |
| PRJNA872862 | SAMN34728795 | BFR-CA-16060 | Vietnam | broiler, feces | CG150 | 10.03.2017 | Campylobacter coli   | 16  | 64  | >128 | >64 | >16  | >16 |      |       |     | 128 | >16 | >512 |                              | CIP, ERY, GEN, NAL, STR, TET | 4-fold                       |        |
| PRJNA872862 | SAMN34728796 | BFR-CA-16062 | Vietnam | broiler, feces | CG154 | 10.03.2017 | Campylobacter coli   | >16 | >64 | >128 | >64 | >16  | >16 | 256  | >1024 |     | 4   |     |      |                              |                              | CIP, ERY, GEN, NAL, STR, TET | 4-fold |
| PRJNA872862 | SAMN34728797 | BFR-CA-16063 | Vietnam | broiler, feces | CG155 | 10.03.2017 | Campylobacter jejuni | >16 | >64 | <=1  | >64 | 1    | 1   |      |       |     |     |     |      |                              | CIP, NAL, TET                | 2-fold                       |        |
| PRJNA872862 | SAMN34728798 | BFR-CA-16064 | Vietnam | broiler, feces | CG156 | 10.03.2017 | Campylobacter jejuni | >16 | >64 | <=1  | >64 | 0,5  | 1   |      |       |     |     |     |      |                              | CIP, NAL, TET                | 2-fold                       |        |
| PRJNA872862 | SAMN34728799 | BFR-CA-16065 | Vietnam | broiler, feces | CG157 | 10.03.2017 | Campylobacter jejuni | >16 | >64 | <=1  | >64 | 0,5  | 1   |      |       |     |     |     |      |                              | CIP, NAL, TET                | 2-fold                       |        |
| PRJNA872862 | SAMN34728800 | BFR-CA-16068 | Vietnam | broiler, feces | CG160 | 10.03.2017 | Campylobacter coli   | 16  | 64  | >128 | >64 | >16  | 2   | >512 | >1024 |     | 64  |     | >512 |                              | CIP, ERY, GEN, NAL, TET      | 4-fold                       |        |
| PRJNA872862 | SAMN34728801 | BFR-CA-16069 | Vietnam | broiler, feces | CG161 | 10.03.2017 | Campylobacter coli   | 16  | 32  | >128 | 64  | >16  | 1   | >512 | >1024 |     | 64  |     | >512 |                              | CIP, ERY, GEN, NAL, TET      | 4-fold                       |        |
| PRJNA872862 | SAMN34728802 | BFR-CA-16070 | Vietnam | broiler, feces | CG162 | 10.03.2017 | Campylobacter jejuni | >16 | >64 | <=1  | >64 | 0,25 | 1   |      |       |     |     |     |      |                              | CIP, NAL, TET                | 2-fold                       |        |
| PRJNA872862 | SAMN34728803 | BFR-CA-16071 | Vietnam | broiler, feces | CG165 | 10.03.2017 | Campylobacter jejuni | >16 | >64 | <=1  | >64 | 0,5  | 1   |      |       |     |     |     |      |                              | CIP, NAL, TET                | 2-fold                       |        |
| PRJNA872862 | SAMN34728804 | BFR-CA-16072 | Vietnam | broiler, feces | CG166 | 10.03.2017 | Campylobacter jejuni | >16 | >64 | <=1  | >64 | 0,5  | 1   |      |       |     |     |     |      |                              | CIP, NAL, TET                | 2-fold                       |        |
| PRJNA872862 | SAMN34728805 | BFR-CA-16073 | Vietnam | broiler, feces | CG167 | 10.03.2017 | Campylobacter coli   | >16 | 64  | >128 | >64 | >16  | >16 | 512  | >1024 |     | 64  |     | >512 |                              | CIP, ERY, GEN, NAL, STR, TET | 4-fold                       |        |

9 Appendix

|             |              |              |         |                |       |            |                      |     |     |      |       |        |     |      |       |     |     |      |     |                         |        |                              |                              |                              |                              |        |
|-------------|--------------|--------------|---------|----------------|-------|------------|----------------------|-----|-----|------|-------|--------|-----|------|-------|-----|-----|------|-----|-------------------------|--------|------------------------------|------------------------------|------------------------------|------------------------------|--------|
|             |              |              |         |                |       | 10.03.2017 |                      |     |     |      |       |        |     |      |       |     |     |      |     | CIP, GEN, NAL, STR, TET | 3-fold |                              |                              |                              |                              |        |
| PRJNA872862 | SAMN34728806 | BFR-CA-16077 | Vietnam | broiler, feces | CG171 |            | Campylobacter jejuni | >16 | 32  | 2    | 64    | >16    | 16  | >512 | >1024 |     |     | 128  |     | >512                    | >128   | CIP, NAL, TET                | 2-fold                       |                              |                              |        |
| PRJNA872862 | SAMN34728807 | BFR-CA-16078 | Vietnam | broiler, feces | CG172 | 09.04.2017 | Campylobacter jejuni | >16 | >64 | <=1  | >64   | 0,5    | 1   |      |       |     |     |      |     |                         |        | CIP, NAL, TET                | 2-fold                       |                              |                              |        |
| PRJNA872862 | SAMN34728808 | BFR-CA-16079 | Vietnam | broiler, feces | CG174 | 09.04.2017 | Campylobacter jejuni | >16 | >64 | 2    | >64   | 0,5    | 1   |      | >1024 |     |     | >128 |     | >16                     |        | >128                         | CIP, NAL, TET                | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34728809 | BFR-CA-16080 | Vietnam | broiler, feces | CG175 | 09.04.2017 | Campylobacter jejuni | 16  | 32  | <=1  | 16    | <=0,12 | 0,5 |      |       |     |     |      |     |                         |        |                              | CIP, NAL, TET                | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34728810 | BFR-CA-16081 | Vietnam | broiler, feces | CG176 | 09.04.2017 | Campylobacter jejuni | >16 | >64 | 2    | >64   | 0,5    | 1   | 512  | >1024 | <=1 |     | >128 |     | >16                     | 8      | >128                         | CIP, NAL, TET                | 2-fold                       |                              |        |
|             |              |              |         |                |       | 09.04.2017 |                      |     |     |      |       |        |     |      |       |     |     |      |     |                         |        | CIP, ERY, GEN, NAL, STR, TET | 4-fold                       |                              |                              |        |
| PRJNA872862 | SAMN34728811 | BFR-CA-16088 | Vietnam | broiler, feces | CG183 |            | Campylobacter jejuni | >16 | 32  | >128 | 64    | >16    | >16 | >512 | >1024 |     |     | 128  |     |                         | >512   | >128                         | CIP, ERY, GEN, NAL, STR, TET | 4-fold                       |                              |        |
| PRJNA872862 | SAMN34728812 | BFR-CA-16089 | Vietnam | broiler, feces | CG184 | 09.04.2017 | Campylobacter jejuni | >16 | >64 | >128 | >64   | >16    | >16 | >512 | >1024 |     |     |      |     |                         |        | >512                         | >128                         | CIP, ERY, GEN, NAL, STR, TET | 4-fold                       |        |
| PRJNA872862 | SAMN34728813 | BFR-CA-16090 | Vietnam | broiler, feces | CG185 | 09.04.2017 | Campylobacter jejuni | >16 | >64 | >128 | >64   | >16    | >16 | >512 | >1024 |     |     | 128  |     |                         |        | >512                         | >128                         | CIP, ERY, GEN, NAL, STR, TET | 4-fold                       |        |
| PRJNA872862 | SAMN34728814 | BFR-CA-16091 | Vietnam | broiler, feces | CG186 | 09.04.2017 | Campylobacter jejuni | >16 | >64 | >128 | >64   | 2      | 4   |      |       |     |     |      |     |                         |        |                              |                              | CIP, ERY, NAL, TET           | 3-fold                       |        |
| PRJNA872862 | SAMN34728815 | BFR-CA-16092 | Vietnam | broiler, feces | CG187 | 09.04.2017 | Campylobacter jejuni | >16 | 64  | >128 | >64   | >16    | >16 | >512 | >1024 | <=1 |     | 128  | 16  |                         | >512   | >128                         | CIP, ERY, GEN, NAL, STR, TET | 4-fold                       |                              |        |
| PRJNA872862 | SAMN34728816 | BFR-CA-16095 | Vietnam | broiler, feces | CG190 | 09.04.2017 | Campylobacter jejuni | >16 | 64  | >128 | 64    | >16    | 16  |      |       |     |     | 128  |     |                         | >512   | >128                         | CIP, ERY, GEN, NAL, STR, TET | 4-fold                       |                              |        |
| PRJNA872862 | SAMN34728817 | BFR-CA-16096 | Vietnam | broiler, feces | CG191 | 09.04.2017 | Campylobacter jejuni | >16 | 32  | >128 | 64    | >16    | >16 |      |       |     |     | 128  |     |                         | >512   | >128                         | CIP, ERY, GEN, NAL, STR, TET | 4-fold                       |                              |        |
| PRJNA872862 | SAMN34728818 | BFR-CA-16099 | Vietnam | broiler, feces | CG194 | 09.04.2017 | Campylobacter jejuni | >16 | 64  | <=1  | >64   | 0,5    | >16 |      |       |     |     |      |     |                         |        |                              |                              | CIP, NAL, STR, TET           | 3-fold                       |        |
| PRJNA872862 | SAMN34728819 | BFR-CA-16103 | Vietnam | broiler, feces | CG97  | 12.01.2017 | Campylobacter coli   | >16 | 64  | 128  | >64   | >16    | >16 | 512  | >1024 |     |     | 64   |     |                         |        | >512                         | >128                         | CIP, ERY, GEN, NAL, STR, TET | 4-fold                       |        |
| PRJNA872862 | SAMN34728820 | BFR-CA-16104 | Vietnam | broiler, feces | CG98  | 12.01.2017 | Campylobacter jejuni | 8   | >64 | 4    | >64   | 0,5    | 2   |      |       |     |     |      |     |                         |        |                              |                              | CIP, NAL, TET                | 2-fold                       |        |
| PRJNA872862 | SAMN34728821 | BFR-CA-16105 | Vietnam | broiler, feces | CG100 | 12.01.2017 | Campylobacter jejuni | >16 | >64 | >128 | >64   | 0,25   | 1   |      |       |     |     |      |     |                         |        |                              |                              | CIP, ERY, NAL, TET           | 3-fold                       |        |
| PRJNA872862 | SAMN34728822 | BFR-CA-16106 | Vietnam | broiler, feces | CG103 | 12.01.2017 | Campylobacter coli   | >16 | 64  | 128  | >64   | >16    | >16 | 512  | >1024 |     |     | 128  |     |                         |        | >512                         | >128                         | CIP, ERY, GEN, NAL, STR, TET | 4-fold                       |        |
| PRJNA872862 | SAMN34728823 | BFR-CA-16107 | Vietnam | broiler, feces | CG104 | 12.01.2017 | Campylobacter coli   | >16 | >64 | >128 | >64   | >16    | >16 | 64   | >1024 | 32  | <=2 | 4    |     |                         | >512   | >128                         | CIP, ERY, GEN, NAL, STR, TET | 4-fold                       |                              |        |
| PRJNA872862 | SAMN34728824 | BFR-CA-16108 | Vietnam | broiler, feces | CG105 | 12.01.2017 | Campylobacter jejuni | >16 | >64 | <=1  | >64   | >16    | 4   | 256  | >1024 |     |     | 64   |     |                         |        | >512                         | >128                         | CIP, ERY, NAL, TET           | 3-fold                       |        |
| PRJNA872862 | SAMN34728825 | BFR-CA-16109 | Vietnam | broiler, feces | CG121 | 13.02.2017 | Campylobacter coli   | >16 | >64 | >128 | >64   | 1      | >16 | >512 | >1024 |     |     | 64   |     |                         |        | >512                         | >128                         | CIP, ERY, NAL, STR, TET      | 4-fold                       |        |
| PRJNA872862 | SAMN34728826 | BFR-CA-16110 | Vietnam | broiler, feces | CG142 | 13.02.2017 | Campylobacter coli   | >16 | 64  | >128 | >64   | 0,5    | >16 |      |       |     |     |      |     |                         |        | >512                         | >128                         | CIP, ERY, NAL, STR, TET      | 4-fold                       |        |
| PRJNA872862 | SAMN34728827 | BFR-CA-16111 | Vietnam | broiler, feces | CG152 | 10.03.2017 | Campylobacter coli   | 16  | >64 | >128 | >64   | >16    | >16 |      |       |     |     | >128 |     | >16                     |        | >512                         | >128                         | CIP, ERY, GEN, NAL, STR, TET | 4-fold                       |        |
| PRJNA872862 | SAMN34728828 | BFR-CA-16112 | Vietnam | broiler, feces | CG153 | 10.03.2017 | Campylobacter jejuni | >16 | >64 | 2    | >64   | 1      | 2   |      |       |     |     |      |     |                         |        |                              |                              | CIP, NAL, TET                | 2-fold                       |        |
| PRJNA872862 | SAMN34728829 | BFR-CA-16190 | Vietnam | broiler, feces | CG198 | 09.04.2017 | Campylobacter jejuni | 8   | 64  | <=1  | 32    | 0,25   | 1   |      |       |     |     |      |     |                         |        |                              |                              | CIP, NAL, TET                | 2-fold                       |        |
| PRJNA872862 | SAMN34728830 | BFR-CA-16191 | Vietnam | broiler, feces | CG200 | 03.05.2017 | Campylobacter coli   | 16  | 64  | >128 | 64    | >16    | >16 | >512 | >1024 |     |     | 32   |     |                         |        | >512                         | >128                         | CIP, ERY, GEN, NAL, STR, TET | 4-fold                       |        |
| PRJNA872862 | SAMN34728831 | BFR-CA-16193 | Vietnam | broiler, feces | CG202 | 03.05.2017 | Campylobacter jejuni | >16 | >64 | <=1  | >64   | >16    | >16 | >512 | >1024 |     |     | 128  |     |                         |        | >512                         | >128                         | CIP, GEN, NAL, STR, TET      | 3-fold                       |        |
| PRJNA872862 | SAMN34728832 | BFR-CA-16196 | Vietnam | broiler, feces | CG205 | 03.05.2017 | Campylobacter coli   | 16  | 64  | >128 | 32    | >16    | >16 | >512 | >1024 | <=1 |     | 128  | >16 |                         |        | >512                         | >128                         | CIP, ERY, GEN, NAL, STR, TET | 4-fold                       |        |
| PRJNA872862 | SAMN34728833 | BFR-CA-16197 | Vietnam | broiler, feces | CG206 | 03.05.2017 | Campylobacter jejuni | >16 | >64 | <=1  | 64    | 0,25   | 0,5 |      |       |     |     |      |     |                         |        |                              |                              | CIP, NAL, TET                | 2-fold                       |        |
| PRJNA872862 | SAMN34728834 | BFR-CA-16198 | Vietnam | broiler, feces | CG208 | 03.05.2017 | Campylobacter jejuni | 16  | 16  | <=1  | 64    | 0,25   | 1   |      |       |     |     |      |     |                         |        |                              |                              |                              | CIP, TET                     | 2-fold |
| PRJNA872862 | SAMN34728835 | BFR-CA-16201 | Vietnam | broiler, feces | CG213 | 03.05.2017 | Campylobacter coli   | >16 | 64  | >128 | >64   | >16    | >16 | 512  | >1024 | <=1 |     | 64   |     |                         | 256    |                              | >512                         | >128                         | CIP, ERY, GEN, NAL, STR, TET | 4-fold |
| PRJNA872862 | SAMN34728836 | BFR-CA-16203 | Vietnam | broiler, feces | CG215 | 03.05.2017 | Campylobacter coli   | >16 | 64  | >128 | >64   | >16    | 16  |      |       |     |     | 128  |     |                         | 512    | >128                         |                              |                              | CIP, ERY, GEN, NAL, STR, TET | 4-fold |
| PRJNA872862 | SAMN34728837 | BFR-CA-16204 | Vietnam | broiler, feces | CG216 | 03.05.2017 | Campylobacter jejuni | >16 | >64 | <=1  | >64   | >16    | 1   | >512 | >1024 |     |     | >128 |     |                         |        | >512                         | >128                         | CIP, GEN, NAL, TET           | 3-fold                       |        |
| PRJNA872862 | SAMN34728838 | BFR-CA-16207 | Vietnam | broiler, feces | CG219 | 03.05.2017 | Campylobacter jejuni | >16 | >64 | <=1  | >64   | 0,25   | 0,5 |      |       |     |     |      |     |                         |        |                              |                              |                              | CIP, NAL, TET                | 2-fold |
| PRJNA872862 | SAMN34728839 | BFR-CA-16208 | Vietnam | broiler, feces | CG220 | 03.05.2017 | Campylobacter jejuni | >16 | 32  | <=1  | 64    | <=0,12 | 0,5 |      | >1024 | <=1 |     |      |     |                         |        |                              |                              |                              | CIP, NAL, TET                | 2-fold |
| PRJNA872862 | SAMN34728840 | BFR-CA-16209 | Vietnam | broiler, feces | CG222 | 03.05.2017 | Campylobacter jejuni | >16 | 64  | <=1  | >64   | 0,25   | 0,5 |      |       |     |     |      |     |                         |        |                              |                              |                              | CIP, NAL, TET                | 2-fold |
| PRJNA872862 | SAMN34728841 | BFR-CA-16210 | Vietnam | broiler, feces | CG223 | 15.05.2017 | Campylobacter jejuni | >16 | >64 | <=1  | >64   | 0,5    | 1   |      |       |     |     |      |     |                         |        |                              |                              |                              | CIP, NAL, TET                | 2-fold |
| PRJNA872862 | SAMN34728842 | BFR-CA-16211 | Vietnam | broiler, feces | CG224 | 15.05.2017 | Campylobacter coli   | >16 | 64  | <=1  | 64    | >16    | 1   |      |       |     |     | 128  |     |                         |        | >512                         | >128                         | CIP, GEN, NAL, TET           | 3-fold                       |        |
| PRJNA872862 | SAMN34728843 | BFR-CA-16215 | Vietnam | broiler, feces | CG229 | 15.05.2017 | Campylobacter jejuni | >16 | >64 | <=1  | >64   | 0,25   | 1   |      |       |     |     |      |     |                         |        |                              |                              |                              | CIP, NAL, TET                | 2-fold |
|             |              |              |         |                |       | 15.05.2017 |                      |     |     |      |       |        |     |      |       |     |     |      |     |                         |        |                              |                              | CIP, NAL                     | 1-fold                       |        |
| PRJNA872862 | SAMN34728844 | BFR-CA-16216 | Vietnam | broiler, feces | CG230 |            | Campylobacter coli   | 16  | 64  | <=1  | <=0,5 | <=0,12 | 0,5 |      |       |     |     |      |     |                         |        |                              |                              |                              | CIP, NAL, TET                | 2-fold |
| PRJNA872862 | SAMN34728845 | BFR-CA-16217 | Vietnam | broiler, feces | CG231 | 15.05.2017 | Campylobacter jejuni | 4   | 64  | <=1  | 64    | 0,25   | 1   |      |       |     |     |      |     |                         |        |                              |                              |                              | CIP, NAL, TET                | 2-fold |
| PRJNA872862 | SAMN34728846 | BFR-CA-16220 | Vietnam | broiler, feces | CG236 | 15.05.2017 | Campylobacter jejuni | >16 | 64  | <=1  | 64    | 0,25   | 16  | >512 | >1024 |     |     | 512  |     |                         |        |                              |                              |                              | CIP, NAL, STR, TET           | 3-fold |
| PRJNA872862 | SAMN34728847 | BFR-CA-16221 | Vietnam | broiler, feces | CG237 | 15.05.2017 | Campylobacter coli   | >16 | >64 | >128 | >64   | >16    | >16 | 64   | >1024 |     |     | 64   |     |                         |        | >512                         | >128                         | CIP, ERY, GEN, NAL, STR, TET | 4-fold                       |        |
| PRJNA872862 | SAMN34728848 | BFR-CA-16222 | Vietnam | broiler, feces | CG238 | 15.05.2017 | Campylobacter jejuni | 16  | 64  | <=1  | >64   | 0,25   | 1   |      |       |     |     |      |     |                         |        |                              |                              |                              | CIP, NAL, TET                | 2-fold |
| PRJNA872862 | SAMN34728849 | BFR-CA-16245 | Vietnam | broiler, feces | CG234 | 15.05.2017 | Campylobacter coli   | >16 | >64 | >128 | >64   | >16    | >16 | 256  | >1024 |     |     | 4    |     |                         |        |                              |                              |                              | CIP, ERY, GEN, NAL, STR, TET | 4-fold |
| PRJNA872862 | SAMN34728850 | BFR-CA-16246 | Vietnam | broiler, feces | CG239 | 15.05.2017 | Campylobacter jejuni | >16 | 64  | <=1  | >64   | 0,25   | 1   |      |       |     |     |      |     |                         |        |                              |                              |                              | CIP, NAL, TET                | 2-fold |
| PRJNA872862 | SAMN34728851 | BFR-CA-16247 | Vietnam | broiler, feces | CG240 | 15.05.2017 | Campylobacter jejuni | >16 | >64 | <=1  | >64   | 0,5    | 2   |      |       |     |     |      |     |                         |        |                              |                              |                              | CIP, NAL, TET                | 2-fold |
| PRJNA872862 | SAMN34728852 | BFR-CA-16248 | Vietnam | broiler, feces | CG241 | 15.05.2017 | Campylobacter jejuni | >16 | 64  | <=1  | >64   | <=0,12 | 1   |      |       |     |     |      |     |                         |        |                              |                              |                              |                              |        |

# 9 Appendix

|             |              |              |         |                |       |            |                      |        |     |      |     |        |        |       |       |  |     |     |      |               |                              |                              |                              |        |
|-------------|--------------|--------------|---------|----------------|-------|------------|----------------------|--------|-----|------|-----|--------|--------|-------|-------|--|-----|-----|------|---------------|------------------------------|------------------------------|------------------------------|--------|
| PRJNA872862 | SAMN34728857 | BFR-CA-16254 | Vietnam | broiler, feces | CG253 | 18.06.2017 | Campylobacter jejuni | >16    | 32  | <=1  | 32  | <=0,12 | <=0,25 |       |       |  |     |     |      | CIP, NAL, TET | 2-fold                       |                              |                              |        |
| PRJNA872862 | SAMN34728858 | BFR-CA-16257 | Vietnam | broiler, feces | CG261 | 16.07.2017 | Campylobacter coli   | >16    | 64  | >128 | >64 | >16    | 2      | 512   | >1024 |  | 8   |     | >512 |               | CIP, ERY, GEN, NAL, TET      | 4-fold                       |                              |        |
|             |              |              |         |                |       | 16.07.2017 |                      |        |     |      |     |        |        |       |       |  |     |     |      |               | CIP, ERY, NAL, STR, TET      | 4-fold                       |                              |        |
| PRJNA872862 | SAMN34728859 | BFR-CA-16258 | Vietnam | broiler, feces | CG263 |            | Campylobacter coli   | >16    | >64 | >128 | >64 | 0,5    | >16    |       | 4     |  |     |     | >512 | 16            |                              |                              |                              |        |
| PRJNA872862 | SAMN34728860 | BFR-CA-16259 | Vietnam | broiler, feces | CG264 | 16.07.2017 | Campylobacter coli   | >16    | >64 | >128 | >64 | >16    | >16    | >512  | >1024 |  |     |     | >512 |               | CIP, ERY, GEN, NAL, STR, TET | 4-fold                       |                              |        |
| PRJNA872862 | SAMN34728861 | BFR-CA-16261 | Vietnam | broiler, feces | CG267 | 16.07.2017 | Campylobacter coli   | >16    | >64 | 64   | >64 | >16    | >16    | >512  | >1024 |  | <=1 | 128 | >16  | >512          | >128                         | CIP, ERY, GEN, NAL, STR, TET | 4-fold                       |        |
| PRJNA872862 | SAMN34728862 | BFR-CA-16264 | Vietnam | broiler, feces | CG270 | 16.07.2017 | Campylobacter coli   | >16    | >64 | >128 | >64 | >16    | 2      |       |       |  | <=1 |     | >512 |               | CIP, ERY, GEN, NAL, TET      | 4-fold                       |                              |        |
| PRJNA872862 | SAMN34728863 | BFR-CA-16265 | Vietnam | broiler, feces | CG271 | 16.07.2017 | Campylobacter jejuni | >16    | >64 | 2    | >64 | 0,5    | 1      |       |       |  |     |     |      |               | CIP, NAL, TET                | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34728864 | BFR-CA-16272 | Vietnam | broiler, feces | CG272 | 16.07.2017 | Campylobacter jejuni | >16    | >64 | <=1  | >64 | 0,5    | 1      |       |       |  |     |     |      |               | CIP, NAL, TET                | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34728865 | BFR-CA-16273 | Vietnam | broiler, feces | CG273 | 16.07.2017 | Campylobacter jejuni | 8      | >64 | <=1  | 64  | 0,5    | 1      |       |       |  |     |     |      |               | CIP, NAL, TET                | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34728866 | BFR-CA-16275 | Vietnam | broiler, feces | CG275 | 14.08.2017 | Campylobacter coli   | 16     | 64  | >128 | >64 | >16    | >16    | 512   | >1024 |  |     |     | >512 |               | CIP, ERY, GEN, NAL, STR, TET | 4-fold                       |                              |        |
| PRJNA872862 | SAMN34728867 | BFR-CA-16277 | Vietnam | broiler, feces | CG277 | 14.08.2017 | Campylobacter jejuni | 16     | 32  | <=1  | 16  | 16     | 1      | 256   | >1024 |  |     |     | >512 |               | CIP, GEN, NAL, TET           | 3-fold                       |                              |        |
| PRJNA872862 | SAMN34728868 | BFR-CA-16278 | Vietnam | broiler, feces | CG278 | 14.08.2017 | Campylobacter jejuni | >16    | >64 | 2    | >64 | >16    | 1      | 256   | >1024 |  |     |     | >512 | 8             |                              | CIP, GEN, NAL, TET           | 3-fold                       |        |
| PRJNA872862 | SAMN34728869 | BFR-CA-16279 | Vietnam | broiler, feces | CG279 | 14.08.2017 | Campylobacter jejuni | 8      | 32  | <=1  | 8   | 0,25   | 1      |       |       |  |     |     |      |               | CIP, NAL, TET                | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34728870 | BFR-CA-16280 | Vietnam | broiler, feces | CG280 | 14.08.2017 | Campylobacter jejuni | >16    | >64 | 2    | >64 | 0,5    | 2      |       |       |  |     |     |      |               | CIP, NAL, TET                | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34728871 | BFR-CA-16281 | Vietnam | broiler, feces | CG281 | 14.08.2017 | Campylobacter jejuni | 16     | 32  | <=1  | 16  | <=0,12 | 0,5    |       |       |  |     |     |      |               | CIP, NAL, TET                | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34728872 | BFR-CA-16282 | Vietnam | broiler, feces | CG282 | 14.08.2017 | Campylobacter coli   | 16     | 64  | 2    | 32  | 0,25   | 1      |       |       |  |     |     |      |               | CIP, NAL, TET                | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34728873 | BFR-CA-16283 | Vietnam | broiler, feces | CG283 | 14.08.2017 | Campylobacter jejuni | 8      | 16  | <=1  | 8   | <=0,12 | 0,5    |       |       |  |     |     |      |               | CIP, TET                     | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34728874 | BFR-CA-16284 | Vietnam | broiler, feces | CG284 | 14.08.2017 | Campylobacter jejuni | 8      | >64 | <=1  | >64 | 0,5    | 1      |       |       |  |     |     |      |               | CIP, NAL, TET                | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34728875 | BFR-CA-16287 | Vietnam | broiler, feces | CG287 | 14.08.2017 | Campylobacter jejuni | 16     | >64 | <=1  | >64 | 0,25   | 2      |       |       |  |     |     |      |               | CIP, NAL, TET                | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34728876 | BFR-CA-16292 | Vietnam | broiler, feces | CG294 | 11.09.2017 | Campylobacter coli   | >16    | 64  | >128 | >64 | >16    | >16    | 32    | >1024 |  |     |     | >512 | 64            | 8                            |                              | CIP, ERY, GEN, NAL, STR, TET | 4-fold |
| PRJNA872862 | SAMN34728877 | BFR-CA-16296 | Vietnam | broiler, feces | CG302 | 11.09.2017 | Campylobacter jejuni | >16    | 64  | <=1  | >64 | 0,25   | 1      |       |       |  |     |     |      |               | CIP, NAL, TET                | 2-fold                       |                              |        |
|             |              |              |         |                |       | 11.09.2017 |                      |        |     |      |     |        |        |       |       |  |     |     |      |               |                              | CIP, ERY, GEN, NAL, STR, TET | 4-fold                       |        |
| PRJNA872862 | SAMN34728878 | BFR-CA-16297 | Vietnam | broiler, feces | CG303 |            | Campylobacter coli   | >16    | 64  | >128 | >64 | >16    | >16    | 512   | >1024 |  |     | 4   |      | >512          |                              |                              |                              |        |
| PRJNA872862 | SAMN34728879 | BFR-CA-16298 | Vietnam | broiler, feces | CG304 | 11.09.2017 | Campylobacter coli   | 8      | 64  | >128 | >64 | 0,5    | >16    |       |       |  | <=1 |     | >512 |               |                              | CIP, ERY, NAL, STR, TET      | 4-fold                       |        |
| PRJNA872862 | SAMN34728880 | BFR-CA-16299 | Vietnam | broiler, feces | CG305 | 11.09.2017 | Campylobacter jejuni | 8      | >64 | <=1  | 32  | 0,5    | 0,5    |       |       |  |     |     |      |               | CIP, NAL, TET                | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34728881 | BFR-CA-16300 | Vietnam | broiler, feces | CG306 | 11.09.2017 | Campylobacter coli   | >16    | 64  | >128 | >64 | 0,5    | 16     |       |       |  | <=1 |     | >512 |               |                              | CIP, ERY, NAL, STR, TET      | 4-fold                       |        |
| PRJNA872862 | SAMN34728882 | BFR-CA-16303 | Vietnam | broiler, feces | CG310 | 09.10.2017 | Campylobacter jejuni | 8      | >64 | <=1  | >64 | 0,5    | 1      |       |       |  |     |     |      |               | CIP, NAL, TET                | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34728883 | BFR-CA-16305 | Vietnam | broiler, feces | CG313 | 09.10.2017 | Campylobacter jejuni | 8      | >64 | <=1  | 32  | 0,5    | 1      |       |       |  |     |     |      |               | CIP, NAL, TET                | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34728884 | BFR-CA-16307 | Vietnam | broiler, feces | CG315 | 09.10.2017 | Campylobacter jejuni | 8      | 64  | <=1  | >64 | 0,25   | 0,5    |       |       |  |     |     |      |               | CIP, NAL, TET                | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34728885 | BFR-CA-16309 | Vietnam | broiler, feces | CG317 | 09.10.2017 | Campylobacter jejuni | 16     | >64 | <=1  | 64  | 1      | 2      |       |       |  |     |     |      |               | CIP, NAL, TET                | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34728886 | BFR-CA-16311 | Vietnam | broiler, feces | CG319 | 09.10.2017 | Campylobacter coli   | 16     | >64 | 4    | >64 | >16    | 2      | 256   | >1024 |  |     |     | >512 | 64            | 2                            |                              | CIP, GEN, NAL, TET           | 3-fold |
| PRJNA872862 | SAMN34728887 | BFR-CA-16329 | Vietnam | broiler, feces | CG292 | 11.09.2017 | Campylobacter jejuni | 8      | >64 | <=1  | >64 | 0,25   | 0,5    |       |       |  |     |     |      |               | CIP, NAL, TET                | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34728888 | BFR-CA-16330 | Vietnam | broiler, feces | CG293 | 11.09.2017 | Campylobacter jejuni | >16    | >64 | <=1  | >64 | 0,5    | 2      |       |       |  |     |     |      |               | CIP, NAL, TET                | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34728889 | BFR-CA-16348 | Vietnam | broiler, feces | CG322 | 09.10.2017 | Campylobacter jejuni | >16    | >64 | 2    | >64 | 1      | 1      |       |       |  |     |     |      |               | CIP, NAL, TET                | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34728890 | BFR-CA-16349 | Vietnam | broiler, feces | CG325 | 09.10.2017 | Campylobacter jejuni | 16     | >64 | <=1  | 64  | 0,5    | 1      |       |       |  |     |     |      |               | CIP, NAL, TET                | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34728891 | BFR-CA-16350 | Vietnam | broiler, feces | CG329 | 09.10.2017 | Campylobacter jejuni | >16    | >64 | <=1  | >64 | 0,5    | 1      |       |       |  |     |     |      |               | CIP, NAL, TET                | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34728892 | BFR-CA-16351 | Vietnam | broiler, feces | CG330 | 09.10.2017 | Campylobacter coli   | 16     | >64 | 4    | >64 | >16    | 2      | 256   | >1024 |  |     |     | >512 | 128           | 2                            |                              | CIP, GEN, NAL, TET           | 3-fold |
| PRJNA872862 | SAMN34728893 | BFR-CA-16352 | Vietnam | broiler, feces | CG338 | 09.10.2017 | Campylobacter coli   | 16     | 64  | >128 | >64 | >16    | >16    | >512  | >1024 |  |     |     | >512 | 64            | 8                            |                              | CIP, ERY, GEN, NAL, STR, TET | 4-fold |
| PRJNA872862 | SAMN34728894 | BFR-CA-16353 | Vietnam | broiler, feces | CG348 | 02.11.2017 | Campylobacter coli   | >16    | >64 | >128 | >64 | >16    | >16    | 64    | >1024 |  |     |     | >512 | 16            |                              |                              | CIP, ERY, GEN, NAL, STR, TET | 4-fold |
| PRJNA872862 | SAMN34728895 | BFR-CA-16354 | Vietnam | broiler, feces | CG352 | 02.11.2017 | Campylobacter coli   | 16     | 64  | 4    | >64 | 1      | 2      |       |       |  |     |     |      |               | CIP, NAL, TET                | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34728896 | BFR-CA-16360 | Vietnam | broiler, feces | CG324 | 09.10.2017 | Campylobacter jejuni | >16    | >64 | 2    | >64 | 1      | 2      |       |       |  |     |     |      |               | CIP, NAL, TET                | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34728897 | BFR-CA-16361 | Vietnam | broiler, feces | CG326 | 09.10.2017 | Campylobacter jejuni | >16    | >64 | <=1  | >64 | 0,5    | 1      |       |       |  |     |     |      |               | CIP, NAL, TET                | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34728898 | BFR-CA-16362 | Vietnam | broiler, feces | CG327 | 09.10.2017 | Campylobacter jejuni | >16    | >64 | <=1  | >64 | 1      | 1      |       |       |  |     |     |      |               | CIP, NAL, TET                | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34728899 | BFR-CA-16365 | Vietnam | broiler, feces | CG332 | 09.10.2017 | Campylobacter jejuni | >16    | >64 | <=1  | >64 | 0,5    | >16    |       | 8     |  |     |     |      | >512          |                              | CIP, NAL, STR, TET           | 3-fold                       |        |
| PRJNA872862 | SAMN34728900 | BFR-CA-16370 | Vietnam | broiler, feces | CG344 | 09.10.2017 | Campylobacter jejuni | >16    | >64 | <=1  | >64 | 0,5    | 1      |       |       |  |     |     |      |               | CIP, NAL, TET                | 2-fold                       |                              |        |
|             |              |              |         |                |       | 09.10.2017 |                      |        |     |      |     |        |        |       |       |  |     |     |      |               |                              | CIP, GEN, NAL, STR, TET      | 3-fold                       |        |
| PRJNA872862 | SAMN34728901 | BFR-CA-16372 | Vietnam | broiler, feces | CG346 |            | Campylobacter coli   | 16     | 64  | 4    | >64 | >16    | >16    | >1024 |       |  |     |     |      |               | 8                            |                              | CIP, NAL, TET                | 2-fold |
| PRJNA872862 | SAMN34728902 | BFR-CA-16373 | Vietnam | broiler, feces | CG349 | 02.11.2017 | Campylobacter jejuni | >16    | >64 | 2    | >64 | 1      | 2      |       |       |  |     |     |      |               |                              | CIP, NAL, TET                | 2-fold                       |        |
| PRJNA872862 | SAMN34728903 | BFR-CA-16374 | Vietnam | broiler, feces | CG350 | 02.11.2017 | Campylobacter jejuni | >16    | >64 | 2    | >64 | 0,5    | 1      |       |       |  |     |     |      |               |                              | CIP, NAL, TET                | 2-fold                       |        |
| PRJNA872862 | SAMN34728904 | BFR-CA-16375 | Vietnam | broiler, feces | CG351 | 02.11.2017 | Campylobacter jejuni | >16    | >64 | <=1  | >64 | 0,5    | 2      |       |       |  |     |     |      |               |                              | CIP, NAL, TET                | 2-fold                       |        |
| PRJNA872862 | SAMN34728905 | BFR-CA-16376 | Vietnam | broiler, feces | CG353 | 02.11.2017 | Campylobacter coli   | 16     | >64 | 2    | >64 | >16    | >16    | 512   | >1024 |  |     |     | >512 | 64            | 4                            |                              | CIP, GEN, NAL, STR, TET      | 3-fold |
| PRJNA872862 | SAMN34728906 | BFR-CA-16378 | Vietnam | broiler, feces | CG355 | 02.11.2017 | Campylobacter jejuni | 8      | >64 | <=1  | >64 | 0,5    | 1      |       |       |  |     |     |      |               |                              | CIP, NAL, TET                | 2-fold                       |        |
| PRJNA872862 | SAMN34728907 | BFR-CA-16381 | Vietnam | broiler, feces | CG361 | 02.11.2017 | Campylobacter jejuni | <=0,12 | 4   | <=1  | >64 | 0,5    | 1      |       |       |  |     |     |      |               |                              | TET                          | 1-fold                       |        |
| PRJNA872862 | SAMN34728908 | BFR-CA-16383 | Vietnam | broiler, feces | CG363 | 02.11.2017 | Campylobacter jejuni | 4      | >64 | <=1  | 64  |        |        |       |       |  |     |     |      |               |                              |                              |                              |        |

# 9 Appendix

|             |              |              |         |                |       |            |                      |        |     |      |       |      |     |      |       |     |     |   |      |                         |           |        |
|-------------|--------------|--------------|---------|----------------|-------|------------|----------------------|--------|-----|------|-------|------|-----|------|-------|-----|-----|---|------|-------------------------|-----------|--------|
| PRJNA872862 | SAMN34728910 | BFR-CA-16387 | Vietnam | broiler, feces | CG341 | 09.10.2017 | Campylobacter jejuni | >16    | >64 | <=1  | >64   | 0,5  | 1   |      |       |     |     |   |      | CIP, NAL, TET           | 2-fold    |        |
| PRJNA872862 | SAMN34728911 | BFR-CA-16389 | Vietnam | broiler, feces | CG357 | 02.11.2017 | Campylobacter jejuni | >16    | >64 | 4    | >64   | >16  | 2   | 256  | >1024 |     | 64  | 8 | >512 | CIP, GEN, NAL, TET      | 3-fold    |        |
| PRJNA872862 | SAMN34728912 | BFR-CA-16491 | Germany | broiler, cecum |       | 20.08.2018 | Campylobacter jejuni | 8      | 64  | <=1  | >64   | 0,5  | 0,5 |      |       |     |     |   |      | CIP, NAL, TET           | 2-fold    |        |
| PRJNA872862 | SAMN34728913 | BFR-CA-16671 | Germany | broiler, meat  |       | 19.09.2018 | Campylobacter coli   | >16    | >64 | >128 | >64   | 0,5  | >16 |      |       |     |     |   |      | CIP, ERY, NAL, STR, TET | 4-fold    |        |
| PRJNA872862 | SAMN34728914 | BFR-CA-16677 | Germany | broiler, cecum |       | 11.10.2018 | Campylobacter jejuni | >16    | 64  | >128 | >64   | 0,25 | >16 | >512 | >1024 | 256 |     |   |      | CIP, ERY, NAL, STR, TET | 4-fold    |        |
| PRJNA872862 | SAMN34728915 | BFR-CA-16708 | Germany | broiler, cecum |       | 25.09.2018 | Campylobacter coli   | 16     | 64  | >128 | >64   | 0,5  | >16 | >512 | >1024 | 128 |     |   |      | CIP, ERY, NAL, STR, TET | 4-fold    |        |
| PRJNA872862 | SAMN34728916 | BFR-CA-16732 | Germany | turkey, cecum  |       | 10.09.2018 | Campylobacter coli   | 16     | 64  | >128 | >64   | 0,5  | >16 |      |       |     |     |   |      | CIP, ERY, NAL, STR, TET | 4-fold    |        |
| PRJNA872862 | SAMN34728917 | BFR-CA-16737 | Germany | turkey, cecum  |       | 18.09.2018 | Campylobacter jejuni | >16    | >64 | <=1  | >64   | 0,25 | 1   | >512 | >1024 | 256 |     |   |      | CIP, NAL, TET           | 2-fold    |        |
| PRJNA872862 | SAMN34728918 | BFR-CA-16743 | Germany | turkey, cecum  |       | 25.09.2018 | Campylobacter coli   | >16    | >64 | >128 | 64    | 1    | >16 |      |       |     |     |   |      | CIP, ERY, NAL, STR, TET | 4-fold    |        |
| PRJNA595957 | SAMN13577915 | BFR-CA-16767 | Germany | turkey, cecum  |       | 18.09.2018 | Campylobacter coli   | >16    | 64  | <=1  | >64   | 0,25 | 0,5 | >512 | >1024 | 8   |     |   |      | CIP, NAL, TET           | 2-fold    |        |
| PRJNA872862 | SAMN34728919 | BFR-CA-16783 | Germany | broiler, cecum |       | 22.10.2018 | Campylobacter jejuni | 8      | >64 | 2    | >64   | 0,5  | 2   |      |       |     |     |   |      | CIP, NAL, TET           | 2-fold    |        |
| PRJNA872862 | SAMN34728920 | BFR-CA-16800 | Germany | broiler, meat  |       | 23.10.2018 | Campylobacter jejuni | <=0,12 | 2   | <=1  | <=0,5 | 0,5  | 1   |      |       |     |     |   |      | sensitive               | sensitive |        |
| PRJNA595957 | SAMN13577916 | BFR-CA-16822 | Germany | turkey, cecum  |       | 08.10.2018 | Campylobacter coli   | 16     | >64 | <=1  | >64   | 0,5  | 1   |      |       |     |     |   |      | CIP, NAL, TET           | 2-fold    |        |
| PRJNA872862 | SAMN34728921 | BFR-CA-16831 | Germany | turkey, cecum  |       | 16.10.2018 | Campylobacter coli   | 16     | 64  | >128 | >64   | 0,5  | >16 | >512 | >1024 | 128 |     |   |      | CIP, ERY, NAL, STR, TET | 4-fold    |        |
|             |              |              |         |                |       | 15.10.2018 |                      |        |     |      |       |      |     |      |       |     |     |   |      | CIP, NAL, TET           | 2-fold    |        |
| PRJNA595957 | SAMN13577917 | BFR-CA-16834 | Germany | turkey, cecum  |       |            | Campylobacter coli   | 16     | >64 | <=1  | >64   | 0,5  | 1   | 512  | >1024 | 512 |     |   |      |                         |           |        |
| PRJNA872862 | SAMN34728922 | BFR-CA-16888 | Germany | broiler, meat  |       | 16.10.2018 | Campylobacter jejuni | 8      | >64 | <=1  | 64    | 0,25 | 0,5 |      |       |     |     |   |      | CIP, NAL, TET           | 2-fold    |        |
| PRJNA872862 | SAMN34728923 | BFR-CA-16930 | Germany | broiler, meat  |       | 22.11.2018 | Campylobacter jejuni | 16     | >64 | <=1  | >64   | 0,5  | 2   |      |       |     |     |   |      | CIP, NAL, TET           | 2-fold    |        |
| PRJNA595957 | SAMN13577918 | BFR-CA-16942 | Germany | turkey, meat   |       | 21.11.2018 | Campylobacter coli   | 16     | 64  | 2    | 2     | 1    | 2   | 512  | 8     | <=1 | <=2 | 2 | 8    | 8                       | CIP, NAL  | 1-fold |
| PRJNA872862 | SAMN34728924 | BFR-CA-17056 | Germany | turkey, cecum  |       | 13.11.2018 | Campylobacter coli   | >16    | 64  | >128 | >64   | 1    | >16 |      |       |     |     |   |      | CIP, ERY, NAL, STR, TET | 4-fold    |        |
| PRJNA872862 | SAMN34728925 | BFR-CA-17071 | Germany | turkey, cecum  |       | 26.11.2018 | Campylobacter coli   | >16    | >64 | >128 | >64   | 0,5  | >16 |      |       | <=1 |     |   | >512 | CIP, ERY, NAL, STR, TET | 4-fold    |        |
|             |              |              |         |                |       | 27.11.2018 |                      |        |     |      |       |      |     |      |       |     |     |   |      | sensitive               | sensitive |        |
| PRJNA595957 | SAMN13577919 | BFR-CA-17078 | Germany | turkey, cecum  |       |            | Campylobacter coli   | 0,5    | 8   | <=1  | <=0,5 | 0,5  | 2   |      |       |     |     |   |      |                         |           |        |
| PRJNA872862 | SAMN34728926 | BFR-CA-17105 | Germany | turkey, meat   |       | 06.12.2018 | Campylobacter jejuni | <=0,12 | 4   | <=1  | >64   | 0,5  | 1   |      |       |     |     |   |      | TET                     | 1-fold    |        |
| PRJNA872862 | SAMN34728927 | BFR-CA-17107 | Germany | turkey, meat   |       | 07.05.2018 | Campylobacter jejuni | >16    | >64 | <=1  | 64    | 0,5  | 1   |      |       |     |     |   |      | CIP, NAL, TET           | 2-fold    |        |
| PRJNA595957 | SAMN13577920 | BFR-CA-17110 | Germany | turkey, meat   |       | 15.05.2018 | Campylobacter coli   | 8      | >64 | <=1  | >64   | 1    | 2   |      |       |     |     |   |      | CIP, NAL, TET           | 2-fold    |        |
| PRJNA872862 | SAMN34728928 | BFR-CA-17153 | Germany | broiler, egg   |       | 08.02.2019 | Campylobacter coli   | 16     | 64  | <=1  | <=0,5 | 0,5  | 2   |      |       |     |     |   |      | CIP, NAL                | 1-fold    |        |
| PRJNA872862 | SAMN34728929 | BFR-CA-17156 | Germany | broiler, meat  |       | 04.02.2019 | Campylobacter jejuni | >16    | >64 | <=1  | >64   | 0,5  | 1   |      |       |     |     |   |      | CIP, NAL, TET           | 2-fold    |        |
| PRJNA872862 | SAMN34728930 | BFR-CA-17157 | Germany | broiler, meat  |       | 24.01.2019 | Campylobacter jejuni | >16    | >64 | <=1  | <=0,5 | 0,5  | 2   |      |       |     |     |   |      | CIP, NAL                | 1-fold    |        |
| PRJNA872862 | SAMN34728931 | BFR-CA-17159 | Germany | broiler, meat  |       | 05.02.2019 | Campylobacter coli   | 8      | >64 | <=1  | >64   | 1    | 2   |      |       |     |     |   |      | CIP, NAL, TET           | 2-fold    |        |
| PRJNA872862 | SAMN34728932 | BFR-CA-17160 | Germany | broiler, meat  |       | 05.02.2019 | Campylobacter jejuni | <=0,12 | 4   | <=1  | <=0,5 | 0,5  | 1   |      |       |     |     |   |      | sensitive               | sensitive |        |
| PRJNA872862 | SAMN34728933 | BFR-CA-17230 | Germany | turkey, cecum  |       | 03.12.2018 | Campylobacter coli   | 16     | 64  | >128 | >64   | 0,5  | >16 | 512  | >1024 | 256 |     |   |      | CIP, ERY, NAL, STR, TET | 4-fold    |        |
| PRJNA872862 | SAMN34728934 | BFR-CA-17247 | Germany | broiler, meat  |       | 11.04.2019 | Campylobacter coli   | 16     | >64 | <=1  | >64   | 1    | 2   |      |       |     |     |   |      | CIP, NAL, TET           | 2-fold    |        |
| PRJNA872862 | SAMN34728935 | BFR-CA-17249 | Germany | broiler, egg   |       | 06.05.2019 | Campylobacter coli   | <=0,12 | 4   | <=1  | >64   | 1    | 2   |      |       |     |     |   |      | TET                     | 1-fold    |        |
| PRJNA872862 | SAMN34728936 | BFR-CA-17288 | Germany | broiler, meat  |       | 11.06.2019 | Campylobacter jejuni | 4      | 2   | <=1  | 32    | 0,25 | 0,5 |      |       |     |     |   |      | CIP, TET                | 2-fold    |        |
| PRJNA872862 | SAMN34728937 | BFR-CA-17356 | Germany | broiler, meat  |       | 15.07.2019 | Campylobacter jejuni | 8      | >64 | <=1  | 16    | 0,5  | 2   |      |       |     |     |   |      | CIP, NAL, TET           | 2-fold    |        |
| PRJNA872862 | SAMN34728938 | BFR-CA-17379 | Germany | broiler, meat  |       | 17.07.2019 | Campylobacter jejuni | >16    | >64 | <=1  | >64   | 0,25 | 1   |      |       |     |     |   |      | CIP, NAL, TET           | 2-fold    |        |
| PRJNA872862 | SAMN34728939 | BFR-CA-17395 | Germany | broiler, meat  |       | 06.08.2019 | Campylobacter jejuni | 16     | >64 | <=1  | >64   | 0,5  | 2   |      |       |     |     |   |      | CIP, NAL, TET           | 2-fold    |        |
| PRJNA872862 | SAMN34728940 | BFR-CA-17405 | Germany | broiler, meat  |       | 15.08.2019 | Campylobacter jejuni | 16     | >64 | <=1  | >64   | 0,5  | >16 | 256  | >1024 | 256 |     |   |      | CIP, NAL, STR, TET      | 3-fold    |        |
| PRJNA872862 | SAMN34728941 | BFR-CA-17407 | Germany | broiler, skin  |       | 19.08.2019 | Campylobacter jejuni | 8      | >64 | <=1  | 64    | 0,25 | 1   |      |       |     |     |   |      | CIP, NAL, TET           | 2-fold    |        |
| PRJNA872862 | SAMN34728942 | BFR-CA-17415 | Germany | broiler, meat  |       | 26.08.2019 | Campylobacter jejuni | >16    | >64 | <=1  | >64   | 0,5  | >16 | 256  | 1024  | 128 |     |   |      | CIP, NAL, STR, TET      | 3-fold    |        |
| PRJNA872862 | SAMN34728943 | BFR-CA-17424 | Germany | broiler, skin  |       | 02.09.2019 | Campylobacter jejuni | 8      | >64 | <=1  | <=0,5 | 0,5  | 2   |      |       |     |     |   |      | CIP, NAL                | 1-fold    |        |
| PRJNA872862 | SAMN34728944 | BFR-CA-17549 | Germany | broiler, meat  |       | 29.10.2019 | Campylobacter jejuni | >16    | >64 | <=1  | >64   | 0,5  | >16 | 256  | >1024 | 256 |     |   |      | CIP, NAL, STR, TET      | 3-fold    |        |
| PRJNA872862 | SAMN34728945 | BFR-CA-17788 | Germany | broiler, meat  |       | 08.01.2020 | Campylobacter jejuni | 16     | >64 | <=1  | >64   | 0,5  | >16 | 256  | >1024 | 256 |     |   |      | CIP, NAL, STR, TET      | 3-fold    |        |
| PRJNA872862 | SAMN34728946 | BFR-CA-17799 | Germany | broiler, meat  |       | 16.01.2020 | Campylobacter jejuni | 8      | >64 | <=1  | >64   | 0,5  | 2   |      |       |     |     |   |      | CIP, NAL, TET           | 2-fold    |        |
| PRJNA872862 | SAMN34728947 | BFR-CA-17805 | Germany | poultry, meat  |       | 29.01.2020 | Campylobacter jejuni | 16     | >64 | <=1  | >64   | 0,5  | 1   |      |       |     |     |   |      | CIP, NAL, TET           | 2-fold    |        |
| PRJNA872862 | SAMN34728948 | BFR-CA-17822 | Germany | broiler, meat  |       | 04.02.2020 | Campylobacter jejuni | 16     | >64 | 2    | >64   | 0,5  | >16 | 256  | >1024 | 256 |     |   |      | CIP, NAL, STR, TET      | 3-fold    |        |
| PRJNA872862 | SAMN34728949 | BFR-CA-17824 | Germany | broiler, meat  |       | 03.02.2020 | Campylobacter jejuni | 16     | >64 | <=1  | >64   | 0,5  | 1   |      |       |     |     |   |      | CIP, NAL, TET           | 2-fold    |        |
| PRJNA872862 | SAMN34728950 | BFR-CA-17827 | Germany | broiler, egg   |       | 29.01.2020 | Campylobacter coli   | 16     | 64  | <=1  | >64   | 0,5  | 1   |      |       |     |     |   |      | CIP, NAL, TET           | 2-fold    |        |
| PRJNA872862 | SAMN34728951 | BFR-CA-17832 | Germany | broiler, meat  |       | 13.02.2020 | Campylobacter jejuni | 8      | >64 | <=1  | 64    | 0,5  | 1   |      |       |     |     |   |      | CIP, NAL, TET           | 2-fold    |        |
| PRJNA872862 | SAMN34728952 | BFR-CA-17836 | Germany | broiler, meat  |       | 18.02.2020 | Campylobacter coli   | 8      | >64 | <=1  | >64   | 0,5  | 2   |      |       |     |     |   |      | CIP, NAL, TET           | 2-fold    |        |
| PRJNA872862 | SAMN34728953 | BFR-CA-17840 | Germany | broiler, meat  |       | 17.02.2020 | Campylobacter jejuni | <=0,12 | 4   | <=1  | <=0,5 | 0,5  | 2   |      |       |     |     |   |      | sensitive               | sensitive |        |
| PRJNA872862 | SAMN34728954 | BFR-CA-17860 | Germany | broiler, meat  |       | 27.02.2020 | Campylobacter jejuni | 16     | >64 | <=1  | 64    | 0,5  | 1   |      |       |     |     |   |      | CIP, NAL, TET           | 2-fold    |        |
| PRJNA872862 | SAMN34728955 | BFR-CA-17861 | Germany | turkey, cecum  |       | 03.03.2020 | Campylobacter coli   | 16     | >64 | >128 | >64   | 1    | 4   |      |       |     |     |   |      | CIP, ERY, NAL, TET      | 3-fold    |        |
| PRJNA872862 | SAMN34728956 | BFR-CA-17869 | Germany | broiler, meat  |       | 10.03.2020 | Campylobacter jejuni | <=0,12 | 4   | <=1  | <=0,5 | 1    | 2   |      |       |     |     |   |      | sensitive               | sensitive |        |

# 9 Appendix

|             |              |              |         |                |       |            |                      |        |     |      |       |      |     |       |       |     |      |     |    |     |                         |                              |                              |        |
|-------------|--------------|--------------|---------|----------------|-------|------------|----------------------|--------|-----|------|-------|------|-----|-------|-------|-----|------|-----|----|-----|-------------------------|------------------------------|------------------------------|--------|
| PRJNA872862 | SAMN34728957 | BFR-CA-17890 | Germany | broiler, meat  |       | 10.03.2020 | Campylobacter jejuni | 16     | >64 | <=1  | >64   | 0,5  | >16 | 256   | >1024 | 256 | <=2  | 2   |    | 8   | CIP, NAL, STR, TET      | 3-fold                       |                              |        |
| PRJNA872862 | SAMN34728958 | BFR-CA-17905 | Germany | turkey, cecum  |       | 17.03.2020 | Campylobacter coli   | 16     | 64  | <=1  | 64    | 1    | 2   |       |       |     |      |     |    |     | CIP, NAL, TET           | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34728959 | BFR-CA-17949 | Germany | broiler, liver |       | 21.04.2020 | Campylobacter coli   | <=0,12 | 4   | <=1  | <=0,5 | 0,5  | 1   |       |       |     |      |     |    |     | sensitive               | sensitive                    |                              |        |
| PRJNA872862 | SAMN34728960 | BFR-CA-17950 | Germany | broiler, liver |       | 21.04.2020 | Campylobacter coli   | <=0,12 | 4   | <=1  | <=0,5 | 0,5  | 2   |       |       |     |      |     |    |     | sensitive               | sensitive                    |                              |        |
| PRJNA872862 | SAMN34728961 | BFR-CA-17951 | Germany | broiler, skin  |       | 08.05.2020 | Campylobacter coli   | <=0,12 | 4   | <=1  | <=0,5 | 0,5  | 2   |       |       |     |      |     |    |     | sensitive               | sensitive                    |                              |        |
| PRJNA872862 | SAMN34728962 | BFR-CA-17959 | Germany | broiler, skin  |       | 08.05.2020 | Campylobacter jejuni | 16     | >64 | <=1  | <=0,5 | 0,5  | 1   |       |       |     |      |     |    |     | CIP, NAL                | 1-fold                       |                              |        |
| PRJNA872862 | SAMN34728963 | BFR-CA-17965 | Germany | broiler, cecum |       | 11.05.2020 | Campylobacter coli   | >16    | 64  | >128 | >64   | 1    | 4   | 512   | >1024 | 128 |      |     |    |     | CIP, ERY, NAL, TET      | 3-fold                       |                              |        |
| PRJNA872862 | SAMN34728964 | BFR-CA-17971 | Germany | turkey, cecum  |       | 21.04.2020 | Campylobacter coli   | 8      | >64 | <=1  | >64   | 1    | 4   |       |       |     |      |     |    |     | CIP, NAL, TET           | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34728965 | BFR-CA-17991 | Germany | broiler, skin  |       | 08.05.2020 | Campylobacter coli   | <=0,12 | 8   | 2    | <=0,5 | 0,25 | 1   |       |       |     |      |     |    |     | sensitive               | sensitive                    |                              |        |
| PRJNA872862 | SAMN34728966 | BFR-CA-17992 | Germany | broiler, cecum |       | 08.05.2020 | Campylobacter coli   | <=0,12 | 4   | <=1  | <=0,5 | 0,5  | 1   |       |       |     |      |     |    |     | sensitive               | sensitive                    |                              |        |
| PRJNA872862 | SAMN34728967 | BFR-CA-18000 | Germany | broiler, skin  |       | 23.05.2020 | Campylobacter coli   | >16    | >64 | >128 | >64   | 0,5  | 2   | >512  | >1024 | 32  |      |     |    |     | CIP, ERY, NAL, TET      | 3-fold                       |                              |        |
| PRJNA872862 | SAMN34728968 | BFR-CA-18036 | Germany | turkey, cecum  |       | 27.05.2020 | Campylobacter coli   | 16     | 64  | >128 | >64   | 0,5  | 2   | 512   | >1024 | 64  |      |     |    |     | CIP, ERY, NAL, TET      | 3-fold                       |                              |        |
| PRJNA872862 | SAMN34728969 | BFR-CA-18037 | Germany | broiler, meat  |       | 26.05.2020 | Campylobacter jejuni | 16     | >64 | <=1  | >64   | 0,25 | 0,5 |       |       |     |      |     |    |     | CIP, NAL, TET           | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34728970 | BFR-CA-18038 | Germany | broiler, meat  |       | 06.05.2020 | Campylobacter jejuni | 16     | >64 | <=1  | <=0,5 | 0,5  | 1   |       |       |     |      |     |    |     | CIP, NAL                | 1-fold                       |                              |        |
| PRJNA872862 | SAMN34728971 | BFR-CA-18039 | Germany | broiler, meat  |       | 02.06.2020 | Campylobacter jejuni | 16     | >64 | <=1  | >64   | 0,5  | 1   |       |       |     |      |     |    |     | CIP, NAL, TET           | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34728972 | BFR-CA-18045 | Germany | broiler, meat  |       | 08.06.2020 | Campylobacter coli   | <=0,12 | 4   | <=1  | <=0,5 | 0,5  | 2   |       |       |     |      |     |    |     | sensitive               | sensitive                    |                              |        |
| PRJNA872862 | SAMN34728973 | BFR-CA-18060 | Germany | broiler, egg   |       | 10.06.2020 | Campylobacter jejuni | <=0,12 | 2   | <=1  | <=0,5 | 0,5  | 1   |       |       |     |      |     |    |     | TET                     | 1-fold                       |                              |        |
| PRJNA872862 | SAMN34728974 | BFR-CA-18061 | Germany | broiler, meat  |       | 15.06.2020 | Campylobacter jejuni | 8      | >64 | <=1  | >64   | 0,5  | 1   |       |       |     |      |     |    |     | CIP, NAL, TET           | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34728975 | BFR-CA-18064 | Germany | broiler, liver |       | 21.04.2020 | Campylobacter jejuni | 16     | >64 | <=1  | >64   | 0,5  | 2   |       |       |     |      |     |    |     | CIP, NAL, TET           | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34728976 | BFR-CA-18096 | Germany | broiler, meat  |       | 24.06.2020 | Campylobacter jejuni | 8      | >64 | <=1  | 8     | 0,5  | 1   |       |       |     |      |     |    |     | CIP, NAL, TET           | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34728977 | BFR-CA-18102 | Germany | broiler, skin  |       | 29.06.2020 | Campylobacter coli   | >16    | >64 | 8    | >64   | 1    | 4   |       |       |     |      |     |    |     | CIP, NAL, TET           | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34728978 | BFR-CA-18104 | Germany | broiler, meat  |       | 22.06.2020 | Campylobacter jejuni | 0,25   | 4   | <=1  | <=0,5 | 1    | 2   |       |       |     |      |     |    |     | sensitive               | sensitive                    |                              |        |
| PRJNA872862 | SAMN34728979 | BFR-CA-18143 | Germany | broiler, meat  |       | 06.07.2020 | Campylobacter jejuni | <=0,12 | 4   | <=1  | <=0,5 | 0,5  | >16 | >512  | >1024 | 128 |      |     |    |     | STR                     | 1-fold                       |                              |        |
| PRJNA872862 | SAMN34728980 | BFR-CA-18167 | Germany | broiler, meat  |       | 06.07.2020 | Campylobacter jejuni | 8      | >64 | <=1  | <=0,5 | 0,5  | 1   |       |       |     |      |     |    |     | CIP, NAL                | 1-fold                       |                              |        |
| PRJNA872862 | SAMN34728981 | BFR-CA-18197 | Germany | broiler, meat  |       | 21.07.2020 | Campylobacter coli   | <=0,12 | 4   | <=1  | <=0,5 | 1    | 2   | 8     | 4     | <=1 | <=2  | 1   | 8  | 4   | sensitive               | sensitive                    |                              |        |
| PRJNA872862 | SAMN34728982 | BFR-CA-18248 | Germany | broiler, meat  |       | 30.06.2020 | Campylobacter jejuni | <=0,12 | 8   | <=1  | <=0,5 | 0,5  | 1   |       |       |     |      |     |    |     | sensitive               | sensitive                    |                              |        |
| PRJNA872862 | SAMN34728983 | BFR-CA-18264 | Germany | broiler, egg   |       | 22.07.2020 | Campylobacter coli   | 0,25   | 8   | 2    | <=0,5 | 1    | 1   |       |       |     |      |     |    |     | sensitive               | sensitive                    |                              |        |
| PRJNA872862 | SAMN34728984 | BFR-CA-18277 | Germany | broiler, meat  |       | 08.07.2020 | Campylobacter jejuni | 16     | >64 | <=1  | >64   | 0,5  | >16 | 512   | >1024 | 128 |      |     |    |     | CIP, NAL, STR, TET      | 3-fold                       |                              |        |
| PRJNA872862 | SAMN34728985 | BFR-CA-18280 | Germany | broiler, meat  |       | 20.07.2020 | Campylobacter jejuni | 16     | >64 | <=1  | >64   | 0,5  | >16 | 512   | >1024 | 128 |      |     |    |     | CIP, NAL, STR, TET      | 3-fold                       |                              |        |
| PRJNA872862 | SAMN34728986 | BFR-CA-18322 | Germany | broiler, meat  |       | 22.07.2020 | Campylobacter jejuni | 0,25   | 4   | <=1  | <=0,5 | 1    | 2   |       |       |     |      |     |    |     | sensitive               | sensitive                    |                              |        |
| PRJNA872862 | SAMN34728987 | BFR-CA-18353 | Germany | turkey, cecum  |       | 27.07.2020 | Campylobacter coli   | 16     | 64  | <=1  | 2     | 0,5  | 1   |       |       |     |      |     |    |     | CIP, NAL                | 1-fold                       |                              |        |
| PRJNA872862 | SAMN34728988 | BFR-CA-18372 | Germany | broiler, meat  |       | 11.08.2020 | Campylobacter jejuni | <=0,12 | 4   | <=1  | >64   | 0,5  | 1   |       |       |     |      |     |    |     | TET                     | 1-fold                       |                              |        |
| PRJNA872862 | SAMN34728989 | BFR-CA-18391 | Germany | broiler, meat  |       | 10.08.2020 | Campylobacter jejuni | 16     | >64 | <=1  | 64    | 0,5  | 1   | 512   |       |     |      |     |    |     | CIP, NAL, TET           | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34728990 | BFR-CA-18407 | Germany | broiler, meat  |       | 11.08.2020 | Campylobacter jejuni | 16     | >64 | <=1  | <=0,5 | 0,5  | 0,5 | 32    | >1024 | 64  |      |     |    |     | CIP, NAL                | 1-fold                       |                              |        |
| PRJNA872862 | SAMN34728991 | BFR-CA-18408 | Germany | duck, meat     |       | 12.08.2020 | Campylobacter coli   | 16     | >64 | <=1  | >64   | 0,5  | 2   | >1024 | <=1   |     |      |     |    |     | CIP, NAL, TET           | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34728992 | BFR-CA-18548 | Germany | broiler, meat  |       | 24.08.2020 | Campylobacter jejuni | 8      | >64 | <=1  | >64   | 0,25 | 0,5 |       |       |     |      |     |    |     | CIP, NAL, TET           | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34728993 | BFR-CA-18564 | Germany | broiler, meat  |       | 31.08.2020 | Campylobacter jejuni | 16     | >64 | <=1  | >64   | 0,5  | 16  | 256   | >1024 | 256 |      |     |    |     | CIP, NAL, STR, TET      | 3-fold                       |                              |        |
| PRJNA872862 | SAMN34728994 | BFR-CA-18580 | Germany | broiler, meat  |       | 08.09.2020 | Campylobacter jejuni | 16     | >64 | <=1  | >64   | 0,5  | 1   |       |       |     |      |     |    |     | CIP, NAL, TET           | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34728995 | BFR-CA-18585 | Germany | broiler, meat  |       | 01.09.2020 | Campylobacter jejuni | 16     | >64 | <=1  | >64   | 0,5  | >16 | 128   | >1024 | 256 | <=2  | 2   | 16 | 8   | CIP, NAL, STR, TET      | 3-fold                       |                              |        |
| PRJNA872862 | SAMN34728996 | BFR-CA-18592 | Germany | broiler, skin  |       | 25.08.2020 | Campylobacter coli   | >16    | >64 | <=1  | >64   | 0,5  | 1   | >512  | >1024 | 32  |      |     |    |     | CIP, NAL, TET           | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34728997 | BFR-CA-18665 | Germany | broiler, meat  |       | 02.09.2020 | Campylobacter jejuni | >16    | >64 | 4    | <=0,5 | 0,5  | >16 | 256   | >1024 | 256 |      |     |    |     | CIP, NAL, STR           | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34728998 | BFR-CA-18683 | Vietnam | broiler, feces | CG96  | 25.12.2016 | Campylobacter jejuni | >16    | >64 | <=1  | >64   | 0,5  | 1   |       |       |     |      |     |    |     | CIP, NAL, TET           | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34728999 | BFR-CA-18684 | Vietnam | broiler, feces | CG110 | 12.01.2017 | Campylobacter jejuni | >16    | >64 | <=1  | >64   | >16  | 1   |       |       |     |      |     |    | 64  | >512                    | CIP, GEN, NAL, TET           | 3-fold                       |        |
| PRJNA872862 | SAMN34729000 | BFR-CA-18685 | Vietnam | broiler, feces | CG118 | 12.01.2017 | Campylobacter coli   | >16    | >64 | >128 | >64   | 0,5  | >16 |       | >1024 | 64  |      |     |    | 64  | >512                    | CIP, ERY, NAL, STR, TET      | 4-fold                       |        |
| PRJNA872862 | SAMN34729001 | BFR-CA-18686 | Vietnam | broiler, feces | CG136 | 13.02.2017 | Campylobacter jejuni | >16    | >64 | <=1  | >64   | 0,5  | 1   |       |       |     |      |     |    |     | CIP, NAL, TET           | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34729002 | BFR-CA-18687 | Vietnam | broiler, feces | CG138 | 13.02.2017 | Campylobacter coli   | >16    | >64 | >128 | >64   | >16  | >16 |       |       |     |      |     |    | 128 | 512                     | CIP, ERY, GEN, NAL, STR, TET | 4-fold                       |        |
| PRJNA872862 | SAMN34729003 | BFR-CA-18689 | Vietnam | broiler, feces | CG146 | 10.03.2017 | Campylobacter jejuni | >16    | >64 | <=1  | >64   | >16  | 0,5 | >512  | >1024 | <=1 | >128 | >16 | 8  | 64  | CIP, GEN, NAL, TET      | 3-fold                       |                              |        |
| PRJNA872862 | SAMN34729004 | BFR-CA-18691 | Vietnam | broiler, feces | CG199 | 09.04.2017 | Campylobacter jejuni | 16     | >64 | <=1  | 32    | 0,5  | 2   |       |       |     |      |     |    |     | CIP, NAL, TET           | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34729005 | BFR-CA-18694 | Vietnam | broiler, feces | CG243 | 15.05.2017 | Campylobacter coli   | 16     | >64 | >128 | >64   | 0,5  | >16 |       | >1024 | 64  |      |     |    |     | CIP, ERY, NAL, STR, TET | 4-fold                       |                              |        |
| PRJNA872862 | SAMN34729006 | BFR-CA-18695 | Vietnam | broiler, feces | CG250 | 18.06.2017 | Campylobacter jejuni | 8      | >64 | <=1  | >64   | 0,5  | 1   |       |       |     |      |     |    |     | CIP, NAL, TET           | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34729007 | BFR-CA-18717 | Vietnam | broiler, feces | CG70  | 24.11.2016 | Campylobacter jejuni | 16     | >64 | <=1  | 64    | 0,5  | 1   |       |       |     |      |     |    |     | CIP, NAL, TET           | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34729008 | BFR-CA-18718 | Vietnam | broiler, feces | CG210 | 03.05.2017 | Campylobacter coli   | >16    | >64 | 2    | >64   | 0,5  | 1   |       |       |     |      |     |    |     | CIP, NAL, TET           | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34729009 | BFR-CA-18719 | Vietnam | broiler, feces | CG211 | 03.05.2017 | Campylobacter jejuni | >16    | >64 | 2    | >64   | 0,5  | 1   |       |       |     |      |     |    |     | CIP, NAL, TET           | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34729010 | BFR-CA-18728 | Vietnam | broiler, feces | CG251 | 18.06.2017 | Campylobacter coli   | >16    | >64 | >128 | >64   | >16  | >16 |       |       |     |      |     |    | 128 | >16                     | >512                         | CIP, ERY, GEN, NAL, STR, TET | 4-fold |
| PRJNA872862 | SAMN34729011 | BFR-CA-18729 | Vietnam | broiler, feces | CG254 | 18.06.2017 | Campylobacter jejuni | >16    | >64 | <=1  | >64   | 0,25 | 16  | 1024  |       |     |      |     |    |     | 128                     | >512                         | CIP, NAL, STR, TET           | 3-fold |
| PRJNA872862 | SAMN34729012 | BFR-CA-18731 | Vietnam | broiler, feces | CG262 | 16.07.2017 | Campylobacter jejuni |        |     |      |       |      |     |       |       |     |      |     |    |     |                         |                              |                              |        |

# 9 Appendix

|             |              |              |         |                |       |            |                      |        |     |      |       |      |     |       |       |      |      |                              |                              |                              |                              |        |
|-------------|--------------|--------------|---------|----------------|-------|------------|----------------------|--------|-----|------|-------|------|-----|-------|-------|------|------|------------------------------|------------------------------|------------------------------|------------------------------|--------|
| PRJNA872862 | SAMN34729014 | BFR-CA-18734 | Vietnam | broiler, feces | CG320 | 09.10.2017 | Campylobacter jejuni | >16    | >64 | <=1  | >64   | >16  | >16 |       |       |      | 128  | >512                         | CIP, GEN, NAL, STR, TET      | 3-fold                       |                              |        |
| PRJNA872862 | SAMN34729015 | BFR-CA-18735 | Vietnam | broiler, feces | CG364 | 02.11.2017 | Campylobacter jejuni | 8      | >64 | <=1  | 64    | 0,5  | 2   |       |       |      |      |                              | CIP, NAL, TET                | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34729016 | BFR-CA-18736 | Vietnam | broiler, feces | CG365 | 02.11.2017 | Campylobacter coli   | >16    | >64 | >128 | >64   | >16  | >16 |       |       | >128 | >512 | CIP, ERY, GEN, NAL, STR, TET | 4-fold                       |                              |                              |        |
| PRJNA872862 | SAMN34729017 | BFR-CA-18737 | Vietnam | broiler, feces | CG366 | 15.11.2017 | Campylobacter coli   | 16     | >64 | >128 | >64   | >16  | >16 | >512  | >1024 |      | 128  | >16                          | >512                         | CIP, ERY, GEN, NAL, STR, TET | 4-fold                       |        |
| PRJNA872862 | SAMN34729018 | BFR-CA-18738 | Vietnam | broiler, feces | CG367 | 15.11.2017 | Campylobacter coli   | >16    | >64 | >128 | >64   | >16  | >16 | >512  | >1024 |      | >128 | >16                          | >512                         | CIP, ERY, GEN, NAL, STR, TET | 4-fold                       |        |
| PRJNA872862 | SAMN34729019 | BFR-CA-18743 | Germany | broiler, meat  |       | 18.08.2020 | Campylobacter jejuni | 16     | >64 | <=1  | >64   | 0,5  | >16 | 256   | >1024 | 256  |      |                              | CIP, NAL, STR, TET           | 3-fold                       |                              |        |
| PRJNA872862 | SAMN34729020 | BFR-CA-18748 | Germany | turkey, cecum  |       | 04.09.2020 | Campylobacter coli   | >16    | >64 | <=1  | >64   | 0,5  | 2   | >512  | >1024 | 16   |      |                              | CIP, NAL, TET                | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34729021 | BFR-CA-18820 | Vietnam | broiler, feces | CG369 | 15.11.2017 | Campylobacter jejuni | >16    | >64 | <=1  | >64   | 0,5  | 1   |       |       |      |      |                              | CIP, NAL, TET                | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34729022 | BFR-CA-18821 | Vietnam | broiler, feces | CG371 | 15.11.2017 | Campylobacter coli   | 16     | >64 | >128 | >64   | >16  | >16 | >512  | >1024 |      | 128  | >16                          | >512                         | CIP, ERY, GEN, NAL, STR, TET | 4-fold                       |        |
| PRJNA872862 | SAMN34729023 | BFR-CA-18822 | Vietnam | broiler, feces | CG372 | 15.11.2017 | Campylobacter coli   | >16    | 64  | 2    | >64   | 1    | 2   |       |       |      |      |                              | CIP, NAL, TET                | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34729024 | BFR-CA-18825 | Vietnam | broiler, feces | CG378 | 15.11.2017 | Campylobacter jejuni | >16    | >64 | <=1  | >64   | 0,5  | 1   |       |       |      |      |                              | CIP, NAL, TET                | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34729025 | BFR-CA-18826 | Vietnam | broiler, feces | CG380 | 15.11.2017 | Campylobacter coli   | 16     | >64 | >128 | >64   | >16  | 2   |       |       | 64   | >512 | CIP, ERY, GEN, NAL, TET      | 4-fold                       |                              |                              |        |
| PRJNA872862 | SAMN34729026 | BFR-CA-18834 | Germany | turkey, cecum  |       | 19.09.2020 | Campylobacter coli   | 8      | 64  | 64   | <=0,5 | 0,5  | 2   |       |       |      |      |                              | CIP, ERY, NAL                | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34729027 | BFR-CA-18839 | Vietnam | broiler, feces | CG368 | 15.11.2017 | Campylobacter jejuni | 16     | >64 | <=1  | >64   | 0,5  | 2   |       |       |      |      |                              | CIP, NAL, TET                | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34729028 | BFR-CA-18840 | Vietnam | broiler, feces | CG370 | 15.11.2017 | Campylobacter jejuni | 8      | >64 | <=1  | >64   | 0,5  | 1   |       |       |      |      |                              | CIP, NAL, TET                | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34729029 | BFR-CA-18841 | Vietnam | broiler, feces | CG373 | 15.11.2017 | Campylobacter jejuni | >16    | >64 | 2    | >64   | 1    | 2   |       |       |      |      |                              | CIP, NAL, TET                | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34729030 | BFR-CA-18842 | Vietnam | broiler, feces | CG375 | 15.11.2017 | Campylobacter coli   | 16     | >64 | 2    | >64   | >16  | >16 |       |       |      |      |                              | CIP, GEN, NAL, STR, TET      | 3-fold                       |                              |        |
| PRJNA872862 | SAMN34729031 | BFR-CA-18843 | Vietnam | broiler, feces | CG377 | 15.11.2017 | Campylobacter coli   | 16     | >64 | >128 | >64   | >16  | >16 |       |       | 128  | >16  | >512                         | CIP, ERY, GEN, NAL, STR, TET | 4-fold                       |                              |        |
| PRJNA872862 | SAMN34729032 | BFR-CA-18844 | Vietnam | broiler, feces | CG379 | 15.11.2017 | Campylobacter jejuni | >16    | >64 | <=1  | >64   | 0,5  | 1   |       |       |      |      |                              | CIP, NAL, TET                | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34729033 | BFR-CA-18869 | Germany | broiler, meat  |       | 28.09.2020 | Campylobacter jejuni | <=0,12 | 16  | 4    | <=0,5 | 0,5  | 2   |       |       |      |      |                              | sensitive                    | sensitive                    |                              |        |
| PRJNA872862 | SAMN34729034 | BFR-CA-18879 | Vietnam | broiler, feces | CG390 | 15.11.2017 | Campylobacter coli   | >16    | 64  | >128 | >64   | >16  | >16 |       | <=1   | 64   | >512 | CIP, ERY, GEN, NAL, STR, TET | 4-fold                       |                              |                              |        |
| PRJNA872862 | SAMN34729035 | BFR-CA-18880 | Vietnam | broiler, feces | CG391 | 01.12.2017 | Campylobacter coli   | >16    | >64 | 8    | >64   | 0,5  | >16 |       |       |      |      |                              | CIP, NAL, STR, TET           | 3-fold                       |                              |        |
| PRJNA872862 | SAMN34729036 | BFR-CA-18881 | Vietnam | broiler, feces | CG392 | 01.12.2017 | Campylobacter jejuni | >16    | >64 | <=1  | >64   | 0,5  | 1   |       |       |      |      |                              | CIP, NAL, TET                | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34729037 | BFR-CA-18883 | Vietnam | broiler, feces | CG396 | 01.12.2017 | Campylobacter coli   | >16    | >64 | >128 | >64   | >16  | >16 |       | <=1   |      | >512 | CIP, ERY, GEN, NAL, STR, TET | 4-fold                       |                              |                              |        |
| PRJNA872862 | SAMN34729038 | BFR-CA-18884 | Vietnam | broiler, feces | CG397 | 01.12.2017 | Campylobacter jejuni | >16    | >64 | <=1  | >64   | 0,5  | 1   |       |       |      |      |                              | CIP, NAL, TET                | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34729039 | BFR-CA-18885 | Vietnam | broiler, feces | CG398 | 01.12.2017 | Campylobacter coli   | >16    | >64 | >128 | >64   | >16  | >16 |       |       | 128  | >512 | CIP, ERY, GEN, NAL, STR, TET | 4-fold                       |                              |                              |        |
| PRJNA872862 | SAMN34729040 | BFR-CA-18886 | Vietnam | broiler, feces | CG399 | 01.12.2017 | Campylobacter coli   | 16     | >64 | >128 | >64   | 0,5  | >16 | 8     | <=1   |      | >512 | CIP, ERY, NAL, STR, TET      | 4-fold                       |                              |                              |        |
| PRJNA872862 | SAMN34729041 | BFR-CA-18887 | Vietnam | broiler, feces | CG400 | 01.12.2017 | Campylobacter coli   | >16    | >64 | 4    | >64   | >16  | >16 |       |       |      |      |                              | CIP, GEN, NAL, STR, TET      | 3-fold                       |                              |        |
| PRJNA872862 | SAMN34729042 | BFR-CA-18889 | Vietnam | broiler, feces | CG404 | 01.12.2017 | Campylobacter jejuni | >16    | >64 | <=1  | >64   | 0,25 | 1   |       |       |      |      |                              | CIP, NAL, TET                | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34729043 | BFR-CA-18891 | Vietnam | broiler, feces | CG406 | 01.12.2017 | Campylobacter jejuni | >16    | >64 | 2    | >64   | 0,5  | 1   |       |       |      |      |                              | CIP, NAL, TET                | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34729044 | BFR-CA-18892 | Vietnam | broiler, feces | CG407 | 01.12.2017 | Campylobacter jejuni | >16    | >64 | <=1  | >64   | 0,5  | 1   |       |       |      |      |                              | CIP, NAL, TET                | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34729045 | BFR-CA-18894 | Germany | broiler, meat  |       | 06.10.2020 | Campylobacter jejuni | <=0,12 | 4   | <=1  | <=0,5 | 0,25 | 1   |       |       |      |      |                              | sensitive                    | sensitive                    |                              |        |
| PRJNA872862 | SAMN34729046 | BFR-CA-18901 | Germany | broiler, meat  |       | 15.10.2020 | Campylobacter jejuni | 16     | >64 | <=1  | <=0,5 | 0,5  | 1   |       |       |      |      |                              | CIP, NAL                     | 1-fold                       |                              |        |
| PRJNA872862 | SAMN34729047 | BFR-CA-19025 | Vietnam | broiler, cecum | CG408 | 15.01.2018 | Campylobacter coli   | 16     | >64 | 2    | >64   | >16  | >16 | 64    |       | 4    | >512 | CIP, GEN, NAL, STR, TET      | 3-fold                       |                              |                              |        |
| PRJNA872862 | SAMN34729048 | BFR-CA-19026 | Vietnam | broiler, cecum | CG409 | 15.01.2018 | Campylobacter coli   | 16     | >64 | 2    | >64   | 0,5  | >16 |       |       | 8    | >512 | CIP, NAL, STR, TET           | 3-fold                       |                              |                              |        |
| PRJNA872862 | SAMN34729049 | BFR-CA-19027 | Vietnam | broiler, cecum | CG410 | 15.01.2018 | Campylobacter coli   | 16     | >64 | >128 | >64   | >16  | >16 |       |       | 128  | >512 | CIP, ERY, GEN, NAL, STR, TET | 4-fold                       |                              |                              |        |
| PRJNA872862 | SAMN34729050 | BFR-CA-19028 | Vietnam | broiler, cecum | CG411 | 15.01.2018 | Campylobacter coli   | 16     | >64 | 2    | >64   | >16  | >16 |       |       |      |      |                              | CIP, ERY, GEN, NAL, STR, TET | 3-fold                       |                              |        |
| PRJNA872862 | SAMN34729051 | BFR-CA-19029 | Vietnam | broiler, cecum | CG412 | 15.01.2018 | Campylobacter coli   | 16     | 64  | 64   | >64   | >16  | >16 |       |       | 64   | >512 | CIP, ERY, GEN, NAL, STR, TET | 4-fold                       |                              |                              |        |
| PRJNA872862 | SAMN34729052 | BFR-CA-19030 | Vietnam | broiler, cecum | CG413 | 15.01.2018 | Campylobacter coli   | 16     | >64 | 64   | >64   | >16  | >16 |       |       | 128  | >512 | CIP, ERY, GEN, NAL, STR, TET | 4-fold                       |                              |                              |        |
| PRJNA872862 | SAMN34729053 | BFR-CA-19031 | Vietnam | broiler, cecum | CG414 | 15.01.2018 | Campylobacter coli   | 16     | 64  | 64   | >64   | >16  | >16 |       |       | 64   | 4    | >512                         | CIP, ERY, GEN, NAL, STR, TET | 4-fold                       |                              |        |
| PRJNA872862 | SAMN34729054 | BFR-CA-19032 | Vietnam | broiler, meat  | CG415 | 15.01.2018 | Campylobacter coli   | >16    | >64 | >128 | >64   | >16  | >16 | 64    |       | 64   | 4    | >512                         | CIP, ERY, GEN, NAL, STR, TET | 4-fold                       |                              |        |
| PRJNA872862 | SAMN34729055 | BFR-CA-19033 | Vietnam | broiler, meat  | CG416 | 15.01.2018 | Campylobacter coli   | >16    | >64 | 64   | >64   | >16  | >16 | 64    | >1024 | 64   | <=2  | 8                            | >512                         | >128                         | CIP, ERY, GEN, NAL, STR, TET | 4-fold |
| PRJNA872862 | SAMN34729056 | BFR-CA-19034 | Vietnam | broiler, meat  | CG417 | 15.01.2018 | Campylobacter coli   | >16    | >64 | >128 | >64   | >16  | >16 |       |       | 128  | 4    | >512                         | CIP, ERY, GEN, NAL, STR, TET | 4-fold                       |                              |        |
| PRJNA872862 | SAMN34729057 | BFR-CA-19035 | Vietnam | broiler, cecum | CG418 | 01.02.2018 | Campylobacter jejuni | >16    | >64 | <=1  | >64   | 0,5  | 1   |       |       |      |      |                              | CIP, NAL, TET                | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34729058 | BFR-CA-19036 | Vietnam | broiler, cecum | CG419 | 01.02.2018 | Campylobacter jejuni | >16    | >64 | <=1  | >64   | 0,5  | 1   |       |       |      |      |                              | CIP, NAL, TET                | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34729059 | BFR-CA-19037 | Vietnam | broiler, cecum | CG420 | 01.02.2018 | Campylobacter coli   | >16    | >64 | >128 | >64   | >16  | >16 |       | >1024 | 64   | >512 | >128                         | CIP, ERY, GEN, NAL, STR, TET | 4-fold                       |                              |        |
| PRJNA872862 | SAMN34729060 | BFR-CA-19038 | Vietnam | broiler, cecum | CG421 | 01.02.2018 | Campylobacter coli   | 16     | 64  | >128 | >64   | >16  | >16 |       |       |      |      |                              | CIP, ERY, GEN, NAL, STR, TET | 4-fold                       |                              |        |
| PRJNA872862 | SAMN34729061 | BFR-CA-19039 | Vietnam | broiler, cecum | CG422 | 01.02.2018 | Campylobacter coli   | 8      | >64 | >128 | >64   | 0,5  | >16 | 8     | <=1   |      | >512 | CIP, ERY, NAL, STR, TET      | 4-fold                       |                              |                              |        |
| PRJNA872862 | SAMN34729062 | BFR-CA-19040 | Vietnam | broiler, meat  | CG423 | 01.02.2018 | Campylobacter coli   | >16    | >64 | >128 | >64   | 0,5  | 16  | 8     | <=1   |      | >512 | CIP, ERY, NAL, STR, TET      | 4-fold                       |                              |                              |        |
| PRJNA872862 | SAMN34729063 | BFR-CA-19044 | Germany | broiler, meat  |       | 28.10.2020 | Campylobacter coli   | 16     | 64  | >128 | >64   | 0,5  | 2   | >1024 | 256   |      | 8    |                              | CIP, ERY, NAL, TET           | 3-fold                       |                              |        |
| PRJNA872862 | SAMN34729064 | BFR-CA-19052 | Germany | broiler, cecum |       | 02.11.2020 | Campylobacter jejuni | 8      | >64 | <=1  | <=0,5 | 0,5  | 0,5 |       |       |      |      |                              | CIP, NAL                     | 1-fold                       |                              |        |
| PRJNA872862 | SAMN34729065 | BFR-CA-19053 | Germany | broiler, cecum |       | 02.11.2020 | Campylobacter jejuni | 8      | 2   | <=1  | <=0,5 | 0,5  | 1   |       |       |      |      |                              | CIP                          | 1-fold                       |                              |        |
| PRJNA872862 | SAMN34729066 | BFR-CA-19055 | Germany | broiler, skin  |       | 02.11.2020 | Campylobacter jejuni | 16     | 2   | <=1  | <=0,5 | 0,5  | 1   |       |       |      |      |                              | CIP                          | 1-fold                       |                              |        |
| PRJNA872862 | SAMN34729067 | BFR-CA-19058 | Germany | turkey, cecum  |       | 12.10.2020 | Campylobacter coli   | >16    | >64 | <=1  | >64   | 0,5  | 1   | >512  | >1024 | 16   |      |                              | CIP, NAL, TET                | 2-fold                       |                              |        |
| PRJNA872862 | SAMN34729068 | BFR-CA-19082 | Germany | broiler, skin  |       | 02.11.2020 | Campylobacter jejuni | >16    | >64 | <=1  | >64   | 0,25 | >16 | 512   | >1024 | 128  |      |                              | CIP, NAL, STR, TET           | 3-fold                       |                              |        |
| PRJNA872862 | SAMN34729069 | BFR-CA-19085 | Germany | broiler, cecum |       | 04.11.2020 | Campylobacter jejuni | 16     | >64 | <=1  | <=0,5 | 0,5  | 1   |       |       |      |      |                              | CIP, NAL                     | 1-fold                       |                              |        |





# 9 Appendix

|             |              |              |         |                |            |                      |        |     |     |       |      |     |      |       |     |  |  |  |  |                    |           |
|-------------|--------------|--------------|---------|----------------|------------|----------------------|--------|-----|-----|-------|------|-----|------|-------|-----|--|--|--|--|--------------------|-----------|
| PRJNA872862 | SAMN34729127 | BFR-CA-19286 | Germany | poultry, meat  | 09.09.2020 | Campylobacter jejuni | 16     | >64 | <=1 | >64   | 0,5  | >16 | 256  | >1024 | 256 |  |  |  |  | CIP, NAL, STR, TET | 3-fold    |
| PRJNA872862 | SAMN34729128 | BFR-CA-19287 | Germany | broiler, meat  | 08.12.2020 | Campylobacter jejuni | <=0,12 | 4   | <=1 | <=0,5 | 0,5  | 1   |      |       |     |  |  |  |  | sensitive          | sensitive |
| PRJNA872862 | SAMN34729129 | BFR-CA-19288 | Germany | broiler, cecum | 08.12.2020 | Campylobacter jejuni | 16     | >64 | <=1 | >64   | 0,5  | 1   |      |       |     |  |  |  |  | CIP, NAL, TET      | 2-fold    |
| PRJNA872862 | SAMN34729130 | BFR-CA-19289 | Germany | broiler, cecum | 07.12.2020 | Campylobacter coli   | <=0,12 | 4   | <=1 | >64   | 0,5  | 2   |      |       |     |  |  |  |  | TET                | 1-fold    |
| PRJNA872862 | SAMN34729131 | BFR-CA-19290 | Germany | broiler, skin  | 08.12.2020 | Campylobacter coli   | <=0,12 | 8   | <=1 | >64   | 0,5  | 2   |      |       |     |  |  |  |  | TET                | 1-fold    |
| PRJNA872862 | SAMN34729132 | BFR-CA-19295 | Germany | broiler, skin  | 17.11.2020 | Campylobacter jejuni | 16     | >64 | <=1 | >64   | 0,5  | 1   |      |       |     |  |  |  |  | CIP, NAL, TET      | 2-fold    |
| PRJNA872862 | SAMN34729133 | BFR-CA-19298 | Germany | broiler, meat  | 29.10.2020 | Campylobacter jejuni | 4      | >64 | <=1 | <=0,5 | 0,25 | 0,5 |      |       |     |  |  |  |  | CIP, NAL           | 1-fold    |
| PRJNA872862 | SAMN34729134 | BFR-CA-19301 | Germany | broiler, meat  | 12.11.2020 | Campylobacter jejuni | 16     | >64 | <=1 | >64   | 0,5  | >16 | 256  | >1024 | 256 |  |  |  |  | CIP, NAL, STR, TET | 3-fold    |
|             |              |              |         |                | 01.12.2020 |                      |        |     |     |       |      |     |      |       |     |  |  |  |  | CIP, NAL, TET      | 2-fold    |
| PRJNA872862 | SAMN34729135 | BFR-CA-19311 | Germany | broiler, cecum |            | Campylobacter jejuni | >16    | 64  | <=1 | >64   | 0,25 | 0,5 | >512 | >1024 | 256 |  |  |  |  |                    |           |
| PRJNA872862 | SAMN34729136 | BFR-CA-19312 | Germany | broiler, skin  | 16.11.2020 | Campylobacter coli   | 16     | 64  | <=1 | >64   | 0,5  | >16 |      |       |     |  |  |  |  | CIP, NAL, STR, TET | 3-fold    |
| PRJNA872862 | SAMN34729137 | BFR-CA-19318 | Germany | broiler, skin  | 02.11.2020 | Campylobacter jejuni | <=0,12 | 8   | <=1 | 32    | 0,5  | 1   |      |       |     |  |  |  |  | TET                | 1-fold    |
| PRJNA872862 | SAMN34729138 | BFR-CA-19320 | Germany | broiler, meat  | 09.11.2020 | Campylobacter jejuni | 8      | >64 | <=1 | <=0,5 | 0,5  | 1   |      |       |     |  |  |  |  | CIP, NAL           | 1-fold    |
| PRJNA872862 | SAMN34729139 | BFR-CA-19328 | Germany | broiler, meat  | 04.11.2020 | Campylobacter coli   | <=0,12 | 4   | <=1 | 64    | 0,5  | 2   |      |       |     |  |  |  |  | TET                | 1-fold    |
| PRJNA872862 | SAMN34729140 | BFR-CA-19329 | Germany | broiler, meat  | 10.11.2020 | Campylobacter jejuni | 8      | >64 | <=1 | >64   | 0,5  | 0,5 |      |       |     |  |  |  |  | CIP, NAL, TET      | 2-fold    |
| PRJNA872862 | SAMN34729141 | BFR-CA-19336 | Germany | broiler, meat  | 12.01.2021 | Campylobacter jejuni | 8      | >64 | <=1 | <=0,5 | 0,5  | 1   |      |       |     |  |  |  |  | CIP, NAL           | 1-fold    |
| PRJNA872862 | SAMN34729142 | BFR-CA-19362 | Germany | broiler, meat  | 16.02.2021 | Campylobacter jejuni | 8      | >64 | <=1 | >64   | 0,5  | 1   |      |       |     |  |  |  |  | CIP, NAL, TET      | 2-fold    |
| PRJNA872862 | SAMN34729143 | BFR-CA-19364 | Germany | broiler, meat  | 17.02.2021 | Campylobacter jejuni | 8      | >64 | <=1 | >64   | 0,5  | 1   |      |       |     |  |  |  |  | CIP, NAL, TET      | 2-fold    |





# 9 Appendix

|              |           |               |        |        |  |                             |    |            |                      |                      |         |           |            |       |        |                       |      |                  |      |    |   |
|--------------|-----------|---------------|--------|--------|--|-----------------------------|----|------------|----------------------|----------------------|---------|-----------|------------|-------|--------|-----------------------|------|------------------|------|----|---|
| BRR-CA-16106 | Gyra_T86  |               | erm(B) |        |  | tel(O/M)                    |    | app(2)*-IF |                      | aaef1                |         |           | app(F)-IHa | aaef1 | catA13 |                       |      | bioDXA-193       |      | 9  | 6 |
| BRR-CA-16107 | Gyra_T86  |               | erm(B) |        |  | tel(O/M/O) + tel(OH)        |    | app(2)*-IF | aac(0)*-h/aph(2)*-la | aaef1, aaef2, A1-169 |         |           | app(F)-IHa | aaef1 | catA13 |                       |      | bioDXA-193       | catA | 10 | 6 |
| BRR-CA-16108 | Gyra_T86  | 505_L22_A103V |        |        |  | tel(O/M/O)                  |    | app(2)*-IF |                      |                      |         |           | app(F)-IHa | aaef1 | catA13 |                       |      | bioDXA-193       |      | 8  | 6 |
| BRR-CA-16109 | Gyra_T86  | 235_A2075G    |        |        |  | tel(O/M/O)                  |    | app(2)*-IF |                      | aaef1                | aaef-Cc |           | app(F)-IHa | aaef1 | catA13 |                       |      | bioDXA-193       |      | 8  | 6 |
| BRR-CA-16110 | Gyra_T86  |               | erm(B) | tel(O) |  |                             |    |            |                      |                      |         |           |            |       |        |                       |      | bioDXA-193       |      | 6  | 6 |
| BRR-CA-16111 | Gyra_T86  | 235_A2075G    |        |        |  | tel(O/M/O) Tawaanentel(1)   |    | app(2)*-IF |                      | aaef1                | aaef-Cc |           | app(F)-IHa | aaef1 | catA13 | optA                  | lexA | bioDXA-489       |      | 13 | 7 |
| BRR-CA-16112 | Gyra_T86V |               |        |        |  | tel(O/M/O)                  |    |            |                      |                      |         |           |            |       |        |                       |      | bioDXA-193       |      | 3  | 3 |
| BRR-CA-16106 | Gyra_T86  | 505_L22_A103V |        |        |  | tel(O)                      |    |            |                      |                      |         |           |            |       |        |                       |      | bioDXA-193       |      | 4  | 4 |
| BRR-CA-16101 | Gyra_T86  | 235_A2075G    |        |        |  | tel(O)                      |    |            |                      | aaef1                |         |           | app(F)-IHa | aaef1 | catA13 |                       |      | bioDXA-193       |      | 3  | 3 |
| BRR-CA-16101 | Gyra_T86  | 235_A2075G    |        |        |  | tel(O/M/O) + tel(OH)        |    | app(2)*-IF | aac(0)*-h/aph(2)*-la | aaef1                |         |           | app(F)-IHa | aaef1 | catA13 |                       |      | bioDXA-193       |      | 9  | 6 |
| BRR-CA-16101 | Gyra_T86  |               |        |        |  | tel(O)                      |    | app(2)*-IF |                      |                      |         | ppul_K43R | app(F)-IHa | aaef1 | catA13 |                       |      | bioDXA-193       |      | 8  | 5 |
| BRR-CA-16106 | Gyra_T86  | 235_A2075G    |        |        |  | tel(O)                      |    | app(2)*-IF |                      |                      |         | ppul_K13R | app(F)-IHa | aaef1 | catA13 | optA                  | lexA | bioDXA-193       |      | 13 | 7 |
| BRR-CA-16107 | Gyra_T86  |               |        |        |  | tel(O)                      |    |            |                      |                      |         |           |            |       |        |                       |      | bioDXA-193       |      | 3  | 3 |
| BRR-CA-16108 | Gyra_T86  | 505_L22_A103V |        |        |  | tel(O/M/O)                  |    |            |                      | aaef1                |         |           | app(F)-IHa | aaef1 | catA13 |                       |      | bioDXA-193       |      | 4  | 4 |
| BRR-CA-16201 | Gyra_T86  | 505_L22_A103V | erm(B) | tel(O) |  |                             |    | app(2)*-IF |                      | aaef1                |         |           | app(F)-IHa | aaef1 | catA13 |                       |      | bioDXA-193       |      | 10 | 6 |
| BRR-CA-16204 | Gyra_T86  |               |        |        |  | tel(O)                      |    | app(2)*-IF |                      | aaef1                |         |           | app(F)-IHa | aaef1 | catA13 |                       |      | bioDXA-193       |      | 10 | 6 |
| BRR-CA-16207 | Gyra_T86  |               |        |        |  | tel(O/M/O)                  |    | app(2)*-IF |                      |                      |         |           | app(F)-IHa | aaef1 | catA13 |                       |      | bioDXA-193       |      | 7  | 5 |
| BRR-CA-16208 | Gyra_T86  | 505_L22_A103V |        |        |  | tel(O)                      |    |            |                      |                      |         |           | app(F)-IHa |       |        |                       |      | bioDXA-91 family |      | 3  | 3 |
| BRR-CA-16209 | Gyra_T86  |               |        |        |  | tel(O)                      |    |            |                      |                      |         |           | app(F)-IHa |       |        |                       |      | bioDXA-193       |      | 5  | 5 |
| BRR-CA-16210 | Gyra_T86  |               |        |        |  | tel(O/M/O)                  |    |            |                      |                      |         |           | app(F)-IHa |       |        |                       |      | bioDXA-184       |      | 3  | 3 |
| BRR-CA-16211 | Gyra_T86  |               |        |        |  | tel(O/M/O)                  |    | app(2)*-IF |                      |                      |         |           | app(F)-IHa | aaef1 | catA13 |                       |      | bioDXA-193       |      | 7  | 5 |
| BRR-CA-16215 | Gyra_T86  |               |        |        |  | tel(O/M/O)                  |    |            |                      |                      |         |           | app(F)-IHa |       |        |                       |      | bioDXA-193       |      | 3  | 3 |
| BRR-CA-16216 | Gyra_T86  | 505_L22_A103V |        |        |  | tel(O/M/O)                  |    |            |                      |                      |         |           |            |       |        |                       |      | bioDXA-193       |      | 3  | 3 |
| BRR-CA-16217 | Gyra_T86  | 505_L22_A103V |        |        |  | tel(O/M/O)                  |    |            |                      |                      |         |           |            |       |        |                       |      | bioDXA-91 family |      | 4  | 4 |
| BRR-CA-16220 | Gyra_T86  |               |        |        |  | tel(O/M/O) + tel(OH)        |    | app(2)*-IF | aac(0)*-h/aph(2)*-la | aaef1, aaef2, A1-415 |         |           | app(F)-IHa | aaef1 | catA13 |                       |      | bioDXA-184       | catA | 7  | 5 |
| BRR-CA-16221 | Gyra_T86  |               | erm(B) |        |  | tel(O/M/O) + tel(OH)        |    | app(2)*-IF | aac(0)*-h/aph(2)*-la | aaef1                |         |           | app(F)-IHa | aaef1 | catA13 |                       |      | bioDXA-489       |      | 9  | 6 |
| BRR-CA-16222 | Gyra_T86  |               |        |        |  | tel(O/M/O)                  |    |            |                      |                      |         |           |            |       |        |                       |      | bioDXA-193       |      | 3  | 3 |
| BRR-CA-16245 | Gyra_T86  | 235_A2075G    |        |        |  | tel(O/M/O) + tel(OH)        |    | app(2)*-IF | aac(0)*-h/aph(2)*-la | aaef1                |         |           |            |       |        |                       |      | bioDXA-193       |      | 3  | 3 |
| BRR-CA-16246 | Gyra_T86  |               |        |        |  | tel(O/M/O)                  |    |            |                      |                      |         |           |            |       |        |                       |      | bioDXA-91 family |      | 3  | 3 |
| BRR-CA-16247 | Gyra_T86  |               |        |        |  | tel(O/M/O)                  |    |            |                      |                      |         |           |            |       |        |                       |      | bioDXA-91 family |      | 3  | 3 |
| BRR-CA-16248 | Gyra_T86  |               |        |        |  | tel(O)                      |    |            |                      | aaef1                |         |           |            |       |        |                       |      | bioDXA-193       |      | 6  | 4 |
| BRR-CA-16248 | Gyra_T86  | 235_A2075G    |        |        |  | tel(O)                      |    |            | aac(0)*-h/aph(2)*-la | aaef1                | aaef-Cc |           |            |       |        |                       |      | bioDXA-489       |      | 7  | 5 |
| BRR-CA-16251 | Gyra_T86  |               |        |        |  | tel(O)                      |    |            |                      |                      |         |           |            |       |        |                       |      | bioDXA-193       |      | 3  | 3 |
| BRR-CA-16252 | Gyra_T86  |               |        |        |  | tel(O)                      |    |            |                      |                      |         |           |            |       |        |                       |      | bioDXA-193       |      | 3  | 3 |
| BRR-CA-16253 | Gyra_T86  |               |        |        |  | tel(O/M/O) + tel(OH)        |    | app(2)*-IF | aac(0)*-h/aph(2)*-la | aaef1                |         |           | app(F)-IHa | aaef1 | catA13 |                       |      | bioDXA-489       |      | 9  | 6 |
| BRR-CA-16254 | Gyra_T86  | 505_L22_A103V | erm(B) | tel(O) |  |                             |    |            |                      |                      |         |           | app(F)-IHa | aaef1 | catA13 |                       |      | bioDXA-91 family |      | 4  | 4 |
| BRR-CA-16257 | Gyra_T86  | 235_A2075G    |        |        |  | tel(O)                      |    | app(2)*-IF |                      |                      |         |           | app(F)-IHa | aaef1 |        |                       |      | bioDXA-193       |      | 7  | 5 |
| BRR-CA-16258 | Gyra_T86  | 505_L22_A103V | erm(B) | tel(O) |  |                             |    |            |                      | aaef1                |         |           |            |       |        |                       |      | bioDXA-193       |      | 7  | 5 |
| BRR-CA-16259 | Gyra_T86  |               | erm(B) |        |  | tel(O/M/O)                  |    | app(2)*-IF |                      | aaef1                |         |           | app(F)-IHa | aaef1 | catA13 | pL180-K207delm180-262 |      | bioDXA-193       |      | 10 | 6 |
| BRR-CA-16260 | Gyra_T86  |               |        |        |  | tel(O/M/O) + tel(OH) tel(1) |    | app(2)*-IF | aac(0)*-h/aph(2)*-la | aaef1                |         |           | app(F)-IHa | aaef1 | catA13 | optA, lexA, optA      |      | bioDXA-193       |      | 10 | 7 |
| BRR-CA-16264 | Gyra_T86  | 235_A2075G    |        |        |  | tel(O)                      |    | app(2)*-IF |                      | aaef1                |         |           | app(F)-IHa | aaef1 |        |                       |      | bioDXA-193       |      | 7  | 5 |
| BRR-CA-16265 | Gyra_T86  | 505_L22_A103V |        |        |  | tel(O)                      |    |            |                      |                      |         |           |            |       |        |                       |      | bioDXA-91 family |      | 4  | 4 |
| BRR-CA-16272 | Gyra_T86  | 505_L22_A103V |        |        |  | tel(O)                      |    |            |                      |                      |         |           |            |       |        |                       |      | bioDXA-91 family |      | 4  | 4 |
| BRR-CA-16273 | Gyra_T86  |               |        |        |  | tel(O)                      |    |            |                      |                      |         |           |            |       |        |                       |      | bioDXA-91 family |      | 4  | 4 |
| BRR-CA-16277 | Gyra_T86  | 235_A2075G    |        |        |  | tel(O/M/O)                  |    |            |                      |                      |         | ppul_K43R | app(F)-IHa | aaef1 | catA13 |                       |      | bioDXA-193       |      | 3  | 3 |
| BRR-CA-16277 | Gyra_T86  | 505_L22_A103V |        |        |  | tel(O)                      |    | app(2)*-IF |                      |                      |         |           | app(F)-IHa | aaef1 | catA13 |                       |      | bioDXA-193       |      | 9  | 6 |
| BRR-CA-16278 | Gyra_T86  | 505_L22_A103V |        |        |  | tel(O)                      |    | app(2)*-IF |                      |                      |         |           | app(F)-IHa | aaef1 | catA13 |                       |      | bioDXA-193       |      | 8  | 6 |
| BRR-CA-16279 | Gyra_T86  |               |        |        |  | tel(O)                      |    |            |                      |                      |         |           | app(F)-IHa | aaef1 | catA13 |                       |      | bioDXA-193       |      | 3  | 3 |
| BRR-CA-16280 | Gyra_T86  |               |        |        |  | tel(O/M/O)                  |    |            |                      |                      |         |           |            |       |        |                       |      | bioDXA-184       |      | 3  | 3 |
| BRR-CA-16281 | Gyra_T86  |               |        |        |  | tel(O/M/O)                  |    |            |                      |                      |         |           |            |       |        |                       |      | bioDXA-193       |      | 3  | 3 |
| BRR-CA-16282 | Gyra_T86  | 505_L22_A103V |        |        |  | tel(O)                      |    |            |                      |                      |         |           |            |       |        |                       |      | bioDXA-193       |      | 4  | 4 |
| BRR-CA-16283 | Gyra_T86  |               |        |        |  | tel(O/M/O)                  |    |            |                      |                      |         |           |            |       |        |                       |      | bioDXA-91 family |      | 3  | 3 |
| BRR-CA-16284 | Gyra_T86  |               |        |        |  | tel(O/M/O)                  |    |            |                      |                      |         |           |            |       |        |                       |      | bioDXA-91 family |      | 3  | 3 |
| BRR-CA-16287 | Gyra_T86  |               |        |        |  | tel(O/M/O)                  |    |            |                      |                      |         |           |            |       |        |                       |      | bioDXA-91 family |      | 3  | 3 |
| BRR-CA-16292 | Gyra_T86  | 235_A2075G    |        |        |  | tel(O/M/O) + tel(OH)        |    | app(2)*-IF | aac(0)*-h/aph(2)*-la | aaef1                |         |           | app(F)-IHa | aaef1 | catA13 |                       |      | bioDXA-193       |      | 9  | 6 |
| BRR-CA-16296 | Gyra_T86  | 505_L22_A103V |        |        |  | tel(O/M/O)                  |    |            |                      |                      |         |           |            |       |        |                       |      | bioDXA-193       |      | 4  | 4 |
| BRR-CA-16297 | Gyra_T86  |               | erm(B) |        |  | tel(O/M/O) + tel(OH)        |    | app(2)*-IF | aac(0)*-h/aph(2)*-la | aaef1                |         |           | app(F)-IHa | aaef1 |        |                       |      | bioDXA-193       |      | 8  | 4 |
| BRR-CA-16298 | Gyra_T86V |               | erm(B) | tel(O) |  |                             |    |            |                      | aaef1                |         |           | app(F)-IHa | aaef1 |        |                       |      | bioDXA-193       |      | 6  | 2 |
| BRR-CA-16299 | Gyra_T86  |               |        |        |  | tel(O)                      |    |            |                      | aaef1                |         |           | app(F)-IHa | aaef1 |        |                       |      | bioDXA-193       |      | 3  | 3 |
| BRR-CA-16300 | Gyra_T86  |               | erm(B) | tel(O) |  |                             |    |            |                      | aaef1                |         |           | app(F)-IHa | aaef1 |        |                       |      | bioDXA-193       |      | 6  | 5 |
| BRR-CA-16303 | Gyra_T86  |               |        |        |  | tel(O/M/O)                  |    |            |                      |                      |         |           |            |       |        |                       |      | bioDXA-91 family |      | 3  | 3 |
| BRR-CA-16305 | Gyra_T86  | 505_L22_A103V |        |        |  | tel(O)                      |    |            |                      |                      |         |           | app(F)-IHa |       |        |                       |      | bioDXA-184       |      | 4  | 4 |
| BRR-CA-16307 | Gyra_T86  |               |        |        |  | tel(O)                      |    |            |                      |                      |         |           | app(F)-IHa |       |        |                       |      | bioDXA-193       |      | 3  | 3 |
| BRR-CA-16309 | Gyra_T86  | 505_L22_A103V |        |        |  | tel(O)                      |    |            |                      |                      |         |           | app(F)-IHa | aaef1 |        |                       |      | bioDXA-91 family |      | 3  | 3 |
| BRR-CA-16311 | Gyra_T86  |               |        |        |  | tel(O)                      |    | app(2)*-IF |                      |                      |         |           | app(F)-IHa | aaef1 | catA13 |                       |      | bioDXA-193       |      | 7  | 5 |
| BRR-CA-16319 | Gyra_T86  |               |        |        |  | tel(O/M/O)                  |    |            |                      |                      |         |           | app(F)-IHa | aaef1 |        |                       |      | bioDXA-91 family |      | 3  | 3 |
| BRR-CA-16330 | Gyra_T86  |               |        |        |  | tel(O)                      |    |            |                      |                      |         |           |            |       |        |                       |      | bioDXA-193       |      | 3  | 3 |
| BRR-CA-16348 | Gyra_T86  | 505_L22_A103V |        |        |  | tel(O/M/O)                  |    |            |                      |                      |         |           |            |       |        |                       |      | bioDXA-91 family |      | 4  | 4 |
| BRR-CA-16349 | Gyra_T86  | 505_L22_A103V |        |        |  | tel(O/M/O)                  |    |            |                      |                      |         |           |            |       |        |                       |      | bioDXA-184       |      | 4  | 4 |
| BRR-CA-16350 | Gyra_T86  | 505_L22_A103V |        |        |  | tel(O)                      |    |            |                      |                      |         |           |            |       |        |                       |      | bioDXA-193       |      | 3  | 3 |
| BRR-CA-16351 | Gyra_T86  |               |        |        |  | tel(O/M/O)                  |    | app(2)*-IF |                      |                      |         |           | app(F)-IHa | aaef1 | catA13 |                       |      | bioDXA-193       |      | 7  | 5 |
| BRR-CA-16352 | Gyra_T86  | 235_A2075G    |        |        |  | tel(O/M/O)                  |    | app(2)*-IF |                      | ppul_K43R            |         |           | app(F)-IHa | aaef1 | catA13 |                       |      | bioDXA-91 family |      | 9  | 5 |
| BRR-CA-16353 | Gyra_T86  |               | erm(B) |        |  | tel(O/M/O) + tel(OH)        |    | app(2)*-IF | aac(0)*-h/aph(2)*-la | aaef1                |         |           | app(F)-IHa | aaef1 |        |                       |      | bioDXA-193       |      | 8  | 5 |
| BRR-CA-16354 | Gyra_T86  | 505_L22_A103V |        |        |  | tel(O)                      |    |            |                      |                      |         |           | app(F)-IHa | aaef1 |        |                       |      | bioDXA-193       |      | 6  | 4 |
| BRR-CA-16360 | Gyra_T86  | 505_L22_A103V |        |        |  | tel(O)                      |    |            |                      |                      |         |           | app(F)-IHa | aaef1 |        |                       |      | bioDXA-184       |      | 4  | 4 |
| BRR-CA-16361 | Gyra_T86  | 505_L22_A103V |        |        |  | tel(O)                      |    |            |                      |                      |         |           | app(F)-IHa | aaef1 |        |                       |      | bioDXA-193       |      | 4  | 4 |
| BRR-CA-16362 | Gyra_T86  | 505_L22_A103V |        |        |  | tel(O)                      |    |            |                      |                      |         |           | app(F)-IHa | aaef1 |        |                       |      | bioDXA-193       |      | 4  | 4 |
| BRR-CA-16365 | Gyra_T86  |               |        |        |  | tel(O)                      |    |            |                      | aaef1                |         |           |            |       |        |                       |      | bioDXA-193       |      | 6  | 4 |
| BRR-CA-16370 | Gyra_T86  |               |        |        |  | tel(O)                      |    |            |                      |                      |         |           |            |       |        |                       |      | bioDXA-193       |      | 3  | 3 |
| BRR-CA-16372 | Gyra_T86  |               |        |        |  | tel(O) + tel(O)             |    |            | aac(0)*-h/aph(2)*-la | aaef1                |         |           |            |       |        |                       |      | bioDXA-193       |      | 5  | 4 |
| BRR-CA-16373 | Gyra_T86  | 505_L22_A103V |        |        |  | tel(O)                      |    |            |                      |                      |         |           | app(F)-IHa | aaef1 |        |                       |      | bioDXA-193       |      | 4  | 4 |
| BRR-CA-16374 | Gyra_T86  |               |        |        |  | tel(O/M/O)                  |    |            |                      |                      |         |           |            |       |        |                       |      | bioDXA-193       |      | 3  | 3 |
| BRR-CA-16375 | Gyra_T86  | 505_L22_A103V |        |        |  | tel(O)                      |    |            |                      |                      |         |           | app(F)-IHa | aaef1 |        |                       |      | bioDXA-193       |      | 3  | 3 |
| BRR-CA-16376 | Gyra_T86  |               |        |        |  | tel(O)                      |    | app(2)*-IF |                      | aaef-Cc              |         |           | app(F)-IHa | aaef1 | catA13 |                       |      | bioDXA-193       |      | 8  | 5 |
| BRR-CA-16378 | Gyra_T86  |               |        |        |  | tel(O/M/O)                  | </ |            |                      |                      |         |           |            |       |        |                       |      |                  |      |    |   |







# 9 Appendix

| sample overview |                            | mobile AMR genes and putative plasmids as predicted by platon v1.6 |                            |                   |                                |                               |                 |    |        |                 |      | Multi-Locus Sequence Typing (MLST)<br>new allele variants and ST-Types are highlighted in yellow |      |     |      |      |              |                |                |                        |                                    | Illumina Short-Read Sequencing overview and QC data |                               |              |         |  |  |  |  |  |  |
|-----------------|----------------------------|--|----------------------------|-------------------|--------------------------------|-------------------------------|-----------------|----|--------|-----------------|------|--|------|-----|------|------|--------------|----------------|----------------|------------------------|------------------------------------|---|-------------------------------|--------------|---------|--|--|--|--|--|--|
| isolate No.     | amr count by mobility      | plasmids contigs   | plasmids cumulative length | plasmids circular | plasmids mobilization elements | plasmids conjugation elements | sum circ. conj. | ST | CC     | aspA            | glnA | gIIA   | gIYA | pgm | hly  | uncA | Total length | coverage depth | number contigs | Read Fraction Majority | Library Preparation Kit            | DNA extraction kit                                  | NGS method                    | cycle number |         |  |  |  |  |  |  |
| BIR-CA-11843    | chromosome (3)             | 0  | 0                          | 0                 | 0                              | 0                             | 0               | 0  | 0      | 0               | 0    | 0  | 0    | 0   | 0    | 0    | 1645861      | 97.5           | 66             | 0.96                   | Nxterera DNA Flex Library prep Kit | PureLink Genomic DNA Mini Kit                       | NextSeq 500                   | 2 x 149      |         |  |  |  |  |  |  |
| BIR-CA-11858    | chromosome (4)             | 1  | 6302                       | 0                 | 1                              | 0                             | 0               | 1  | 7159   | ST-828 complex  | 33   | 39   | 418  | 82  | 104  | 85   | 17           | 1664776        | 97.7           | 38                     | 0.98                               | Nxterera DNA Flex Library prep Kit                  | PureLink Genomic DNA Mini Kit | NextSeq 500  | 2 x 149 |  |  |  |  |  |  |
| BIR-CA-11892    | chromosome (3)             | 0  | 0                          | 0                 | 0                              | 0                             | 0               | 0  | 0      | 0               | 0    | 0  | 0    | 0   | 0    | 0    | 1682675      | 97.2           | 53             | 0.97                   | Nxterera DNA Flex Library prep Kit | PureLink Genomic DNA Mini Kit                       | NextSeq 500                   | 2 x 149      |         |  |  |  |  |  |  |
| BIR-CA-11893    | chromosome (2)             | 0  | 0                          | 0                 | 0                              | 0                             | 0               | 0  | 0      | 0               | 0    | 0  | 0    | 0   | 0    | 0    | 1685169      | 97.2           | 68             | 0.96                   | Nxterera DNA Flex Library prep Kit | PureLink Genomic DNA Mini Kit                       | NextSeq 500                   | 2 x 149      |         |  |  |  |  |  |  |
| BIR-CA-11930    | chromosome (6)             | 0  | 0                          | 0                 | 0                              | 0                             | 0               | 0  | 0      | 0               | 0    | 0  | 0    | 0   | 0    | 0    | 1687230      | 97.5           | 40             | 0.97                   | Nxterera DNA Flex Library prep Kit | PureLink Genomic DNA Mini Kit                       | NextSeq 500                   | 2 x 149      |         |  |  |  |  |  |  |
| BIR-CA-12208    | chromosome (2)             | 1  | 5417                       | 0                 | 1                              | 0                             | 0               | 0  | 18053  | ST-828 complex  | 33   | 39   | 30   | 79  | 113  | 625  | 17           | 1602385        | 97.2           | 66                     | 0.93                               | Nxterera DNA Flex Library prep Kit                  | PureLink Genomic DNA Mini Kit | NextSeq 500  | 2 x 149 |  |  |  |  |  |  |
| BIR-CA-12658    | chromosome (5)             | 2  | 8696                       | 0                 | 1                              | 0                             | 0               | 0  | 1832   | ST-828 complex  | 33   | 39   | 30   | 79  | 113  | 43   | 17           | 1602031        | 96.4           | 46                     | 0.95                               | Nxterera DNA Flex Library prep Kit                  | PureLink Genomic DNA Mini Kit | NextSeq 500  | 2 x 149 |  |  |  |  |  |  |
| BIR-CA-12887    | chromosome (2)             | 0  | 0                          | 0                 | 0                              | 0                             | 0               | 0  | 0      | 0               | 0    | 0  | 0    | 0   | 0    | 0    | 1700507      | 97.1           | 45             | 0.99                   | Nxterera DNA Flex Library prep Kit | PureLink Genomic DNA Mini Kit                       | NextSeq 500                   | 2 x 149      |         |  |  |  |  |  |  |
| BIR-CA-12897    | chromosome (3)             | 2  | 32454                      | 0                 | 2                              | 10                            | 0               | 0  | 121595 | ST-828 complex  | 33   | 39   | 30   | 79  | 104  | 43   | 17           | 1801810        | 96.8           | 40                     | 0.96                               | Nxterera DNA Flex Library prep Kit                  | PureLink Genomic DNA Mini Kit | NextSeq 500  | 2 x 149 |  |  |  |  |  |  |
| BIR-CA-12906    | chromosome (3)             | 0  | 0                          | 0                 | 0                              | 0                             | 0               | 0  | 8509   | ST-828 complex  | 292  | 66   | 30   | 79  | 113  | 43   | 17           | 1651791        | 97.3           | 38                     | 0.95                               | Nxterera DNA Flex Library prep Kit                  | PureLink Genomic DNA Mini Kit | NextSeq 500  | 2 x 149 |  |  |  |  |  |  |
| BIR-CA-12973    | chromosome (2)             | 0  | 0                          | 0                 | 0                              | 0                             | 0               | 0  | 0      | 0               | 0    | 0  | 0    | 0   | 0    | 0    | 1683652      | 97.4           | 24             | 0.92                   | Nxterera DNA Flex Library prep Kit | PureLink Genomic DNA Mini Kit                       | NextSeq 500                   | 2 x 149      |         |  |  |  |  |  |  |
| BIR-CA-13310    | chromosome (2)             | 0  | 0                          | 0                 | 0                              | 0                             | 0               | 0  | 0      | 0               | 0    | 0  | 0    | 0   | 0    | 0    | 1636608      | 97.3           | 52             | 0.95                   | Nxterera DNA Flex Library prep Kit | PureLink Genomic DNA Mini Kit                       | NextSeq 500                   | 2 x 149      |         |  |  |  |  |  |  |
| BIR-CA-13470    | chromosome (4)             | 0  | 0                          | 0                 | 0                              | 0                             | 0               | 0  | 0      | 0               | 0    | 0  | 0    | 0   | 0    | 0    | 1707966      | 97.1           | 43             | 0.96                   | Nxterera DNA Flex Library prep Kit | PureLink Genomic DNA Mini Kit                       | NextSeq 500                   | 2 x 149      |         |  |  |  |  |  |  |
| BIR-CA-13525    | chromosome (5)             | 1  | 30564                      | 0                 | 1                              | 10                            | 0               | 0  | 11872  | ST-828 complex  | 33   | 39   | 30   | 82  | 113  | 64   | 17           | 1734510        | 97.4           | 53                     | 0.96                               | Nxterera DNA Flex Library prep Kit                  | PureLink Genomic DNA Mini Kit | NextSeq 500  | 2 x 149 |  |  |  |  |  |  |
| BIR-CA-13528    | chromosome (3)             | 0  | 0                          | 0                 | 0                              | 0                             | 0               | 0  | 0      | 0               | 0    | 0  | 0    | 0   | 0    | 0    | 1695006      | 97.9           | 31             | 0.97                   | Nxterera DNA Flex Library prep Kit | PureLink Genomic DNA Mini Kit                       | NextSeq 500                   | 2 x 149      |         |  |  |  |  |  |  |
| BIR-CA-13537    | chromosome (3)             | 1  | 29421                      | 0                 | 1                              | 10                            | 0               | 0  | 11829  | ST-828 complex  | 33   | 39   | 30   | 82  | 113  | 43   | 17           | 1750236        | 92.9           | 114                    | 0.97                               | Nxterera DNA Flex Library prep Kit                  | PureLink Genomic DNA Mini Kit | NextSeq 500  | 2 x 149 |  |  |  |  |  |  |
| BIR-CA-13895    | chromosome (1)             | 0  | 0                          | 0                 | 0                              | 0                             | 0               | 0  | 0      | 0               | 0    | 0  | 0    | 0   | 0    | 0    | 1735529      | 94.9           | 40             | 0.75                   | Nxterera DNA Flex Library prep Kit | PureLink Genomic DNA Mini Kit                       | MISeq                         | 2 x 251      |         |  |  |  |  |  |  |
| BIR-CA-13918    | chromosome (1)             | 0  | 0                          | 0                 | 0                              | 0                             | 0               | 0  | 0      | 0               | 0    | 0  | 0    | 0   | 0    | 0    | 1620744      | 99.8           | 20             | 0.98                   | Nxterera DNA Flex Library prep Kit | PureLink Genomic DNA Mini Kit                       | MISeq                         | 2 x 251      |         |  |  |  |  |  |  |
| BIR-CA-13919    | chromosome (2)             | 0  | 0                          | 0                 | 0                              | 0                             | 0               | 0  | 0      | 0               | 0    | 0  | 0    | 0   | 0    | 0    | 1734346      | 93.4           | 43             | 0.75                   | Nxterera DNA Flex Library prep Kit | PureLink Genomic DNA Mini Kit                       | MISeq                         | 2 x 251      |         |  |  |  |  |  |  |
| BIR-CA-13937    | chromosome (2)             | 0  | 0                          | 0                 | 0                              | 0                             | 0               | 0  | 0      | 0               | 0    | 0  | 0    | 0   | 0    | 0    | 1649010      | 84.6           | 29             | 0.99                   | Nxterera DNA Flex Library prep Kit | PureLink Genomic DNA Mini Kit                       | MISeq                         | 2 x 301      |         |  |  |  |  |  |  |
| BIR-CA-13938    | chromosome (4)             | 0  | 0                          | 0                 | 0                              | 0                             | 0               | 0  | 0      | 0               | 0    | 0  | 0    | 0   | 0    | 0    | 1732290      | 71.2           | 29             | 0.94                   | Nxterera DNA Flex Library prep Kit | PureLink Genomic DNA Mini Kit                       | MISeq                         | 2 x 151      |         |  |  |  |  |  |  |
| BIR-CA-13978    | chromosome (2)             | 0  | 0                          | 0                 | 0                              | 0                             | 0               | 0  | 0      | 0               | 0    | 0  | 0    | 0   | 0    | 0    | 1794954      | 92.5           | 40             | 0.99                   | Nxterera DNA Flex Library prep Kit | PureLink Genomic DNA Mini Kit                       | MISeq                         | 2 x 251      |         |  |  |  |  |  |  |
| BIR-CA-13953    | chromosome (5)             | 1  | 6963                       | 0                 | 1                              | 0                             | 0               | 0  | 110183 | ST-607 complex  | 114  | 110  | 30   | 140 | 104  | 625  | 79           | 1762543        | 94.7           | 25                     | 0.72                               | Nxterera DNA Flex Library prep Kit                  | PureLink Genomic DNA Mini Kit | MISeq        | 2 x 251 |  |  |  |  |  |  |
| BIR-CA-13971    | chromosome (4)             | 5  | 71302                      | 1                 | 2                              | 16                            | 0               | 0  | 198509 | ST-828 complex  | 292  | 66   | 30   | 79  | 113  | 43   | 17           | 1808400        | 94.7           | 68                     | 0.93                               | Nxterera DNA Flex Library prep Kit                  | PureLink Genomic DNA Mini Kit | MISeq        | 2 x 301 |  |  |  |  |  |  |
| BIR-CA-13985    | chromosome (5)             | 3  | 12866                      | 0                 | 1                              | 0                             | 0               | 0  | 14754  | ST-828 complex  | 37   | 61   | 57   | 64  | 519  | 430  | 23           | 1805268        | 95.4           | 109                    | 0.96                               | Nxterera DNA Flex Library prep Kit                  | PureLink Genomic DNA Mini Kit | MISeq        | 2 x 251 |  |  |  |  |  |  |
| BIR-CA-14088    | chromosome (2)             | 0  | 0                          | 0                 | 0                              | 0                             | 0               | 0  | 0      | 0               | 0    | 0  | 0    | 0   | 0    | 0    | 1644285      | 96.5           | 18             | 1.00                   | Nxterera DNA Flex Library prep Kit | PureLink Genomic DNA Mini Kit                       | MISeq                         | 2 x 251      |         |  |  |  |  |  |  |
| BIR-CA-14109    | chromosome (1)             | 0  | 0                          | 0                 | 0                              | 0                             | 0               | 0  | 0      | 0               | 0    | 0  | 0    | 0   | 0    | 0    | 1663767      | 101.1          | 17             | 1.00                   | Nxterera DNA Flex Library prep Kit | PureLink Genomic DNA Mini Kit                       | MISeq                         | 2 x 251      |         |  |  |  |  |  |  |
| BIR-CA-14180    | chromosome (3)             | 0  | 0                          | 0                 | 0                              | 0                             | 0               | 0  | 0      | 0               | 0    | 0  | 0    | 0   | 0    | 0    | 1703363      | 93.1           | 17             | 0.99                   | Nxterera DNA Flex Library prep Kit | PureLink Genomic DNA Mini Kit                       | MISeq                         | 2 x 251      |         |  |  |  |  |  |  |
| BIR-CA-14181    | chromosome (1)             | 0  | 0                          | 0                 | 0                              | 0                             | 0               | 0  | 0      | 0               | 0    | 0  | 0    | 0   | 0    | 0    | 1474344      | 95.2           | 44             | 0.99                   | Nxterera DNA Flex Library prep Kit | PureLink Genomic DNA Mini Kit                       | MISeq                         | 2 x 251      |         |  |  |  |  |  |  |
| BIR-CA-14216    | chromosome (3)             | 0  | 0                          | 0                 | 0                              | 0                             | 0               | 0  | 0      | 0               | 0    | 0  | 0    | 0   | 0    | 0    | 1669723      | 80             | 41             | 0.96                   | Nxterera DNA Flex Library prep Kit | PureLink Genomic DNA Mini Kit                       | MISeq                         | 2 x 301      |         |  |  |  |  |  |  |
| BIR-CA-14226    | chromosome (3)             | 0  | 0                          | 0                 | 0                              | 0                             | 0               | 0  | 0      | 0               | 0    | 0  | 0    | 0   | 0    | 0    | 1730283      | 94.3           | 45             | 0.75                   | Nxterera DNA Flex Library prep Kit | PureLink Genomic DNA Mini Kit                       | MISeq                         | 2 x 251      |         |  |  |  |  |  |  |
| BIR-CA-14373    | chromosome (3)             | 0  | 0                          | 0                 | 0                              | 0                             | 0               | 0  | 0      | 0               | 0    | 0  | 0    | 0   | 0    | 0    | 1691214      | 95.5           | 73             | 0.99                   | Nxterera DNA Flex Library prep Kit | PureLink Genomic DNA Mini Kit                       | MISeq                         | 2 x 301      |         |  |  |  |  |  |  |
| BIR-CA-14430    | chromosome (2) plasmid (1) | 1  | 41863                      | 0                 | 1                              | 6                             | 0               | 0  | 744    | ST-21 complex   | 8    | 1  | 6    | 3   | 2    | 1    | 1            | 1667778        | 96.8           | 33                     | 0.99                               | Nxterera DNA Flex Library prep Kit                  | PureLink Genomic DNA Mini Kit | MISeq        | 2 x 251 |  |  |  |  |  |  |
| BIR-CA-14582    | chromosome (1)             | 1  | 13935                      | 1                 | 0                              | 0                             | 0               | 0  | 1830   | ST-828 complex  | 33   | 39   | 30   | 79  | 104  | 47   | 17           | 1656703        | 92.5           | 46                     | 0.95                               | Nxterera DNA Flex Library prep Kit                  | PureLink Genomic DNA Mini Kit | MISeq        | 2 x 251 |  |  |  |  |  |  |
| BIR-CA-14583    | chromosome (2)             | 0  | 0                          | 0                 | 0                              | 0                             | 0               | 0  | 0      | 0               | 0    | 0  | 0    | 0   | 0    | 0    | 1696609      | 77.9           | 21             | 0.96                   | Nxterera DNA Flex Library prep Kit | PureLink Genomic DNA Mini Kit                       | MISeq                         | 2 x 301      |         |  |  |  |  |  |  |
| BIR-CA-14610    | chromosome (1)             | 2  | 13706                      | 0                 | 1                              | 1                             | 0               | 0  | 21586  | ST-828 complex  | 33   | 39   | 30   | 82  | 113  | 43   | 17           | 1693819        | 96.7           | 88                     | 0.97                               | Nxterera DNA Flex Library prep Kit                  | PureLink Genomic DNA Mini Kit | MISeq        | 2 x 251 |  |  |  |  |  |  |
| BIR-CA-14731    | chromosome (1)             | 1  | 5506                       | 1                 | 0                              | 0                             | 0               | 0  | 110185 | ST-828 complex  | 265  | 195  | 103  | 140 | 113  | 164  | 79           | 1725152        | 81.3           | 21                     | 0.79                               | Nxterera DNA Flex Library prep Kit                  | PureLink Genomic DNA Mini Kit | MISeq        | 2 x 251 |  |  |  |  |  |  |
| BIR-CA-14781    | chromosome (3)             | 0  | 0                          | 0                 | 0                              | 0                             | 0               | 0  | 0      | 0               | 0    | 0  | 0    | 0   | 0    | 0    | 1701481      | 91.5           | 29             | 0.99                   | Nxterera DNA Flex Library prep Kit | PureLink Genomic DNA Mini Kit                       | MISeq                         | 2 x 251      |         |  |  |  |  |  |  |
| BIR-CA-14810    | chromosome (3)             | 1  | 3495                       | 1                 | 0                              | 0                             | 0               | 0  | 110186 | ST-828 complex  | 541  | 195  | 30   | 140 | 1041 | 164  | 79           | 1751482        | 93.5           | 45                     | 0.74                               | Nxterera DNA Flex Library prep Kit                  | PureLink Genomic DNA Mini Kit | MISeq        | 2 x 251 |  |  |  |  |  |  |
| BIR-CA-14815    | chromosome (3)             | 1  | 21379                      | 1                 | 0                              | 0                             | 0               | 0  | 121595 | ST-828 complex  | 33   | 39   | 30   | 79  | 104  | 43   | 17           | 1767921        | 84.1           | 28                     | 0.97                               | Nxterera DNA Flex Library prep Kit                  | PureLink Genomic DNA Mini Kit | MISeq        | 2 x 301 |  |  |  |  |  |  |
| BIR-CA-14825    | chromosome (2)             | 1  | 3588                       | 1                 | 0                              | 0                             | 0               | 0  | 14148  | ST-828 complex  | 114  | 195  | 103  | 140 | 459  | 164  | 79           | 1734756        | 91.4           | 34                     | 0.73                               | Nxterera DNA Flex Library prep Kit                  | PureLink Genomic DNA Mini Kit | MISeq        | 2 x 251 |  |  |  |  |  |  |
| BIR-CA-14833    | chromosome (1)             | 1  | 5517                       | 1                 | 0                              | 0                             | 0               | 0  | 15439  | ST-828 complex  | 114  | 406  | 103  | 140 | 459  | 164  | 79           | 1735554        | 90.6           | 33                     | 0.74                               | Nxterera DNA Flex Library prep Kit                  | PureLink Genomic DNA Mini Kit | MISeq        | 2 x 251 |  |  |  |  |  |  |
| BIR-CA-14857    | chromosome (3)             | 2  | 13243                      | 0                 | 0                              | 0                             | 0               | 0  | 0      | 0               | 0    | 0  | 0    | 0   | 0    | 0    | 1748928      | 79.4           | 62             | 0.98                   | Nxterera DNA Flex Library prep Kit | PureLink Genomic DNA Mini Kit                       | MISeq                         | 2 x 251      |         |  |  |  |  |  |  |
| BIR-CA-14872    | chromosome (2)             | 0  | 0                          | 0                 | 0                              | 0                             | 0               | 0  | 0      | 0               | 0    | 0  | 0    | 0   | 0    | 0    | 1623935      | 93.2           | 35             | 0.97                   | Nxterera DNA Flex Library prep Kit | PureLink Genomic DNA Mini Kit                       | MISeq                         | 2 x 251      |         |  |  |  |  |  |  |
| BIR-CA-14940    | chromosome (1)             | 0  | 0                          | 0                 | 0                              | 0                             | 0               | 0  | 0      | 0               | 0    | 0  | 0    | 0   | 0    | 0    | 1597079      | 90.3           | 10             | 1.00                   | Nxterera DNA Flex Library prep Kit | PureLink Genomic DNA Mini Kit                       | MISeq                         | 2 x 301      |         |  |  |  |  |  |  |
| BIR-CA-14943    | chromosome (2)             | 1  | 3605                       | 1                 | 0                              | 0                             | 0               | 0  | 0      | 0               | 0    | 0  | 0    | 0   | 0    | 0    | 1747482      | 89.2           | 35             | 0.77                   | Nxterera DNA Flex Library prep Kit | PureLink Genomic DNA Mini Kit                       | MISeq                         | 2 x 251      |         |  |  |  |  |  |  |
| BIR-CA-14958    | chromosome (3)             | 0  | 0                          | 0                 | 0                              | 0                             | 0               | 0  | 0      | 0               | 0    | 0  | 0    | 0   | 0    | 0    | 1682809      | 86.6           | 34             | 0.95                   | Nxterera DNA Flex Library prep Kit | PureLink Genomic DNA Mini Kit                       | MISeq                         | 2 x 151      |         |  |  |  |  |  |  |
| BIR-CA-14973    | chromosome (4)             | 0  | 0                          | 0                 | 0                              | 0                             | 0               | 0  | 0      | 0               | 0    | 0  | 0    | 0   | 0    | 0    | 1783799      | 93.1           | 88             | 0.74                   | Nxterera DNA Flex Library prep Kit | PureLink Genomic DNA Mini Kit                       | MISeq                         | 2 x 251      |         |  |  |  |  |  |  |
| BIR-CA-14974    | chromosome (2)             | 1  | 4704                       | 0                 | 1                              | 0                             | 0               | 0  | 113858 | ST-1150 complex | 103  | 110  | 30   | 140 | 188  | 99   | 17           | 1705768        | 68.2           | 24                     | 0.78                               | Nxterera DNA Flex Library prep Kit                  | PureLink Genomic DNA Mini Kit | MISeq        | 2 x 151 |  |  |  |  |  |  |
| BIR-CA-15005    | chromosome (1)             | 0  | 0                          | 0                 | 0                              | 0                             | 0               | 0  | 0      | 0               | 0    | 0  | 0    | 0   | 0    | 0    | 1703192      | 91.5           | 51             | 0.97                   | Nxterera DNA Flex Library prep Kit | PureLink Genomic DNA Mini Kit                       | MISeq                         | 2 x 251      |         |  |  |  |  |  |  |
| BIR-CA-15035    | chromosome (1)             | 1  | 43026                      | 0                 | 1                              | 6                             | 0               | 0  | 7520   |                 |      |  |      |     |      |      |              |                |                |                        |                                    |   |                               |              |         |  |  |  |  |  |  |



# 9 Appendix

|              |                            |   |       |   |   |    |         |                |                |     |     |     |      |     |     |         |         |      |       |                                      |                                      |                               |         |         |
|--------------|----------------------------|---|-------|---|---|----|---------|----------------|----------------|-----|-----|-----|------|-----|-----|---------|---------|------|-------|--------------------------------------|--------------------------------------|-------------------------------|---------|---------|
| BIR-CA-15403 | chromosome (4)             | 1 | 5543  | 0 | 1 | 0  | 0       | 1 832          | ST-828 complex | 33  | 39  | 30  | 79   | 113 | 43  | 17      | 1743826 | 82,7 | 29    | 0,84                                 | Nxtera DNA Flex Library prep Kit     | PureLink Genomic DNA Mini Kit | MISeq   | 2 x 301 |
| BIR-CA-15426 | chromosome (3)             | 0 | 0     | 0 | 0 | 0  | 0       | 0 1595         | ST-828 complex | 33  | 38  | 30  | 79   | 104 | 43  | 17      | 1680091 | 85,9 | 25    | 0,97                                 | Nxtera DNA Flex Library prep Kit     | PureLink Genomic DNA Mini Kit | MISeq   | 2 x 301 |
| BIR-CA-15489 | chromosome (3)             | 0 | 0     | 0 | 0 | 0  | 0       | 0 10180        | ST-828 complex | 33  | 39  | 30  | 79   | 104 | 35  | 170     | 1720317 | 85,3 | 39    | 0,88                                 | Nxtera DNA Flex Library prep Kit     | PureLink Genomic DNA Mini Kit | MISeq   | 2 x 301 |
| BIR-CA-15500 | chromosome (2)             | 4 | 13931 | 0 | 0 | 0  | 0       | 0 905          |                | 2   | 15  | 4   | 3    | 154 | 25  | 35      | 1702965 | 96,8 | 47    | 0,97                                 | Nxtera DNA Flex Library prep Kit     | PureLink Genomic DNA Mini Kit | NextSeq | 2 x 151 |
| BIR-CA-15532 | chromosome (3)             | 0 | 0     | 0 | 0 | 0  | 0       | 0 9926         | ST-21 complex  | 2   | 2   | 12  | 3    | 2   | 1   | 5       | 1658043 | 89,2 | 25    | 0,99                                 | Nxtera DNA Flex Library prep Kit     | PureLink Genomic DNA Mini Kit | MISeq   | 2 x 301 |
| BIR-CA-15533 | chromosome (3)             | 0 | 0     | 0 | 0 | 0  | 0       | 0 1595         | ST-828 complex | 33  | 38  | 30  | 79   | 104 | 43  | 17      | 1658800 | 97,5 | 26    | 0,97                                 | Nxtera DNA Flex Library prep Kit     | PureLink Genomic DNA Mini Kit | NextSeq | 2 x 151 |
| BIR-CA-15629 | chromosome (2)             | 1 | 26872 | 1 | 1 | 10 | 12 3016 | ST-828 complex | 33             | 39  | 103 | 79  | 104  | 35  | 17  | 1732393 | 85,4    | 31   | 0,97  | Nxtera DNA Flex Library prep Kit     | PureLink Genomic DNA Mini Kit        | MISeq                         | 2 x 301 |         |
| BIR-CA-15630 | chromosome (5)             | 0 | 0     | 0 | 0 | 0  | 0 10183 |                | 114            | 110 | 30  | 140 | 104  | 625 | 79  | 1759614 | 102,5   | 31   | 0,71  | Nxtera DNA Flex Library prep Kit     | PureLink Genomic DNA Mini Kit        | NextSeq                       | 2 x 151 |         |
| BIR-CA-15687 | chromosome (3)             | 1 | 42602 | 0 | 1 | 6  | 7 10049 |                | 292            | 66  | 30  | 79  | 113  | 47  | 17  | 1736253 | 91,8    | 41   | 0,96  | Nxtera DNA Flex Library prep Kit     | PureLink Genomic DNA Mini Kit        | MISeq                         | 2 x 149 |         |
| BIR-CA-15692 | chromosome (5)             | 0 | 0     | 0 | 0 | 0  | 0 10183 |                | 114            | 110 | 30  | 140 | 104  | 625 | 79  | 1770276 | 84,2    | 443  | 0,66  | Nxtera DNA Flex Library prep Kit     | PureLink Genomic DNA Mini Kit        | MISeq                         | 2 x 149 |         |
| BIR-CA-15913 | chromosome (3)             | 1 | 27120 | 1 | 1 | 10 | 12 1595 | ST-828 complex | 33             | 38  | 30  | 79  | 104  | 43  | 17  | 1767715 | 83,3    | 42   | 0,97  | Nxtera DNA Flex Library prep Kit     | PureLink Genomic DNA Mini Kit        | MISeq                         | 2 x 301 |         |
| BIR-CA-15969 | chromosome (4)             | 1 | 5543  | 0 | 1 | 0  | 1 832   | ST-828 complex | 33             | 39  | 30  | 79  | 113  | 43  | 17  | 1720479 | 85,1    | 23   | 0,90  | Nxtera DNA Flex Library prep Kit     | PureLink Genomic DNA Mini Kit        | MISeq                         | 2 x 301 |         |
| BIR-CA-15978 | chromosome (2)             | 0 | 0     | 0 | 0 | 0  | 0 825   | ST-828 complex | 33             | 39  | 30  | 82  | 113  | 47  | 17  | 1660644 | 83      | 33   | 0,98  | Nxtera DNA Flex Library prep Kit     | PureLink Genomic DNA Mini Kit        | MISeq                         | 2 x 301 |         |
| BIR-CA-15985 | chromosome (3)             | 1 | 3701  | 0 | 0 | 0  | 0 8920  |                | 9              | 2   | 2   | 10  | 11   | 253 | 147 | 1739716 | 97,5    | 119  | 0,98  | Illumina* DNA Prep, (M) Tagmentation | PureLink Genomic DNA Mini Kit        | MISeq                         | 2 x 151 |         |
| BIR-CA-15986 | chromosome (7)             | 1 | 39258 | 1 | 1 | 6  | 8 11994 | ST-828 complex | 33             | 176 | 30  | 82  | 1198 | 43  | 17  | 1699972 | 98,5    | 32   | 0,96  | Illumina* DNA Prep, (M) Tagmentation | PureLink Genomic DNA Mini Kit        | MISeq                         | 2 x 151 |         |
| BIR-CA-15987 | chromosome (5)             | 3 | 23389 | 0 | 1 | 10 | 11 860  | ST-828 complex | 33             | 39  | 30  | 79  | 113  | 47  | 17  | 1737477 | 98,3    | 32   | 0,95  | Illumina* DNA Prep, (M) Tagmentation | PureLink Genomic DNA Mini Kit        | MISeq                         | 2 x 151 |         |
| BIR-CA-15988 | chromosome (5)             | 1 | 38986 | 0 | 1 | 6  | 7 3119  | ST-828 complex | 33             | 39  | 30  | 82  | 188  | 47  | 17  | 1739357 | 103,3   | 68   | 0,96  | Illumina* DNA Prep, (M) Tagmentation | PureLink Genomic DNA Mini Kit        | MISeq                         | 2 x 151 |         |
| BIR-CA-15989 | chromosome (12)            | 0 | 0     | 0 | 0 | 0  | 0 3753  | ST-828 complex | 33             | 176 | 30  | 82  | 104  | 43  | 17  | 1714244 | 99      | 27   | 0,96  | Illumina* DNA Prep, (M) Tagmentation | PureLink Genomic DNA Mini Kit        | MISeq                         | 2 x 151 |         |
| BIR-CA-15990 | chromosome (13)            | 3 | 40135 | 0 | 1 | 6  | 7 860   | ST-828 complex | 33             | 39  | 30  | 79  | 113  | 47  | 17  | 1754549 | 104,1   | 74   | 0,94  | Illumina* DNA Prep, (M) Tagmentation | PureLink Genomic DNA Mini Kit        | NextSeq 500                   | 2 x 149 |         |
| BIR-CA-15991 | chromosome (10)            | 0 | 0     | 0 | 0 | 0  | 0 829   | ST-828 complex | 33             | 39  | 30  | 82  | 113  | 43  | 17  | 1668403 | 97,3    | 46   | 0,96  | Illumina* DNA Prep, (M) Tagmentation | PureLink Genomic DNA Mini Kit        | NextSeq 500                   | 2 x 149 |         |
| BIR-CA-15992 | chromosome (7)             | 4 | 42057 | 0 | 1 | 6  | 7 9867  | ST-828 complex | 33             | 393 | 30  | 82  | 113  | 43  | 17  | 1745071 | 97,3    | 80   | 0,93  | Illumina* DNA Prep, (M) Tagmentation | PureLink Genomic DNA Mini Kit        | NextSeq 500                   | 2 x 149 |         |
| BIR-CA-15993 | chromosome (3)             | 1 | 8952  | 0 | 0 | 0  | 0 1723  | ST-354 complex | 8              | 17  | 2   | 2   | 11   | 12  | 6   | 1700597 | 97,7    | 51   | 0,99  | Illumina* DNA Prep, (M) Tagmentation | PureLink Genomic DNA Mini Kit        | NextSeq 500                   | 2 x 149 |         |
| BIR-CA-15994 | chromosome (9)             | 0 | 0     | 0 | 0 | 0  | 0 3753  | ST-828 complex | 33             | 176 | 30  | 82  | 104  | 43  | 17  | 1708486 | 96,2    | 16   | 0,96  | Illumina* DNA Prep, (M) Tagmentation | PureLink Genomic DNA Mini Kit        | MISeq                         | 2 x 201 |         |
| BIR-CA-15995 | chromosome (3) plasmid (1) | 2 | 44205 | 2 | 1 | 6  | 9 5799  | ST-443 complex | 7              | 2   | 2   | 15  | 23   | 3   | 12  | 1675983 | 95,8    | 28   | 1,00  | Illumina* DNA Prep, (M) Tagmentation | PureLink Genomic DNA Mini Kit        | MISeq                         | 2 x 201 |         |
| BIR-CA-15996 | chromosome (8)             | 0 | 0     | 0 | 0 | 0  | 0 7280  |                | 406            | 114 | 2   | 2   | 2    | 3   | 147 | 1681172 | 98,9    | 25   | 0,97  | Illumina* DNA Prep, (M) Tagmentation | PureLink Genomic DNA Mini Kit        | MISeq                         | 2 x 151 |         |
| BIR-CA-15997 | chromosome (2)             | 2 | 7713  | 0 | 0 | 0  | 0 5229  |                | 8              | 2   | 2   | 212 | 10   | 253 | 147 | 1777563 | 97,4    | 127  | 0,99  | Illumina* DNA Prep, (M) Tagmentation | PureLink Genomic DNA Mini Kit        | MISeq                         | 2 x 151 |         |
| BIR-CA-15998 | chromosome (4)             | 3 | 9361  | 0 | 0 | 0  | 0 5229  |                | 8              | 2   | 2   | 212 | 10   | 253 | 147 | 1768992 | 97,7    | 109  | 0,97  | Illumina* DNA Prep, (M) Tagmentation | PureLink Genomic DNA Mini Kit        | MISeq                         | 2 x 151 |         |
| BIR-CA-15999 | chromosome (4) plasmid (1) | 1 | 50476 | 0 | 1 | 6  | 7 11851 |                | 7              | 114 | 12  | 2   | 2    | 6   | 676 | 1683341 | 99,1    | 15   | 0,97  | Illumina* DNA Prep, (M) Tagmentation | PureLink Genomic DNA Mini Kit        | MISeq                         | 2 x 151 |         |
| BIR-CA-16000 | chromosome (3)             | 0 | 0     | 0 | 0 | 0  | 0 11999 | ST-354 complex | 8              | 812 | 2   | 2   | 11   | 12  | 6   | 1700252 | 98,4    | 37   | 0,99  | Illumina* DNA Prep, (M) Tagmentation | PureLink Genomic DNA Mini Kit        | MISeq                         | 2 x 151 |         |
| BIR-CA-16001 | chromosome (4)             | 3 | 10538 | 0 | 0 | 0  | 0 4395  | ST-353 complex | 24             | 17  | 1   | 2   | 11   | 3   | 8   | 1791477 | 98,9    | 42   | 0,99  | Illumina* DNA Prep, (M) Tagmentation | PureLink Genomic DNA Mini Kit        | MISeq                         | 2 x 151 |         |
| BIR-CA-16002 | chromosome (2) plasmid (1) | 1 | 39815 | 1 | 1 | 6  | 8 1831  |                | 1166           | 2   | 1   | 10  | 151  | 3   | 1   | 1825018 | 98,1    | 60   | 0,99  | Illumina* DNA Prep, (M) Tagmentation | PureLink Genomic DNA Mini Kit        | MISeq                         | 2 x 151 |         |
| BIR-CA-16003 | chromosome (4)             | 0 | 0     | 0 | 0 | 0  | 0 9649  | ST-464 complex | 24             | 2   | 2   | 2   | 10   | 3   | 629 | 1709988 | 98,3    | 57   | 0,99  | Illumina* DNA Prep, (M) Tagmentation | PureLink Genomic DNA Mini Kit        | MISeq                         | 2 x 151 |         |
| BIR-CA-16004 | chromosome (3) plasmid (1) | 7 | 48676 | 0 | 1 | 6  | 9 9131  | ST-460 complex | 24             | 30  | 2   | 2   | 11   | 716 | 6   | 1790494 | 98,5    | 50   | 1,00  | Illumina* DNA Prep, (M) Tagmentation | PureLink Genomic DNA Mini Kit        | MISeq                         | 2 x 151 |         |
| BIR-CA-16005 | chromosome (9)             | 0 | 0     | 0 | 0 | 0  | 0 829   | ST-828 complex | 33             | 39  | 30  | 82  | 113  | 43  | 17  | 1761239 | 94,8    | 44   | 0,95  | Illumina* DNA Prep, (M) Tagmentation | PureLink Genomic DNA Mini Kit        | MISeq                         | 2 x 201 |         |
| BIR-CA-16007 | chromosome (6) plasmid (1) | 1 | 43331 | 0 | 1 | 6  | 7 11870 | ST-354 complex | 8              | 364 | 2   | 2   | 11   | 12  | 6   | 1645840 | 99,7    | 36   | 0,99  | Illumina* DNA Prep, (M) Tagmentation | PureLink Genomic DNA Mini Kit        | NextSeq 500                   | 2 x 149 |         |
| BIR-CA-16008 | chromosome (3) plasmid (2) | 4 | 47864 | 0 | 1 | 6  | 7 11852 | ST-574 complex | 9              | 30  | 2   | 2   | 11   | 3   | 3   | 1738828 | 98,3    | 47   | 0,99  | Illumina* DNA Prep, (M) Tagmentation | PureLink Genomic DNA Mini Kit        | NextSeq 500                   | 2 x 149 |         |
| BIR-CA-16010 | chromosome (5)             | 0 | 0     | 0 | 0 | 0  | 0 966   | ST-828 complex | 33             | 39  | 30  | 79  | 112  | 47  | 17  | 1709102 | 98,2    | 45   | 0,96  | Illumina* DNA Prep, (M) Tagmentation | PureLink Genomic DNA Mini Kit        | NextSeq 500                   | 2 x 149 |         |
| BIR-CA-16011 | chromosome (2)             | 0 | 0     | 0 | 0 | 0  | 0 11864 |                | 9              | 17  | 5   | 10  | 23   | 3   | 3   | 1776183 | 97,2    | 35   | 0,987 | Illumina* DNA Prep, (M) Tagmentation | PureLink Genomic DNA Mini Kit        | NextSeq 500                   | 2 x 149 |         |
| BIR-CA-16012 | chromosome (3)             | 1 | 2645  | 0 | 0 | 0  | 0 8920  |                | 9              | 2   | 2   | 10  | 11   | 253 | 147 | 1734619 | 96,1    | 184  | 0,985 | Illumina* DNA Prep, (M) Tagmentation | PureLink Genomic DNA Mini Kit        | NextSeq 500                   | 2 x 149 |         |
| BIR-CA-16013 | chromosome (9)             | 1 | 4390  | 0 | 1 | 0  | 0 11899 |                | 22             | 15  | 4   | 64  | 777  | 2   | 35  | 1620163 | 96,9    | 46   | 0,916 | Illumina* DNA Prep, (M) Tagmentation | PureLink Genomic DNA Mini Kit        | NextSeq 500                   | 2 x 149 |         |
| BIR-CA-16014 | chromosome (2) plasmid (1) | 1 | 43491 | 0 | 1 | 6  | 7 11878 | ST-354 complex | 8              | 3   | 2   | 2   | 11   | 12  | 6   | 1637237 | 97,3    | 34   | 0,988 | Illumina* DNA Prep, (M) Tagmentation | PureLink Genomic DNA Mini Kit        | NextSeq 500                   | 2 x 149 |         |
| BIR-CA-16015 | chromosome (4)             | 1 | 2564  | 0 | 0 | 0  | 0 9649  | ST-464 complex | 24             | 2   | 2   | 2   | 10   | 3   | 629 | 1741845 | 99,2    | 78   | 0,99  | Illumina* DNA Prep, (M) Tagmentation | PureLink Genomic DNA Mini Kit        | NextSeq 500                   | 2 x 149 |         |
| BIR-CA-16016 | chromosome (9)             | 0 | 0     | 0 | 0 | 0  | 0 3753  | ST-828 complex | 33             | 176 | 30  | 82  | 104  | 43  | 17  | 1710925 | 85,1    | 33   | 0,97  | Illumina* DNA Prep, (M) Tagmentation | PureLink Genomic DNA Mini Kit        | NextSeq 500                   | 2 x 149 |         |
| BIR-CA-16021 | chromosome (7) plasmid (1) | 1 | 46291 | 0 | 1 | 5  | 6 7268  | ST-464 complex | 7              | 2   | 2   | 958 | 2    | 188 | 3   | 1       | 1764944 | 98,3 | 56    | 0,99                                 | Illumina* DNA Prep, (M) Tagmentation | PureLink Genomic DNA Mini Kit | MISeq   | 2 x 151 |
| BIR-CA-16022 | chromosome (4)             | 0 | 0     | 0 | 0 | 0  | 0 4108  | ST-464 complex | 24             | 2   | 2   | 2   | 11   | 3   | 1   | 1695926 | 78,7    | 77   | 0,99  | Illumina* DNA Prep, (M) Tagmentation | PureLink Genomic DNA Mini Kit        | NextSeq 500                   | 2 x 149 |         |
| BIR-CA-16023 | chromosome (6)             | 0 | 0     | 0 | 0 | 0  | 0 829   | ST-828 complex | 33             | 39  | 30  | 82  | 113  | 43  | 17  | 1658852 | 97,9    | 56   | 0,98  | Illumina* DNA Prep, (M) Tagmentation | PureLink Genomic DNA Mini Kit        | NextSeq 500                   | 2 x 149 |         |
| BIR-CA-16024 | chromosome (5)             | 3 | 11289 | 0 | 0 | 0  | 0 1919  | ST-52 complex  | 9              | 2   | 2   | 10  | 10   | 3   | 5   | 1722252 | 96,8    | 138  | 0,99  | Illumina* DNA Prep, (M) Tagmentation | PureLink Genomic DNA Mini Kit        | NextSeq 500                   | 2 x 149 |         |
| BIR-CA-16026 | chromosome (3)             | 0 | 0     | 0 | 0 | 0  | 0 3930  |                | 78             | 42  | 4   | 10  | 11   | 3   | 8   | 1639741 | 98,1    | 23   | 0,98  | Illumina* DNA Prep, (M) Tagmentation | PureLink Genomic DNA Mini Kit        | NextSeq 500                   | 2 x 149 |         |
| BIR-CA-16027 | chromosome (9)             | 0 | 0     | 0 | 0 | 0  | 0 1586  | ST-828 complex | 33             | 176 | 30  | 82  | 113  | 43  | 17  | 1696026 | 92,9    | 19   | 0,98  | Illumina* DNA Prep, (M) Tagmentation | PureLink Genomic DNA Mini Kit        | NextSeq 500                   | 2 x 201 |         |
| BIR-CA-16028 | chromosome (6)             | 0 | 0     | 0 | 0 | 0  | 0 829   | ST-828 complex | 33             | 39  | 30  | 82  | 113  | 43  | 17  | 1658840 | 98      | 55   | 0,97  | Illumina* DNA Prep, (M) Tagmentation | PureLink Genomic DNA Mini Kit        | NextSeq 500                   | 2 x 149 |         |
| BIR-CA-16029 | chromosome (7) plasmid (1) | 2 | 45256 | 0 | 1 | 6  | 7 1433  | ST-353 complex | 7              | 17  | 358 | 2   | 22   | 3   | 1   | 1761129 | 97,5    | 72   | 0,99  | Illumina* DNA Prep, (M) Tagmentation | PureLink Genomic DNA Mini Kit        | NextSeq 500                   | 2 x 149 |         |
| BIR-CA-16030 | chromosome (7)             | 2 | 5817  |   |   |    |         |                |                |     |     |     |      |     |     |         |         |      |       |                                      |                                      |                               |         |         |

# 9 Appendix

|              |                            |   |       |   |    |    |         |                 |     |     |     |     |      |     |     |           |           |       |       |  |  |             |         |
|--------------|----------------------------|---|-------|---|----|----|---------|-----------------|-----|-----|-----|-----|------|-----|-----|-----------|-----------|-------|-------|--|--|-------------|---------|
| BIR-CA-16058 | chromosome (3)             | 1 | 1909  | 0 | 0  | 0  | 0.8920  | 9               | 2   | 2   | 10  | 11  | 253  | 147 |     | 1731585   | 86        | 163   | 0.98  | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500  | 2 x 149     |         |
| BIR-CA-16059 | chromosome (13)            | 2 | 39436 | 0 | 1  | 6  | 7.10873 | 33              | 284 | 30  | 79  | 113 | 47   | 17  |     | 1765524   | 98        | 50    | 0.92  | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | MiSeq  | 2 x 151     |         |
| BIR-CA-16060 | chromosome (13)            | 1 | 39269 | 0 | 1  | 6  | 7.10873 | 33              | 284 | 30  | 79  | 113 | 47   | 17  |     | 1,764,096 | 96,4      | 51    | 0.945 | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500  | 2 x 149     |         |
| BIR-CA-16062 | chromosome (6)             | 2 | 4815  | 0 | 0  | 0  | 0.9976  | ST-828 complex  | 33  | 39  | 631 | 544 | 104  | 43  | 17  |           | 1719703   | 96,2  | 59    | 0.86   | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500 | 2 x 149 |
| BIR-CA-16063 | chromosome (3),plasmid (1) | 1 | 48607 | 0 | 1  | 6  | 7.855   | ST-354 complex  | 9   | 17  | 2   | 11  | 12   | 6   |     | 1,651,130 | 97,6      | 36    | 0.993 | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500  | 2 x 149     |         |
| BIR-CA-16064 | chromosome (4)             | 0 | 0     | 0 | 0  | 0  | 0.9943  |                 | 7   | 2   | 5   | 10  | 13   | 608 | 1   |           | 1,098,584 | 96,3  | 67    | 0.99   | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500 | 2 x 149 |
| BIR-CA-16065 | chromosome (3)             | 0 | 0     | 0 | 0  | 0  | 0.13866 | ST-354 complex  | 9   | 10  | 2   | 37  | 11   | 12  | 6   |           | 1,654,034 | 97,8  | 29    | 0.99   | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500 | 2 x 149 |
| BIR-CA-16068 | chromosome (8)             | 0 | 0     | 0 | 0  | 0  | 0.10873 |                 | 33  | 284 | 30  | 79  | 113  | 47  | 17  |           | 1735989   | 98,2  | 29    | 0.96   | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | MiSeq       | 2 x 151 |
| BIR-CA-16069 | chromosome (8)             | 0 | 0     | 0 | 0  | 0  | 0.10873 |                 | 33  | 284 | 30  | 79  | 113  | 47  | 17  |           | 1732226   | 97,6  | 51    | 0.96   | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500 | 2 x 149 |
| BIR-CA-16070 | chromosome (3)             | 0 | 0     | 0 | 0  | 0  | 0.3611  | ST-607 complex  | 9   | 2   | 5   | 333 | 11   | 3   | 1   |           | 1762900   | 101,8 | 52    | 0.99   | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500 | 2 x 149 |
| BIR-CA-16071 | chromosome (3)             | 0 | 0     | 0 | 0  | 0  | 0.3611  | ST-607 complex  | 9   | 2   | 5   | 333 | 11   | 3   | 1   |           | 1789401   | 98,7  | 43    | 0.99   | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500 | 2 x 149 |
| BIR-CA-16072 | chromosome (3)             | 2 | 7035  | 0 | 0  | 0  | 0.5229  |                 | 8   | 2   | 2   | 212 | 10   | 253 | 147 |           | 1763746   | 72,9  | 117   | 0.97   | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500 | 2 x 149 |
| BIR-CA-16073 | chromosome (10)            | 0 | 0     | 0 | 0  | 0  | 0.4300  | ST-1150 complex | 103 | 110 | 30  | 140 | 210  | 164 | 79  |           | 1762769   | 90    | 54    | 0.74   | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500 | 2 x 149 |
| BIR-CA-16077 | chromosome (8),plasmid (1) | 1 | 1082  | 0 | 0  | 0  | 0.8089  |                 | 8   | 455 | 291 | 668 | 127  | 24  | 19  |           | 1588879   | 97,5  | 42    | 0.95   | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500 | 2 x 149 |
| BIR-CA-16078 | chromosome (3),plasmid (1) | 3 | 46747 | 0 | 1  | 6  | 7.1213  | ST-460 complex  | 7   | 30  | 2   | 2   | 89   | 59  | 6   |           | 1,830,802 | 97,3  | 52    | 0.996  | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500 | 2 x 149 |
| BIR-CA-16079 | chromosome (9)             | 0 | 0     | 0 | 0  | 0  | 0.9560  |                 | 8   | 455 | 291 | 494 | 127  | 24  | 35  |           | 1,611,117 | 96,9  | 35    | 0.953  | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500 | 2 x 149 |
| BIR-CA-16080 | chromosome (3),plasmid (1) | 4 | 49386 | 0 | 1  | 6  | 7.1213  | ST-460 complex  | 7   | 30  | 2   | 2   | 89   | 59  | 6   |           | 1,831,519 | 97,1  | 46    | 0.996  | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500 | 2 x 149 |
| BIR-CA-16081 | chromosome (9)             | 0 | 0     | 0 | 0  | 0  | 0.9560  |                 | 8   | 455 | 291 | 494 | 127  | 24  | 35  |           | 1653984   | 96,8  | 20    | 0.94   | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | MiSeq       | 2 x 151 |
| BIR-CA-16088 | chromosome (9),plasmid (1) | 1 | 1082  | 0 | 0  | 0  | 0.8089  |                 | 8   | 455 | 291 | 668 | 127  | 24  | 19  |           | 1636320   | 97    | 43    | 0.95   | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500 | 2 x 149 |
| BIR-CA-16089 | chromosome (9),plasmid (1) | 1 | 1082  | 0 | 0  | 0  | 0.8089  |                 | 8   | 455 | 291 | 668 | 127  | 24  | 19  |           | 1659776   | 97,5  | 38    | 0.95   | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500 | 2 x 149 |
| BIR-CA-16090 | chromosome (9)             | 0 | 0     | 0 | 0  | 0  | 0.7476  |                 | 37  | 476 | 4   | 28  | 682  | 25  | 147 |           | 1675797   | 98,2  | 57    | 0.94   | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500 | 2 x 149 |
| BIR-CA-16091 | chromosome (5)             | 1 | 4410  | 1 | 1  | 0  | 2.8887  |                 | 22  | 15  | 4   | 735 | 777  | 3   | 35  |           | 1591057   | 98,5  | 35    | 0.93   | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | MiSeq       | 2 x 151 |
| BIR-CA-16092 | chromosome (9),plasmid (1) | 1 | 1082  | 0 | 0  | 0  | 0.8089  |                 | 8   | 455 | 291 | 668 | 127  | 24  | 19  |           | 1637736   | 98,1  | 35    | 0.95   | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | MiSeq       | 2 x 151 |
| BIR-CA-16095 | chromosome (9)             | 0 | 0     | 0 | 0  | 0  | 0.7476  |                 | 37  | 476 | 4   | 28  | 682  | 25  | 147 |           | 1,676,203 | 96,3  | 55    | 0.942  | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500 | 2 x 149 |
| BIR-CA-16096 | chromosome (9),plasmid (1) | 1 | 1082  | 0 | 0  | 0  | 0.8089  |                 | 8   | 455 | 291 | 668 | 127  | 24  | 19  |           | 1,636,365 | 96,7  | 35    | 0.953  | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500 | 2 x 149 |
| BIR-CA-16099 | chromosome (4)             | 0 | 0     | 0 | 0  | 0  | 0.7280  |                 | 406 | 114 | 2   | 2   | 2    | 3   | 147 |           | 1654493   | 98,7  | 26    | 0.97   | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | MiSeq       | 2 x 151 |
| BIR-CA-16103 | chromosome (9)             | 0 | 0     | 0 | 0  | 0  | 0.3753  | ST-828 complex  | 33  | 176 | 30  | 82  | 104  | 43  | 17  |           | 1703929   | 97,6  | 37    | 0.97   | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500 | 2 x 149 |
| BIR-CA-16104 | chromosome (4)             | 0 | 0     | 0 | 0  | 0  | 0.4108  | ST-464 complex  | 24  | 2   | 2   | 2   | 11   | 3   | 1   |           | 1693316   | 91,9  | 66    | 0.99   | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500 | 2 x 149 |
| BIR-CA-16105 | chromosome (4),plasmid (1) | 1 | 50457 | 0 | 1  | 6  | 7.13851 |                 | 7   | 114 | 12  | 2   | 2    | 676 | 6   |           | 1,681,038 | 98,4  | 27    | 0.97   | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | MiSeq       | 2 x 151 |
| BIR-CA-16106 | chromosome (9)             | 0 | 0     | 0 | 0  | 0  | 0.3753  | ST-828 complex  | 33  | 176 | 30  | 82  | 104  | 43  | 17  |           | 1704957   | 97,6  | 32    | 0.97   | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500 | 2 x 149 |
| BIR-CA-16107 | chromosome (10)            | 1 | 30063 | 1 | 10 | 12 | 11,145  | ST-828 complex  | 33  | 39  | 30  | 82  | 104  | 44  | 17  |           | 1688312   | 98,5  | 54    | 0.97   | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | MiSeq       | 2 x 151 |
| BIR-CA-16108 | chromosome (7),plasmid (1) | 2 | 45256 | 0 | 1  | 6  | 7.7433  | ST-353 complex  | 7   | 17  | 358 | 2   | 22   | 3   | 1   |           | 1761790   | 91,8  | 73    | 0.99   | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500 | 2 x 149 |
| BIR-CA-16109 | chromosome (8)             | 1 | 47200 | 0 | 1  | 6  | 7.829   | ST-828 complex  | 33  | 39  | 30  | 82  | 113  | 43  | 17  |           | 1764366   | 97,9  | 42    | 0.94   | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | MiSeq       | 2 x 151 |
| BIR-CA-16110 | chromosome (9)             | 0 | 0     | 0 | 0  | 0  | 0.5507  | ST-828 complex  | 33  | 39  | 30  | 79  | 112  | 64  | 17  |           | 1655911   | 98,2  | 25    | 0.98   | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | MiSeq       | 2 x 151 |
| BIR-CA-16112 | chromosome (13)            | 0 | 0     | 0 | 0  | 0  | 0.3020  | ST-828 complex  | 33  | 176 | 30  | 79  | 113  | 43  | 17  |           | 1,668,361 | 96,9  | 46    | 0.976  | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500 | 2 x 149 |
| BIR-CA-16112 | chromosome (3)             | 0 | 0     | 0 | 0  | 0  | 0.3611  | ST-607 complex  | 9   | 2   | 5   | 333 | 11   | 3   | 1   |           | 1787311   | 97    | 66    | 0.99   | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500 | 2 x 149 |
| BIR-CA-16190 | chromosome (4)             | 0 | 0     | 0 | 0  | 0  | 0.6607  |                 | 9   | 22  | 173 | 146 | 11   | 3   | 6   |           | 1,743,017 | 97,6  | 26    | 0.992  | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500 | 2 x 149 |
| BIR-CA-16191 | chromosome (9)             | 2 | 34194 | 1 | 1  | 10 | 12,629  | ST-828 complex  | 33  | 39  | 30  | 82  | 113  | 43  | 17  |           | 1818813   | 97,3  | 61    | 0.99   | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500 | 2 x 149 |
| BIR-CA-16193 | chromosome (8)             | 0 | 0     | 0 | 0  | 0  | 0.7280  |                 | 406 | 114 | 2   | 2   | 2    | 3   | 147 |           | 1672985   | 97,4  | 35    | 0.97   | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500 | 2 x 149 |
| BIR-CA-16196 | chromosome (13)            | 0 | 0     | 0 | 0  | 0  | 0.10873 |                 | 33  | 284 | 30  | 79  | 113  | 47  | 17  |           | 1725376   | 98,2  | 40    | 0.95   | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | MiSeq       | 2 x 151 |
| BIR-CA-16197 | chromosome (3)             | 1 | 3479  | 0 | 0  | 0  | 0.8920  |                 | 9   | 2   | 2   | 10  | 11   | 253 | 147 |           | 1,734,834 | 95,9  | 177   | 0.984  | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500 | 2 x 149 |
| BIR-CA-16198 | chromosome (3),plasmid (1) | 8 | 53960 | 0 | 1  | 6  | 7.1113  | ST-460 complex  | 24  | 30  | 2   | 2   | 11   | 5   | 8   |           | 1,744,409 | 97,1  | 76    | 0.993  | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500 | 2 x 149 |
| BIR-CA-16201 | chromosome (10)            | 0 | 0     | 0 | 0  | 0  | 0.1121  | ST-1150 complex | 103 | 110 | 30  | 140 | 188  | 164 | 79  |           | 1723247   | 98    | 34    | 0.75   | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | MiSeq       | 2 x 149 |
| BIR-CA-16203 | chromosome (10)            | 0 | 0     | 0 | 0  | 0  | 0.1121  | ST-1150 complex | 103 | 110 | 30  | 140 | 188  | 164 | 79  |           | 1720584   | 97,6  | 45    | 0.72   | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500 | 2 x 149 |
| BIR-CA-16204 | chromosome (7)             | 0 | 2328  | 0 | 0  | 0  | 0.2328  |                 | 8   | 2   | 2   | 212 | 153  | 253 | 147 |           | 1761160   | 96,3  | 153   | 0.98   | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500 | 2 x 149 |
| BIR-CA-16207 | chromosome (2),plasmid (1) | 1 | 47704 | 0 | 1  | 6  | 7.161   | ST-52 complex   | 14  | 21  | 2   | 10  | 86   | 3   | 6   |           | 1,750,596 | 97,5  | 59    | 0.991  | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500 | 2 x 149 |
| BIR-CA-16208 | chromosome (5)             | 2 | 9733  | 0 | 0  | 0  | 0.4258  |                 | 2   | 2   | 2   | 10  | 10   | 59  | 5   |           | 1,777,718 | 96,7  | 137   | 0.989  | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500 | 2 x 149 |
| BIR-CA-16209 | chromosome (2),plasmid (1) | 1 | 47587 | 0 | 1  | 6  | 7.161   | ST-52 complex   | 14  | 21  | 2   | 10  | 86   | 3   | 6   |           | 1,750,337 | 97,5  | 65    | 0.992  | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500 | 2 x 149 |
| BIR-CA-16210 | chromosome (3)             | 0 | 0     | 0 | 0  | 0  | 0.13867 |                 | 2   | 10  | 292 | 26  | 127  | 25  | 541 |           | 1,589,588 | 94,6  | 24    | 0.97   | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | MiSeq       | 2 x 151 |
| BIR-CA-16211 | chromosome (7)             | 1 | 70890 | 0 | 2  | 16 | 18.1993 | ST-828 complex  | 33  | 176 | 30  | 82  | 1200 | 43  | 17  |           | 1,758,639 | 96,9  | 72    | 0.962  | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500 | 2 x 149 |
| BIR-CA-16215 | chromosome (3)             | 1 | 3299  | 0 | 0  | 0  | 0.3228  |                 | 8   | 2   | 2   | 212 | 153  | 253 | 147 |           | 1,748,154 | 99,8  | 142   | 0.979  | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit |             |         |





# 9 Appendix

|             |                             |   |       |   |   |    |         |                 |     |     |     |     |      |     |     |           |       |     |       |  |                               |             |         |
|-------------|-----------------------------|---|-------|---|---|----|---------|-----------------|-----|-----|-----|-----|------|-----|-----|-----------|-------|-----|-------|--|-------------------------------|-------------|---------|
| BR-CA-18717 | chromosome (4)              | 1 | 2564  | 0 | 0 | 0  | 0.464   | ST-464 complex  | 24  | 2   | 2   | 2   | 10   | 3   | 1   | 1750138   | 98.6  | 67  | 0.99  | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | MISeq                         | 2 x 151     |         |
| BR-CA-18718 | chromosome (4)              | 0 | 0     | 0 | 0 | 0  | 0.1121  | ST-1150 complex | 103 | 110 | 30  | 140 | 188  | 164 | 79  | 1762205   | 97.1  | 28  | 0.74  | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500                   | 2 x 149     |         |
| BR-CA-18719 | chromosome (4)              | 0 | 0     | 0 | 0 | 0  | 0.4108  | ST-464 complex  | 24  | 2   | 2   | 2   | 11   | 3   | 1   | 1769277   | 97.7  | 82  | 0.99  | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500                   | 2 x 149     |         |
| BR-CA-18728 | chromosome (11),plasmid (1) | 2 | 8966  | 0 | 1 | 0  | 1.860   | ST-828 complex  | 33  | 39  | 30  | 79  | 113  | 47  | 17  | 1.722.356 | 96.5  | 50  | 0.941 | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500                   | 2 x 149     |         |
| BR-CA-18729 | chromosome (7)              | 2 | 5720  | 0 | 0 | 0  | 0.11365 |                 | 8   | 2   | 2   | 212 | 2    | 253 | 147 | 1.790.286 | 96.6  | 146 | 0.976 | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500                   | 2 x 149     |         |
| BR-CA-18731 | chromosome (9),plasmid (1)  | 2 | 2543  | 0 | 0 | 0  | 0.11872 |                 | 363 | 17  | 292 | 48  | 694  | 24  | 541 | 1.591.274 | 97.7  | 72  | 0.957 | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500                   | 2 x 149     |         |
| BR-CA-18732 | chromosome (6)              | 2 | 6407  | 0 | 0 | 0  | 0.2328  |                 | 8   | 2   | 2   | 212 | 153  | 253 | 147 | 1.777.578 | 96.5  | 142 | 0.98  | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500                   | 2 x 149     |         |
| BR-CA-18734 | chromosome (8)              | 0 | 0     | 0 | 0 | 0  | 0.11879 |                 | 37  | 476 | 4   | 28  | 682  | 3   | 23  | 1.675.715 | 97.1  | 65  | 0.941 | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500                   | 2 x 149     |         |
| BR-CA-18735 | chromosome (3),plasmid (1)  | 1 | 40660 | 0 | 1 | 6  | 7.5799  | ST-463 complex  | 7   | 2   | 2   | 15  | 23   | 3   | 12  | 1.677.127 | 89.9  | 33  | 0.995 | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500                   | 2 x 149     |         |
| BR-CA-18736 | chromosome (10)             | 1 | 6724  | 1 | 0 | 0  | 1.1121  | ST-1150 complex | 103 | 110 | 30  | 140 | 188  | 164 | 79  | 1.723.959 | 97.2  | 20  | 0.771 | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500                   | 2 x 149     |         |
| BR-CA-18737 | chromosome (13)             | 0 | 0     | 0 | 0 | 0  | 0.10873 |                 | 33  | 284 | 30  | 79  | 113  | 47  | 17  | 1.721.858 | 97.1  | 53  | 0.95  | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500                   | 2 x 149     |         |
| BR-CA-18738 | chromosome (14)             | 0 | 0     | 0 | 0 | 0  | 0.860   | ST-828 complex  | 33  | 39  | 30  | 79  | 113  | 47  | 17  | 1.714.634 | 97.2  | 40  | 0.94  | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500                   | 2 x 149     |         |
| BR-CA-18743 | chromosome (9)              | 1 | 27948 | 0 | 1 | 10 | 11.7355 | ST-353 complex  | 8   | 17  | 5   | 2   | 10   | 59  | 23  | 1.805515  | 97.6  | 48  | 0.99  | Nextera DNA Flex Library prep Kit                                  | PureLink Genomic DNA Mini Kit | NextSeq 500 | 2 x 149 |
| BR-CA-18748 | chromosome (5)              | 0 | 0     | 0 | 0 | 0  | 0.10183 |                 | 114 | 110 | 30  | 140 | 104  | 625 | 79  | 1.753005  | 97.6  | 43  | 0.75  | Nextera DNA Flex Library prep Kit                                  | PureLink Genomic DNA Mini Kit | NextSeq 500 | 2 x 149 |
| BR-CA-18820 | chromosome (4)              | 3 | 11511 | 0 | 0 | 0  | 0.1919  | ST-52 complex   | 9   | 2   | 2   | 10  | 10   | 3   | 5   | 1.764653  | 96.5  | 152 | 0.99  | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500                   | 2 x 149     |         |
| BR-CA-18821 | chromosome (13)             | 0 | 0     | 0 | 0 | 0  | 0.10873 |                 | 33  | 284 | 30  | 79  | 113  | 47  | 17  | 1.720989  | 97.5  | 63  | 0.95  | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500                   | 2 x 149     |         |
| BR-CA-18822 | chromosome (4)              | 3 | 6829  | 0 | 0 | 0  | 0.2172  | ST-828 complex  | 32  | 38  | 30  | 82  | 104  | 35  | 17  | 1.786500  | 97.4  | 62  | 0.91  | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500                   | 2 x 149     |         |
| BR-CA-18825 | chromosome (4)              | 2 | 5774  | 0 | 0 | 0  | 0.4258  |                 | 2   | 2   | 2   | 10  | 10   | 59  | 5   | 1.772.835 | 85.4  | 134 | 0.988 | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500                   | 2 x 149     |         |
| BR-CA-18834 | chromosome (4)              | 2 | 12650 | 1 | 1 | 0  | 2.832   | ST-828 complex  | 33  | 39  | 30  | 79  | 113  | 43  | 17  | 1.757.129 | 85.2  | 26  | 0.927 | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500                   | 2 x 149     |         |
| BR-CA-18839 | chromosome (3)              | 1 | 3160  | 0 | 0 | 0  | 0.354   | ST-354 complex  | 8   | 10  | 2   | 2   | 11   | 12  | 6   | 1.702392  | 98.1  | 39  | 0.99  | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500                   | 2 x 149     |         |
| BR-CA-18840 | chromosome (3)              | 0 | 0     | 0 | 0 | 0  | 0.354   | ST-354 complex  | 8   | 10  | 2   | 2   | 11   | 12  | 6   | 1.701781  | 88.6  | 33  | 0.99  | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500                   | 2 x 149     |         |
| BR-CA-18841 | chromosome (4)              | 1 | 2564  | 0 | 0 | 0  | 0.9649  | ST-464 complex  | 24  | 2   | 2   | 2   | 10   | 3   | 629 | 1.70225   | 103.6 | 92  | 0.99  | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500                   | 2 x 149     |         |
| BR-CA-18842 | chromosome (3)              | 0 | 0     | 0 | 0 | 0  | 0.829   | ST-828 complex  | 33  | 39  | 30  | 82  | 113  | 43  | 17  | 1.701.389 | 96.7  | 49  | 0.962 | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500                   | 2 x 149     |         |
| BR-CA-18843 | chromosome (13)             | 0 | 0     | 0 | 0 | 0  | 0.10873 |                 | 33  | 284 | 30  | 79  | 113  | 47  | 17  | 1.724.012 | 91.5  | 47  | 0.955 | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500                   | 2 x 149     |         |
| BR-CA-18844 | chromosome (4)              | 0 | 0     | 0 | 0 | 0  | 0.4108  | ST-464 complex  | 24  | 2   | 2   | 2   | 11   | 3   | 1   | 1.781.769 | 70.8  | 61  | 0.592 | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500                   | 2 x 149     |         |
| BR-CA-18849 | chromosome (1)              | 0 | 0     | 0 | 0 | 0  | 0.50    | ST-21 complex   | 2   | 1   | 12  | 3   | 2    | 1   | 1   | 1.709930  | 97.8  | 59  | 1.00  | Nextera DNA Flex Library prep Kit                                  | PureLink Genomic DNA Mini Kit | NextSeq 500 | 2 x 149 |
| BR-CA-18879 | chromosome (10)             | 1 | 3415  | 0 | 0 | 0  | 1.4360  | ST-1150 complex | 103 | 110 | 30  | 140 | 210  | 164 | 79  | 1.754.476 | 71.8  | 35  | 0.765 | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500                   | 2 x 149     |         |
| BR-CA-18880 | chromosome (4)              | 0 | 0     | 0 | 0 | 0  | 0.1121  | ST-1150 complex | 103 | 110 | 30  | 140 | 188  | 164 | 79  | 1.706833  | 95.5  | 25  | 0.81  | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500                   | 2 x 149     |         |
| BR-CA-18881 | chromosome (4)              | 0 | 0     | 0 | 0 | 0  | 0.4108  | ST-464 complex  | 24  | 2   | 2   | 2   | 11   | 3   | 1   | 1.739745  | 99.6  | 75  | 0.99  | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500                   | 2 x 149     |         |
| BR-CA-18883 | chromosome (9)              | 1 | 2509  | 1 | 0 | 0  | 1.6267  | ST-828 complex  | 33  | 42  | 30  | 82  | 189  | 35  | 17  | 1.691.304 | 76.7  | 48  | 0.954 | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500                   | 2 x 149     |         |
| BR-CA-18884 | chromosome (4)              | 0 | 0     | 0 | 0 | 0  | 0.4108  | ST-464 complex  | 24  | 2   | 2   | 2   | 11   | 3   | 1   | 1.748.304 | 88.2  | 59  | 0.994 | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500                   | 2 x 149     |         |
| BR-CA-18885 | chromosome (9)              | 1 | 26971 | 0 | 1 | 10 | 11.5586 | ST-828 complex  | 33  | 176 | 30  | 82  | 113  | 43  | 17  | 1.765.192 | 99.3  | 49  | 0.961 | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500                   | 2 x 149     |         |
| BR-CA-18886 | chromosome (1),plasmid (3)  | 4 | 40335 | 3 | 2 | 10 | 15.860  | ST-828 complex  | 33  | 39  | 30  | 79  | 113  | 47  | 17  | 1.743.220 | 100.3 | 68  | 0.957 | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500                   | 2 x 149     |         |
| BR-CA-18887 | chromosome (5)              | 0 | 0     | 0 | 0 | 0  | 0.11993 | ST-828 complex  | 33  | 176 | 30  | 82  | 1203 | 43  | 17  | 1.749.655 | 98.9  | 43  | 0.960 | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500                   | 2 x 149     |         |
| BR-CA-18889 | chromosome (3)              | 0 | 0     | 0 | 0 | 0  | 0.11861 |                 | 166 | 1   | 1   | 10  | 151  | 3   | 1   | 1.759273  | 98    | 46  | 0.99  | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500                   | 2 x 149     |         |
| BR-CA-18891 | chromosome (3),plasmid (1)  | 2 | 52410 | 2 | 1 | 6  | 9.6493  |                 | 7   | 2   | 6   | 10  | 465  | 37  | 1   | 1.732.881 | 93    | 47  | 0.99  | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500                   | 2 x 149     |         |
| BR-CA-18892 | chromosome (2),plasmid (1)  | 1 | 43511 | 0 | 1 | 6  | 7.11878 | ST-354 complex  | 8   | 3   | 2   | 2   | 11   | 12  | 6   | 1.638.627 | 56.4  | 25  | 0.987 | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500                   | 2 x 149     |         |
| BR-CA-18894 | chromosome (1)              | 0 | 0     | 0 | 0 | 0  | 0.4800  |                 | 275 | 84  | 5   | 10  | 119  | 178 | 16  | 1.729875  | 96.8  | 50  | 1.00  | Nextera DNA Flex Library prep Kit                                  | PureLink Genomic DNA Mini Kit | NextSeq 500 | 2 x 149 |
| BR-CA-18901 | chromosome (2)              | 0 | 0     | 0 | 0 | 0  | 0.658   | ST-658 complex  | 4   | 2   | 4   | 2   | 19   | 3   | 6   | 1.656440  | 96.6  | 121 | 0.99  | Nextera DNA Flex Library prep Kit                                  | PureLink Genomic DNA Mini Kit | NextSeq 500 | 2 x 149 |
| BR-CA-19025 | chromosome (6)              | 0 | 0     | 0 | 0 | 0  | 0.829   | ST-828 complex  | 33  | 39  | 30  | 82  | 113  | 43  | 17  | 1.658725  | 97.7  | 58  | 0.97  | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500                   | 2 x 149     |         |
| BR-CA-19026 | chromosome (5)              | 0 | 0     | 0 | 0 | 0  | 0.3119  | ST-828 complex  | 33  | 39  | 30  | 82  | 188  | 47  | 17  | 1.726041  | 97.7  | 50  | 0.96  | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500                   | 2 x 149     |         |
| BR-CA-19027 | chromosome (9)              | 0 | 0     | 0 | 0 | 0  | 0.1586  | ST-828 complex  | 33  | 176 | 30  | 82  | 113  | 43  | 17  | 1.691.222 | 60.3  | 32  | 0.983 | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500                   | 2 x 149     |         |
| BR-CA-19028 | chromosome (6)              | 0 | 0     | 0 | 0 | 0  | 0.829   | ST-828 complex  | 33  | 39  | 30  | 82  | 113  | 43  | 17  | 1.658.011 | 57    | 64  | 0.973 | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500                   | 2 x 149     |         |
| BR-CA-19029 | chromosome (8),plasmid (1)  | 1 | 2126  | 0 | 0 | 0  | 0.1586  | ST-828 complex  | 33  | 176 | 30  | 82  | 113  | 43  | 17  | 1.711.039 | 96.3  | 121 | 0.962 | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500                   | 2 x 149     |         |
| BR-CA-19030 | chromosome (8),plasmid (1)  | 1 | 2126  | 0 | 0 | 0  | 0.1586  | ST-828 complex  | 33  | 176 | 30  | 82  | 113  | 43  | 17  | 1.711.903 | 96.5  | 120 | 0.961 | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500                   | 2 x 149     |         |
| BR-CA-19031 | chromosome (8),plasmid (1)  | 1 | 2126  | 0 | 0 | 0  | 0.1586  | ST-828 complex  | 33  | 176 | 30  | 82  | 113  | 43  | 17  | 1.712.442 | 96.7  | 118 | 0.961 | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500                   | 2 x 149     |         |
| BR-CA-19032 | chromosome (9)              | 0 | 0     | 0 | 0 | 0  | 0.1586  | ST-828 complex  | 33  | 176 | 30  | 82  | 113  | 43  | 17  | 1.709873  | 97.2  | 128 | 0.96  | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500                   | 2 x 149     |         |
| BR-CA-19033 | chromosome (10)             | 0 | 0     | 0 | 0 | 0  | 0.1145  | ST-828 complex  | 33  | 39  | 30  | 82  | 104  | 44  | 17  | 1.667999  | 97    | 55  | 0.97  | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500                   | 2 x 149     |         |
| BR-CA-19034 | chromosome (8),plasmid (1)  | 1 | 2126  | 0 | 0 | 0  | 0.1586  | ST-828 complex  | 33  | 176 | 30  | 82  | 113  | 43  | 17  | 1.711.834 | 96.4  | 109 | 0.961 | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500                   | 2 x 149     |         |
| BR-CA-19035 | chromosome (4)              | 0 | 0     | 0 | 0 | 0  | 0.8649  | ST-464 complex  | 24  | 2   | 2   | 2   | 10   | 3   | 629 | 1.741924  | 97.8  | 74  | 0.99  | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500                   | 2 x 149     |         |
| BR-CA-19036 | chromosome (3),plasmid (1)  | 1 | 47257 | 0 | 1 | 6  | 9.1311  | ST-460 complex  | 24  | 2   | 2   | 11  | 716  | 30  | 1   | 1.750782  | 96.2  | 57  | 0.99  | illumina* DNA Prep, (M) Tagmentation PureLink Genomic DNA Mini Kit | NextSeq 500                   | 2 x 149     |         |
| BR-CA-19037 | chromosome (8)              | 0 | 0     | 0 |   |    |         |                 |     |     |     |     |      |     |     |           |       |     |       |  |                               |             |         |



# 9 Appendix

| Sample overview<br>Samples highlighted in blue were additionally sequenced with Oxford Nanopore Technology |             |         |                  |                 |                    | <b>Unicycler hybrid assembly and BakCharak AMR prediction</b><br>Genes not found are highlighted in <b>red</b><br>Genes that were falsely annotated are struck through (e.g. <del>tet(O)</del> )<br>Genes found in close proximity to transposases are in <b>bold</b> .<br>Partial genes correctly found are annotated as such (e.g. aadE2_Δ1-415)<br>aad9 falsely annotated as truncated due to frame-shifting in the poly-C tract are shown in <b>bold and blue</b> |   |   |
|--|-------------|---------|------------------|-----------------|--------------------|---|---|---|
|  | isolate No. | contigs | circular contigs | plasmid contigs | plasmids cumlength | largest contig size   | Point mutations and AMR genes on chromosome<br>Bold highlighting indicates close proximity of AMR genes to AMR-associated transposase | amr genes plasmid localization  |
| Bfr-CA-15687   | 2           | 2       | 1                | 44826           | 1657372            | blaOXA-489;tet(O);GyrA_T86I   | tet(O)  |   |
| Bfr-CA-15991   | 1           | 1       | 0                |                 | 1691542            | <del>tet(O/M/O)</del> <sub>Flavanese variant</sub> -catA9-tnp <sub>S1216</sub> family-fexA-optrA-tnp <sub>S1216</sub> family-tet(L);aac(6')-le/aph(2'')-la-aadE1-tet(O) <sub>XΔC-terminus</sub> ;blaOXA-193; 23S_A2075G; 23S_A2075G;23S_A2075G; GyrA_T86I   |   | IS6-like element IS1216 family transposase (x2)   |
| Bfr-CA-16040   | 1           | 1       | 0                |                 | 1760061            | tnp <sub>IScCo2</sub> family-catA13-aph(3')-IIla-aad9;tet(O/M/O)-aad9-erm(B)-aadE1;aac(6')-le/aph(2'')-la-aadE1-tet(O) <sub>XΔC-terminus</sub> ;blaOXA-489;GyrA_T86I  |   | IS1595-like element IScCo2 family transposase   |
| Bfr-CA-16046   | 2           | 2       | 1                | 39212           | 1819504            | tnp <sub>IScCo2</sub> family-CatA13-aph(3')-IIla-aad9-aph(2'')-If-blaOXA-193;tet(O/M/O)-aad9-erm(B)-aadE1;aadE-Cc;GyrA_T86I   |   | IS1595-like element IScCo2 family transposase   |
| Bfr-CA-16077   | 1           | 1       | 0                |                 | 1620666            | tnp <sub>IScCo2</sub> family-CatA13-aph(3')-IIla-aad9-aph(2'')-If;tet(O/M/O) <sub>Henan variant</sub> -aadE1-tet(O) <sub>XΔN-terminus</sub> ;aph(3')-IIla-tnp <sub>IScCaj6</sub> family;blaOXA-184 family;tnp <sub>IScCo2</sub> family-Inu(C);GyrA_T86I   |   | IS1595-like element IScCo2 family transposase (x2);<br>IS1595-like element IScCaj6 family transposase |
| Bfr-CA-16088   | 1           | 1       | 0                |                 | 1657686            | tnp <sub>IScCo2</sub> family-CatA13-aph(3')-IIla-aad9-aph(2'')-If;tet(O/M/O) <sub>Henan variant</sub> -aadE1-tet(O) <sub>XΔN-terminus</sub> ;aph(3')-IIla-tnp <sub>IScCaj6</sub> family;blaOXA-184 family;tnp <sub>IScCo2</sub> family-Inu(C); GyrA_T86I;23S_A2075G; 23S_A2075G;23S_A2075G  |   | IS1595-like element IScCo2 family transposase (x2);<br>IS1595-like element IScCaj6 family transposase |
| Bfr-CA-16110   | 1           | 1       | 0                |                 | 1673552            | tet(O)-aad9-erm(B)-aadE1;blaOXA-193;GyrA_T86I   |   |   |
| Bfr-CA-16201   | 2           | 2       | 1                | 3333            | 1734659            | tet(O)-aad9-erm(B)-aadE1;tnp <sub>IScCo2</sub> family-catA13-aph(3')-IIla-aad9-aph(2'')-If-blaOXA-193;tet(O);GyrA_T86I;50S_L22_A103V  |   | IS1595-like element IScCo2 family transposase   |
| Bfr-CA-16258   | 2           | 2       | 1                | 3310            | 1724953            | tet(O)-aad9-erm(B)-aadE1;tet(O);blaOXA-193;GyrA_T86I;50S_L22_A103V  |   |   |
| Bfr-CA-16297   | 1           | 1       | 0                |                 | 1703146            | tet(O/M/O)-aad9-erm(B)-aadE1;aph(2'')-If-aph(3')-IIla-tnp <sub>IScCaj6</sub> family;aac(6')-le/aph(2'')-la-aadE1-tet(O) <sub>XΔC-terminus</sub> ;tet(O)-tnp <sub>IS607</sub> family;blaOXA-193;GyrA_T86I  |   | IS1595-like element IScCaj6 family transposase; IS607 Family transposase                              |
| Bfr-CA-16737   | 2           | 2       | 1                | 52704           | 1817905            | blaOXA-185 like;tet(O);GyrA_T86I  | tet(O);tet(O/32/O)-aadE2_Δ1-415-sat4-aph(3')-IIla   |   |
| Bfr-CA-18842   | 1           | 1       | 0                |                 | 1725012            | aac(6')-le/aph(2'')-la-aadE1-tet(O) <sub>XΔC-terminus</sub> ;tet(O/M/O);blaOXA-193;GyrA_T86I  |   |   |
| Bfr-CA-19087   | 2           | 2       | 1                | 4097            | 1733474            | tnp <sub>IS607</sub> family-tet(O);tet(O/32/O)-aph(2'')-If-aph(3')-IIla-aad9-aadE1-tet(O) <sub>XΔN-terminus</sub> ; tet(O/32/O);aadE-Cc;GyrA_T86I   |   | IS607 Family transposase  |

# 9 Appendix

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|              |   |   |   |         |   |  |
|--------------|---|---|---|---------|---|--|
| BfR-CA-19301 | 1 | 1 | 0 | 1801855 | <b>aadE3-sat4-aph(3')-IIIa-trp</b> <sub>IS1216 family</sub> ; <b>tet(O)</b> <b>tet(O/32/O)</b> ; blaOXA-193; GyrA_T86I; SOS_L22_A103V | IS6-like element IS1216 family transposase |
|--------------|---|---|---|---------|---|--|



# 9 Appendix

**Proof of principle comparison of AMR gene detection based on SKESA or shovill genome assemblies**  
 Resistance determinants as reported by AMRFinderPlus  
 The coverage of the gene is expressed as a percentage of the full-length translated protein sequence  
 Genes not found are highlighted in red  
 differences in AMR detection based on SKESA vs. Shovill are highlighted with red shading

| SAMPLE # | SAMPLE ID    | Assembler | enhanced performance shovill vs. SKESA | blaOXA     | blaOXA coverage | gyrA      | tet(O) | tet(O) coverage | tet(O/M/O) | tet(O/M/O) coverage | aph(3')-IIIa | aph(3')-IIIa coverage | 50S_L22       | aad9 | aad9 coverage | catA13 | catA13 coverage | aadE1 | aadE1 coverage | 23S        | aph(2'')-IIf | aph(2'')-IIf coverage | sat4 | sat4 coverage |
|----------|--------------|-----------|--|------------|-----------------|-----------|--------|-----------------|------------|---------------------|--------------|-----------------------|---------------|------|---------------|--------|-----------------|-------|----------------|------------|--------------|-----------------------|------|---------------|
| 1        | BFR-CA-15687 | Shovill   | no                                     | blaOXA-489 | 100             | gyrA_T86i | tet(O) | 61,03           |            | 0                   |              | 0                     |               |      | 0             |        | 0               |       | 0              |            |              | 0                     |      | 0             |
|          |              | SKESA     |  | blaOXA-489 | 100             | gyrA_T86i | tet(O) | 59,94           |            | 0                   |              | 0                     |               |      |               | 0      |                 | 0     |                | 0          |              |                       | 0    |               |
| 2        | BFR-CA-16062 | Shovill   | no                                     | blaOXA-193 | 100             | gyrA_T86i | tet(O) | 70,27           |            | 0                   |              | 0                     |               |      | 0             |        | 0               | aadE  | 100            | 23S_A2075G |              | 0                     |      | 0             |
|          |              | SKESA     |  | blaOXA-193 | 100             | gyrA_T86i | tet(O) | 69,17           |            | 0                   |              | 0                     |               |      |               | 0      |                 | 0     | aadE           | 100        | 23S_A2075G   |                       | 0    |               |
| 3        | BFR-CA-16088 | Shovill   | no                                     | blaOXA     | 100             | gyrA_T86i | tet(O) | 0               | tet(O/M/O) | 56,65               | aph(3')-IIIa | 100                   |               | aad9 | 83,72         | catA13 | 100             | aadE  | 100            | 23S_A2075G | aph(2'')-IIf | 100                   |      | 0             |
|          |              | SKESA     |  | blaOXA     | 100             | gyrA_T86i | tet(O) | 54,77           | tet(O/M/O) | 51,8                | aph(3')-IIIa | 100                   |               | aad9 | 87,98         | catA13 | 100             | aadE  | 100            | 23S_A2075G | aph(2'')-IIf | 100                   |      | 0             |
| 4        | BFR-CA-16220 | Shovill   | no                                     | blaOXA-184 | 100             | gyrA_T86i | tet(O) | 0               | tet(O/M/O) | 56,65               | aph(3')-IIIa | 100                   |               |      | 0             |        | 0               | aadE  | 100            |            |              | 0                     | sat4 | 100           |
|          |              | SKESA     |  | blaOXA-184 | 100             | gyrA_T86i | tet(O) | 0               | tet(O/M/O) | 51,8                | aph(3')-IIIa | 100                   |               |      |               | 0      |                 | 0     | aadE           | 100        |              |                       | 0    | sat4          |
| 5        | BFR-CA-16249 | Shovill   | no                                     | blaOXA-489 | 100             | gyrA_T86i | tet(O) | 70,27           |            | 0                   |              | 0                     |               |      | 0             |        | 0               | aadE  | 100            | 23S_A2075G |              | 0                     |      | 0             |
|          |              | SKESA     |  | blaOXA-489 | 100             | gyrA_T86i | tet(O) | 69,17           |            | 0                   |              | 0                     |               |      |               | 0      |                 | 0     | aadE           | 100        | 23S_A2075G   |                       | 0    |               |
| 6        | BFR-CA-16258 | Shovill   | yes                                    | blaOXA-193 | 100             | gyrA_T86i | tet(O) | 63,22           |            | 0                   |              | 0                     | 50S_L22_A103V | aad9 | 100           |        | 0               | aadE  | 100            |            |              | 0                     |      | 0             |
|          |              | SKESA     |  | blaOXA-193 | 100             | gyrA_T86i | tet(O) | 62,13           |            | 0                   |              | 0                     | 50S_L22_A103V | aad9 | 83,72         |        | 0               | aadE  | 100            |            |              | 0                     |      | 0             |
| 7        | BFR-CA-17105 | Shovill   | no                                     | blaOXA-630 | 100             |           | tet(O) | 100             |            | 0                   |              | 0                     |               |      | 0             |        | 0               |       | 0              |            |              | 0                     |      | 0             |
|          |              | SKESA     |  | blaOXA-630 | 100             |           | tet(O) | 100             |            | 0                   |              | 0                     |               |      |               | 0      |                 | 0     |                | 0          |              |                       | 0    |               |
| 8        | BFR-CA-18886 | Shovill   | no                                     |            |                 | gyrA_T86i | tet(O) | 0               | tet(O/M/O) | 0                   |              | 0                     |               | aad9 | 83,72         |        | 0               | aadE  | 100            |            |              | 0                     |      | 0             |
|          |              | SKESA     |  |            |                 | gyrA_T86i | tet(O) | 0               | tet(O/M/O) | 0                   |              | 0                     |               | aad9 | 81,4          |        | 0               | aadE  | 100            |            |              | 0                     |      | 0             |
| 9        | BFR-CA-19033 | Shovill   | no                                     | blaOXA-193 | 100             | gyrA_T86i | tet(O) | 0               | tet(O/M/O) | 69,17               | aph(3')-IIIa | 100                   |               | aad9 | 100           |        | 0               | aadE  | 100            |            |              | 0                     | sat4 | 100           |
|          |              | SKESA     |  | blaOXA-193 | 100             | gyrA_T86i | tet(O) | 0               | tet(O/M/O) | 64,32               | aph(3')-IIIa | 100                   |               | aad9 | 100           |        | 0               | aadE  | 100            |            |              | 0                     | sat4 | 100           |
| 10       | BFR-CA-19112 | Shovill   | no                                     | blaOXA-193 | 100             | gyrA_T86i | tet(O) | 0               | tet(O/M/O) | 90,61               | aph(3')-IIIa | 100                   |               | aad9 | 84,5          |        | 0               | aadE  | 88,89          |            |              | 0                     | sat4 | 100           |
|          |              | SKESA     |  | blaOXA-193 | 100             | gyrA_T86i | tet(O) | 0               | tet(O/M/O) | 64,32               | aph(3')-IIIa | 100                   |               | aad9 | 81,4          |        | 0               | aadE  | 86,46          |            |              | 0                     | sat4 | 100           |

# 9 Appendix

## Proof of principle comparison of AMR gene detection based on SKESA or shovill genome assemblies

Resistance determinants as reported by AMRFinderPlus

The coverage of the gene is expressed as a percentage of the full-length translated protein sequence

Genes not found are highlighted in **red**

differences in AMR detection based on SKESA vs. Shovill are highlighted with red shading

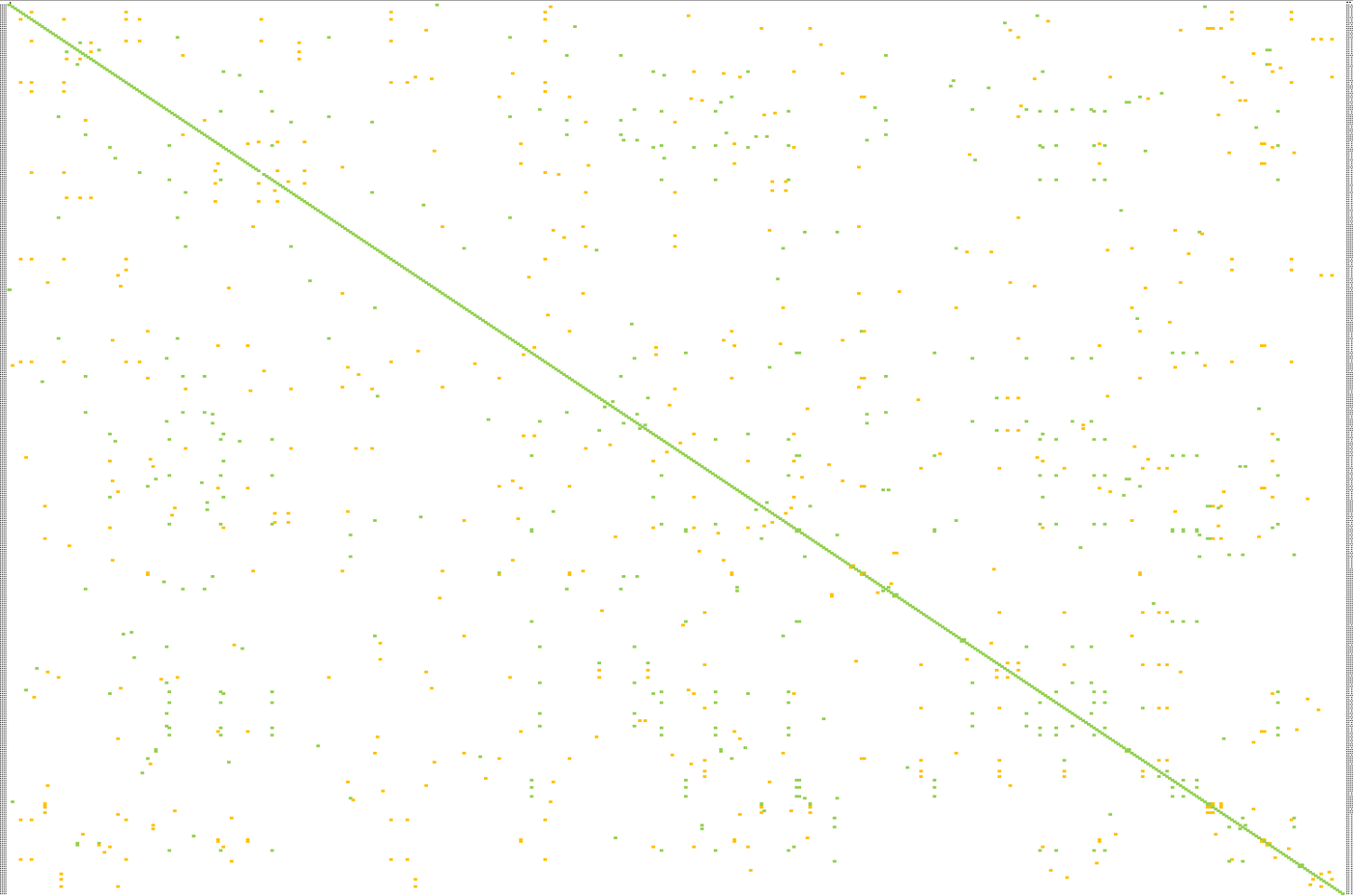
| SAMPLE # | SAMPLE ID    | Assembler | enhanced performance shovill vs. SKESA | erm(B) | erm(B) coverage | aac(6)-Ie/aph(2 <sup>+</sup> )-Ia | aac(6)-Ie/aph(2 <sup>+</sup> )-Ia coverage | aadE-Cc | aadE-Cc coverage | tet(L) | tet(L) coverage | catA | catA coverage | optrA | optrA coverage | fexA | fexA coverage | rpsL_K43R | lnu(C) | lnu(C) coverage | ant(6)-Ia | ant(6)-Ia coverage | cat-TC    | cat-TC coverage | rpsL_K88R | aph(2 <sup>+</sup> )-II1 | aph(2 <sup>+</sup> )-II1 coverage | tet(W) | tet(W) coverage |        |                 |                                   |
|----------|--------------|-----------|--|--------|-----------------|-----------------------------------|--|---------|------------------|--------|-----------------|------|---------------|-------|----------------|------|---------------|-----------|--------|-----------------|-----------|--------------------|-----------|-----------------|-----------|--------------------------|-----------------------------------|--------|-----------------|--------|-----------------|-----------------------------------|
|          |              |           |  |        |                 |                                   |  |         |                  |        |                 |      |               |       |                |      |               |           |        |                 |           |                    |           |                 |           |                          |                                   |        |                 | erm(B) | erm(B) coverage | aac(6)-Ie/aph(2 <sup>+</sup> )-Ia |
| 1        | BFR-CA-15687 | Shovill   | no                                     |        | 0               |                                   | 0  |         | 0                |        | 0               |      | 0             |       | 0              |      | 0             |           | 0      |                 | 0         |                    | 0         |                 | 0         |                          | 0                                 |        | 0               |        | 0               |                                   |
|          |              | SKESA     |  |        | 0               |                                   | 0  |         | 0                |        | 0               |      | 0             |       | 0              |      | 0             |           | 0      |                 | 0         |                    | 0         |                 | 0         |                          | 0                                 |        | 0               |        | 0               |                                   |
| 2        | BFR-CA-16062 | Shovill   | no                                     |        | 0               | aac(6)-Ie/aph(2 <sup>+</sup> )-Ia | 100  |         | 0                |        | 0               |      | 0             |       | 0              |      | 0             |           | 0      |                 | 0         |                    | 0         |                 | 0         |                          | 0                                 |        | 0               |        | 0               |                                   |
|          |              | SKESA     |  |        | 0               | aac(6)-Ie/aph(2 <sup>+</sup> )-Ia | 100  |         | 0                |        | 0               |      | 0             |       | 0              |      | 0             |           | 0      |                 | 0         |                    | 0         |                 | 0         |                          | 0                                 |        | 0               |        | 0               |                                   |
| 3        | BFR-CA-16088 | Shovill   | no                                     |        | 0               |                                   | 0  |         | 0                |        | 0               |      | 0             |       | 0              |      | 0             |           | lnu(C) | 100             |           | 0                  |           | 0               |           | 0                        |                                   | 0      |                 | 0      |                 | 0                                 |
|          |              | SKESA     |  |        | 0               |                                   | 0  |         | 0                |        | 0               |      | 0             |       | 0              |      | 0             |           | 0      |                 | lnu(C)    | 100                |           | 0               |           | 0                        |                                   | 0      |                 | 0      |                 | 0                                 |
| 4        | BFR-CA-16220 | Shovill   | no                                     |        | 0               |                                   | 0  |         | 0                |        | 0               |      | 0             |       | 0              |      | 0             |           | 0      |                 | ant(6)-Ia | 53.97              |           | 0               |           | 0                        |                                   | 0      |                 | 0      |                 | 0                                 |
|          |              | SKESA     |  |        | 0               |                                   | 0  |         | 0                |        | 0               |      | 0             |       | 0              |      | 0             |           | 0      |                 | 0         |                    | ant(6)-Ia | 53.97           |           | 0                        |                                   | 0      |                 | 0      |                 | 0                                 |
| 5        | BFR-CA-16249 | Shovill   | no                                     |        | 0               | aac(6)-Ie/aph(2 <sup>+</sup> )-Ia | 100  | aadE-Cc | 100              |        | 0               |      | 0             |       | 0              |      | 0             |           | 0      |                 | 0         |                    | 0         |                 | 0         |                          | 0                                 |        | 0               |        | 0               |                                   |
|          |              | SKESA     |  |        | 0               | aac(6)-Ie/aph(2 <sup>+</sup> )-Ia | 100  | aadE-Cc | 100              |        | 0               |      | 0             |       | 0              |      | 0             |           | 0      |                 | 0         |                    | 0         |                 | 0         |                          | 0                                 |        | 0               |        | 0               |                                   |
| 6        | BFR-CA-16258 | Shovill   | yes                                    | erm(B) | 100             |                                   | 0  |         | 0                |        | 0               |      | 0             |       | 0              |      | 0             |           | 0      |                 | 0         |                    | 0         |                 | 0         |                          | 0                                 |        | 0               |        | 0               |                                   |
|          |              | SKESA     |  |        | erm(B)          | 100                               |  | 0       |                  | 0      |                 | 0    |               | 0     |                | 0    |               | 0         |        | 0               |           | 0                  |           | 0               |           | 0                        |                                   | 0      |                 | 0      |                 | 0                                 |
| 7        | BFR-CA-17105 | Shovill   | no                                     |        | 0               |                                   | 0  |         | 0                |        | 0               |      | 0             |       | 0              |      | 0             |           | 0      |                 | 0         |                    | 0         |                 | 0         |                          | 0                                 |        | 0               |        | 0               |                                   |
|          |              | SKESA     |  |        | 0               |                                   | 0  |         | 0                |        | 0               |      | 0             |       | 0              |      | 0             |           | 0      |                 | 0         |                    | 0         |                 | 0         |                          | 0                                 |        | 0               |        | 0               |                                   |
| 8        | BFR-CA-18886 | Shovill   | no                                     | erm(B) | 100             |                                   | 0  |         | 0                |        | 0               |      | 0             |       | 0              |      | 0             |           | 0      |                 | 0         |                    | 0         |                 | 0         |                          | 0                                 |        | 0               |        | 0               |                                   |
|          |              | SKESA     |  |        | erm(B)          | 100                               |  | 0       |                  | 0      |                 | 0    |               | 0     |                | 0    |               | 0         |        | 0               |           | 0                  |           | 0               |           | 0                        |                                   | 0      |                 | 0      |                 | 0                                 |
| 9        | BFR-CA-19033 | Shovill   | no                                     | erm(B) | 100             | aac(6)-Ie/aph(2 <sup>+</sup> )-Ia | 100  |         | 0                |        | 0               |      | 0             |       | 0              |      | 0             |           | 0      |                 | ant(6)-Ia | 87.75              |           | 0               |           | 0                        |                                   | 0      |                 | 0      |                 | 0                                 |
|          |              | SKESA     |  |        | erm(B)          | 100                               | aac(6)-Ie/aph(2 <sup>+</sup> )-Ia          | 100     |                  | 0      |                 | 0    |               | 0     |                | 0    |               | 0         |        | 0               |           | 0                  |           | ant(6)-Ia       | 87.75     |                          | 0                                 |        | 0               |        | 0               |                                   |
| 10       | BFR-CA-19112 | Shovill   | no                                     | erm(B) | 100             | aac(6)-Ie/aph(2 <sup>+</sup> )-Ia | 100  |         | 0                |        | 0               |      | 0             |       | 0              |      | 0             |           | 0      |                 | ant(6)-Ia | 87.75              |           | 0               |           | 0                        |                                   | 0      |                 | 0      |                 | 0                                 |
|          |              | SKESA     |  |        | erm(B)          | 100                               | aac(6)-Ie/aph(2 <sup>+</sup> )-Ia          | 100     |                  | 0      |                 | 0    |               | 0     |                | 0    |               | 0         |        | 0               |           | ant(6)-Ia          | 87.75     |                 | 0         |                          | 0                                 |        | 0               |        | 0               |                                   |

## 9 Appendix

| Antibiotic class   | Resistance determinant              | Accession of protein reference sequence(s)  | Accession of nucleotide reference sequence(s)   |
|--------------------|-------------------------------------|---|---|
| Aminoglycoside     | <i>aph(2'')-I<sub>f</sub></i>       | WP_021424053.1  | NG_047405.1   |
|                    | <i>aac(6)-Ie/aph(2'')-Ia</i>        | WP_001028144.1  | NG_047055.1   |
|                    | <i>aph(2'')-I<sub>i</sub></i>       | WP_052776520.1  | KX931104.1  |
|                    | <i>aadE 1</i>                       | WP_001255868.1  | NZ_CP109819.1:317969-318835   |
|                    | <i>aadE 2</i>                       | WP_001255866.1  | NG_047393.1   |
|                    | <i>aadE 3</i>                       | WP_057035408.1 (missing in AMRFinder database)  | NZ_RYYM01000001.1:c70287-69379  |
|                    | <i>aadE-Cc</i>                      | WP_002785795.1  | CP013733.1:c230242-229343   |
|                    | RpsL_K43R; RpsL_K88R                | n.a. - point mutation   | n.a. - point mutation   |
|                    | <i>aph(3'')-IIIa</i>                | WP_001096887.1  | NG_047418.1   |
| <i>aad9</i>        | WP_057031337.1                      | CP091310.1:1750066-1750845  |   |
| Beta-lactam        | <i>bla<sub>OXA-61</sub></i> family  | WP_002783228.1 (blaOXA-193 as example sequence);<br>variants: blaOXA-61, blaOXA-450, blaOXA-460,<br>blaOXA-461, blaOXA-489, blaOXA-577, blaOXA-579, blaOXA-584, blaOXA-<br>591, blaOXA-594, blaOXA-595  | NG_049489.1   |
|                    | <i>bla<sub>OXA-184</sub></i> family | WP_002872405.1 (blaOXA-184 as example sequence);<br>variants: blaOXA-185, blaOXA-447, blaOXA-448,<br>blaOXA-449, blaOXA-452, blaOXA-465, blaOXA-625, blaOXA-630, blaOXA-<br>631, blaOXA-632, blaOXA-633 | NG_049485.1   |
| (Fluoro-)Quinolone | GyrA_T86I; GyrA_T86V                | n.a. - point mutation   | n.a. - point mutation   |
| Lincosamide        | <i>lnu(C)</i>                       | WP_002837187.1  | NZ_CP114883.1:456702-457196   |
| Macrolide          | 23S_A2075G                          | n.a. - point mutation   | n.a. - point mutation   |
|                    | 50S_L22_A103V                       | n.a. - point mutation (to be deleted in AMRFinder database, since no<br>macrolide resistance is conferred)  | n.a. - point mutation (to be deleted in AMRFinder database, since no<br>macrolide resistance is conferred)  |
|                    | <i>erm(B)</i>                       | WP_002321849.1  | NC_012926.1:1031089-1031826   |
| Phenicol           | <i>catA9</i>                        | WP_001010387.1  | NG_047564.1   |
|                    | <i>catA13</i>                       | WP_040564913.1  | NG_047588.1   |
|                    | <i>optrA</i>                        | WP_063854496.1  | NZ_CP081833.1:c1246663-1244696  |
|                    | <i>flexA</i>                        | WP_015585966.1  | NG_047857.1   |
| Tetracycline       | <i>tet(O)</i>                       | WP_032490535.1  | AY190525.1  |
|                    | <i>tet(O/32/O)</i>                  | WP_215475009.1 (missing in AMRFinder database)  | MT176412.1  |
|                    |                                     | WP_002872163.1;<br>WP_185886429.1 (Henan variant);<br>WP_216170378.1 (Taiwanese variant);<br>AVY51757.1 (Shanghai variant)  | NG_048259.1;<br>NZ_JACMID010000002.1:c171398-169479 (Henan variant);<br>CP076508.1:43486-45405 (Taiwanese variant);<br>MF037585.1:c18890-16971 (Shanghai variant) |
|                    | <i>tet(O/M/O)</i>                   |   |   |
|                    | <i>tet(L)</i>                       | WP_002294500.1  | NG_048206.1   |
|                    | <i>tet(W)</i>                       | WP_000691721.1  | NG_048291.1   |
|                    | <i>tet(M)</i>                       | WP_002364936  | EU182585.1  |
| Streptothricin     | <i>sat4</i>                         | WP_063854935.1, WP_000627290.1  | NG_048073.1, NG_048072.1  |

## 9 Appendix

| Odds ratio (OR) with 95 % confidence interval (CI)                  |                     |                 |  |  |                        |
|---|---------------------|-----------------|--|--|------------------------|
| Odds ratios with p-values of less than 0.05 are highlighted in bold |                     |                 |  |  |                        |
| Comparison  | Antimicrobial       | Odds ratio (OR) | 95% confidence interval (CI) lower bound | 95% confidence interval (CI) upper bound | significance level (p) |
| <i>C. coli</i> vs. <i>C. jejuni</i> (VN)                            | CIP                 | 4,5672          | 0,2334                                   | 89,3794                                  | 0,3168                 |
|   | ERY                 | <b>26,8235</b>  | 13,5002                                  | 53,2957                                  | 0,0001                 |
|   | GEN                 | <b>13,2185</b>  | 7,154                                    | 24,4237                                  | 0,0001                 |
|   | NAL                 | 8,6522          | 0,482                                    | 155,3158                                 | 0,143                  |
|   | STR                 | <b>40,9821</b>  | 19,6524                                  | 85,4621                                  | 0,0001                 |
|   | TET                 | 0,6364          | 0,0393                                   | 10,2925                                  | 0,7503                 |
|   | 3-4 fold resistance | <b>45,9556</b>  | 17,5241                                  | 120,5146                                 | 0,0001                 |
| <i>C. coli</i> vs. <i>C. jejuni</i> (DE)                            | CIP                 | 1,1647          | 0,62                                     | 2,1877                                   | 0,6356                 |
|   | ERY                 | <b>27,7021</b>  | 3,6603                                   | 209,6546                                 | 0,0013                 |
|   | GEN                 | 5,5286          | 0,2626                                   | 116,391                                  | 0,2714                 |
|   | NAL                 | 1,4348          | 0,7829                                   | 2,6295                                   | 0,2428                 |
|   | STR                 | 0,6652          | 0,3282                                   | 1,3484                                   | 0,2581                 |
|   | TET                 | 1,2857          | 0,7495                                   | 2,2054                                   | 0,3613                 |
|   | 3-4 fold resistance | 1,3757          | 0,7216                                   | 2,6224                                   | 0,3326                 |
| VN vs. DE ( <i>C. coli</i> )  | CIP                 | <b>47,8877</b>  | 2,8639                                   | 800,7344                                 | 0,0071                 |
|   | ERY                 | <b>14,7909</b>  | 7,6114                                   | 28,7424                                  | 0,0001                 |
|   | GEN                 | <b>209,8571</b> | 47,8266                                  | 920,8264                                 | 0,0001                 |
|   | NAL                 | <b>50,5568</b>  | 3,0272                                   | 844,3407                                 | 0,0063                 |
|   | STR                 | <b>40,4762</b>  | 18,4868                                  | 88,6212                                  | 0,0001                 |
|   | TET                 | <b>42,875</b>   | 5,7469                                   | 319,8688                                 | 0,0002                 |
|   | 3-4 fold resistance | <b>67,68</b>    | 24,8293                                  | 184,4826                                 | 0,0001                 |
| VN vs. DE ( <i>C. jejuni</i> )                                      | CIP                 | <b>13,9592</b>  | 4,1231                                   | 47,2608                                  | 0,0001                 |
|   | ERY                 | <b>15,2754</b>  | 2,0035                                   | 116,4654                                 | 0,0085                 |
|   | GEN                 | <b>71,2716</b>  | 4,3211                                   | 1175,5515                                | 0,0029                 |
|   | NAL                 | <b>8,9076</b>   | 3,5932                                   | 22,0822                                  | 0,0001                 |
|   | STR                 | 0,657           | 0,3423                                   | 1,2612                                   | 0,2067                 |
|   | TET                 | <b>86,625</b>   | 11,7236                                  | 640,0665                                 | 0,0001                 |
|   | 3-4 fold resistance | <b>2,026</b>    | 1,1307                                   | 3,6302                                   | 0,0177                 |
| VN vs. DE ( <i>C. spp.</i> )  | 3-4 fold resistance | <b>5,0975</b>   | 3,3985                                   | 7,6459                                   | 0,0001                 |



Distance matrix from cgMLST analysis using 1343 core genes and pairwise ignoring missing loci. Created with Ridom SeqSphere+ 8.4.2.

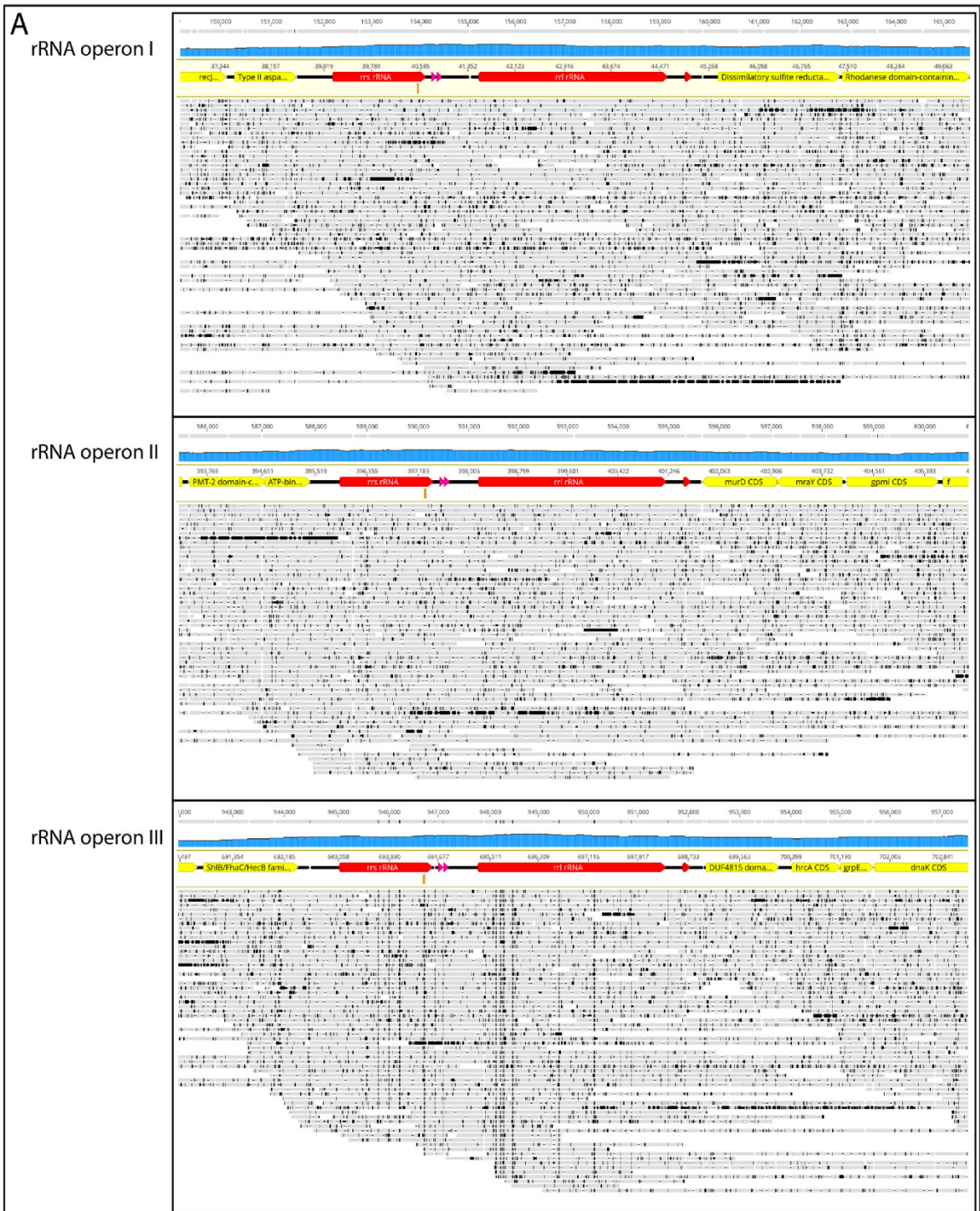
isolates with  $\leq 10$  allele distance are in green  
isolates with 11-100 allele distance are in orange  
isolates with  $>100$  allele distance are in white

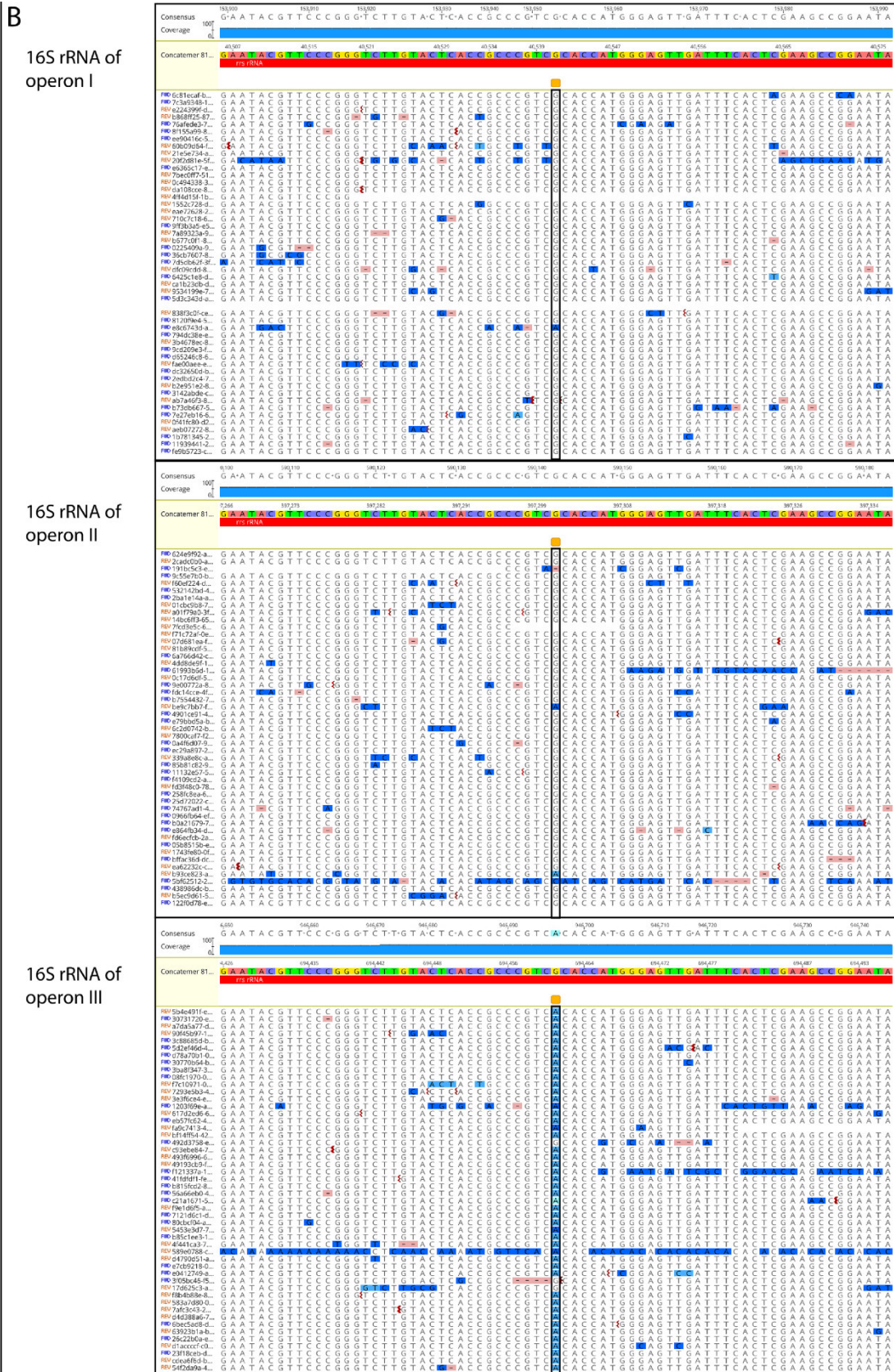
**9.2.4 Publication 4: *The point mutation A1387G in the 16S rRNA gene confers aminoglycoside resistance in C. jejuni and C. coli***

**Supplementary Figures**

The point mutation A1387G in the 16S rRNA gene confers aminoglycoside resistance in *C. jejuni* and *C. coli*

Michael Zarske, Christiane Werckenthin, Julia Golz, Kerstin Stingl





**Figure S1.** The transformant 81-176-TF15687-K8 harbored the A1387G point mutation in the rRNA operon I and II but remained wildtype A1387 in operon III. Trimmed ONT long reads of 81-176-TF15687-K8 were mapped to its unicycler hybrid assembly. Overview of the different gene contexts of rRNA operon I-II confirmed that long reads covered 5' and 3' regions specifically flanking the respective rRNA operon (A). The sequence surrounding position 1387 (highlighted with a black frame) in the respective 16S rRNA genes is depicted in (B). Red arrows, rRNA genes; yellow arrows,



## 9 Appendix

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Supplementary Tables

**The point mutation A1387G in the 16S rRNA gene confers aminoglycoside resistance in *C. jejuni* and *C. coli***

Michael Zarske, Christiane Werckenthin, Julia C. Golz, Kerstin Stingl

Results from mapping of trimmed reads from nine APR-GEN-KAN-TOB resistant isolates to its isogenic sensitive recipient (Bfr-CA-11057) after transformation of gDNA of Bfr-CA-1568 and subsequent mapping of unused reads to the donor Bfr-CA-1568

Table S1A SNP raw data: Sequence variants (raw data) exported from Geneious Prime Software identified between nine Bfr-CA-11057-TF15687 transformants relative to the recipient strain (Bfr-CA-11057);  
Table S1B SNP summary: Summary of number of sequence variants based on Table S1  
Table S2 Read coverage < 5 exported from Geneious Prime Software identified upon mapping of nine Bfr-CA-11057-TF15687 transformants to the recipient strain (Bfr-CA-11057);  
Table S3 Sequences with a coverage  $\geq 20$  exported from Geneious Prime Software from unused reads of each transformant after analysis in Table S1A mapped to the donor Bfr-CA-1568











# 9 Appendix

| Category | Accession | Gene   | Feature        | Start        | End       | Strand        | Score  | Annotations                   | Gene | Strand | Score | Annotations                 |
|----------|-----------|--------|----------------|--------------|-----------|---------------|--|-------------------------------|------|--------|-------|-----------------------------|
| C        | 532048    | 532048 | 1 A > C        | SNP (Strand) | 92        | 100.0%        | 15-278 Variants: BRC-CA-1057-TF-15687-11.R   | Concatemer BRC-CA-1057- Unkns |      |        |       | gaa4 CDS                    |
| A        | 532054    | 532054 | 1 G > A        | SNP (Strand) | 89        | 100.0%        | 1,26-275 Variants: BRC-CA-1057-TF-15687-11.R | Concatemer BRC-CA-1057- Unkns |      |        |       | gaa4 CDS                    |
| S        | 532057    | 532057 | 1 G > A        | SNP (Strand) | 87        | 100.0%        | 20-269 Variants: BRC-CA-1057-TF-15687-11.R   | Concatemer BRC-CA-1057- Unkns |      |        |       | gaa4 CDS                    |
| A        | 532064    | 532064 | 1 G > A        | SNP (Strand) | 78        | 98.7%         | 3,85-223 Variants: BRC-CA-1057-TF-15687-11.R | Concatemer BRC-CA-1057- Unkns |      |        |       | gaa4 CDS                    |
| G        | 532064    | 532064 | 1 A > G        | SNP (Strand) | 78        | 97.5%         | 3,11-227 Variants: BRC-CA-1057-TF-15687-11.R | Concatemer BRC-CA-1057- Unkns |      |        |       | gaa4 CDS                    |
| S        | 532068    | 532068 | 1 T > C        | SNP (Strand) | 85        | 100.0%        | 3,00-263 Variants: BRC-CA-1057-TF-15687-11.R | Concatemer BRC-CA-1057- Unkns |      |        |       | 1->Y gaa4 CDS               |
| C        | 532069    | 532069 | 1 C > A        | SNP (Strand) | 83        | 100.0%        | 52-247 Variants: BRC-CA-1057-TF-15687-11.R   | Concatemer BRC-CA-1057- Unkns |      |        |       | gaa4 CDS                    |
| S        | 532126    | 532126 | 1 A > G        | SNP (Strand) | 102       | 99.0%         | 0 Variants: BRC-CA-1057-TF-15687-11.R        | Concatemer BRC-CA-1057- Unkns |      |        |       | gaa4 CDS                    |
| AMG      | 532128    | 532131 | 2 TAA > AAG    | Substitution | 105 > 306 | 100.0%        | 3,20-394 Variants: BRC-CA-1057-TF-15687-11.R | Concatemer BRC-CA-1057- Unkns |      |        |       | gaa4 CDS                    |
| T        | 532133    | 532133 | 1 A > T        | SNP (Strand) | 110       | 100.0%        | 0 Variants: BRC-CA-1057-TF-15687-11.R        | Concatemer BRC-CA-1057- Unkns |      |        |       | F->Y gaa4 CDS               |
| T        | 532138    | 532138 | 1 C > T        | SNP (Strand) | 113       | 99.1%         | 0 Variants: BRC-CA-1057-TF-15687-11.R        | Concatemer BRC-CA-1057- Unkns |      |        |       | gaa4 CDS                    |
| A        | 532147    | 532147 | 1 G > A        | SNP (Strand) | 114       | 100.0%        | 0 Variants: BRC-CA-1057-TF-15687-11.R        | Concatemer BRC-CA-1057- Unkns |      |        |       | gaa4 CDS                    |
| S        | 532156    | 532156 | 1 A > T        | SNP (Strand) | 110       | 99.1%         | 0 Variants: BRC-CA-1057-TF-15687-11.R        | Concatemer BRC-CA-1057- Unkns |      |        |       | gaa4 CDS                    |
| C        | 532223    | 532223 | 1 T > C        | SNP (Strand) | 121       | 99.3%         | 0 Variants: BRC-CA-1057-TF-15687-11.R        | Concatemer BRC-CA-1057- Unkns |      |        |       | 1->Y gaa4 CDS               |
| TCT      | 532201    | 532203 | 2 GCG > TCT    | Substitution | 107 > 108 | 100.0%        | 3,52-399 Variants: BRC-CA-1057-TF-15687-11.R | Concatemer BRC-CA-1057- Unkns |      |        |       | gaa4 CDS                    |
| T        | 532210    | 532210 | 1 C > T        | SNP (Strand) | 112       | 97.3%         | 0 Variants: BRC-CA-1057-TF-15687-11.R        | Concatemer BRC-CA-1057- Unkns |      |        |       | gaa4 CDS                    |
| T        | 532218    | 532218 | 1 A > T        | SNP (Strand) | 113       | 99.1%         | 0 Variants: BRC-CA-1057-TF-15687-11.R        | Concatemer BRC-CA-1057- Unkns |      |        |       | gaa4 CDS                    |
| G        | 532222    | 532222 | 1 A > G        | SNP (Strand) | 115       | 100.0%        | 0 Variants: BRC-CA-1057-TF-15687-11.R        | Concatemer BRC-CA-1057- Unkns |      |        |       | gaa4 CDS                    |
| HTA      | 532228    | 532228 | 4 TCT > ATTA   | Substitution | 114       | 98.2%         | 0 Variants: BRC-CA-1057-TF-15687-11.R        | Concatemer BRC-CA-1057- Unkns |      |        |       | AS > AN gaa4 CDS            |
| CT       | 532238    | 532238 | 2 TC > CT      | Substitution | 111 > 113 | 100.0%        | 0 Variants: BRC-CA-1057-TF-15687-11.R        | Concatemer BRC-CA-1057- Unkns |      |        |       | D->S gaa4 CDS               |
| A        | 532242    | 532242 | 1 G > A        | SNP (Strand) | 117       | 99.3%         | 0 Variants: BRC-CA-1057-TF-15687-11.R        | Concatemer BRC-CA-1057- Unkns |      |        |       | gaa4 CDS                    |
| TEA      | 532248    | 532252 | 4 CTTC > TCA   | Substitution | 117       | 98.3%         | 0 Variants: BRC-CA-1057-TF-15687-11.R        | Concatemer BRC-CA-1057- Unkns |      |        |       | SK > SE gaa4 CDS            |
| G        | 532272    | 532272 | 1 A > G        | SNP (Strand) | 117       | 100.0%        | 0 Variants: BRC-CA-1057-TF-15687-11.R        | Concatemer BRC-CA-1057- Unkns |      |        |       | gaa4 CDS                    |
| CG       | 532275    | 532275 | 2 TA > CG      | Substitution | 109 > 112 | 100.0%        | 0 Variants: BRC-CA-1057-TF-15687-11.R        | Concatemer BRC-CA-1057- Unkns |      |        |       | IN > ID gaa4 CDS            |
| T        | 532279    | 532279 | 1 C > G        | SNP (Strand) | 108       | 99.3%         | 0 Variants: BRC-CA-1057-TF-15687-11.R        | Concatemer BRC-CA-1057- Unkns |      |        |       | gaa4 CDS                    |
| TE       | 532321    | 532322 | 2 GGT > TC     | Substitution | 118 > 120 | 100.0%        | 0 Variants: BRC-CA-1057-TF-15687-11.R        | Concatemer BRC-CA-1057- Unkns |      |        |       | G->G gaa4 CDS               |
| T        | 532362    | 532362 | 1 C > T        | SNP (Strand) | 117       | 99.1%         | 0 Variants: BRC-CA-1057-TF-15687-11.R        | Concatemer BRC-CA-1057- Unkns |      |        |       | V->I gaa4 CDS               |
| C        | 532366    | 532366 | 1 A > C        | SNP (Strand) | 111       | 100.0%        | 0 Variants: BRC-CA-1057-TF-15687-11.R        | Concatemer BRC-CA-1057- Unkns |      |        |       | D->T gaa4 CDS               |
| T        | 532372    | 532372 | 1 G > T        | SNP (Strand) | 118       | 99.2%         | 0 Variants: BRC-CA-1057-TF-15687-11.R        | Concatemer BRC-CA-1057- Unkns |      |        |       | gaa4 CDS                    |
| A        | 532375    | 532375 | 1 T > A        | SNP (Strand) | 117       | 100.0%        | 0 Variants: BRC-CA-1057-TF-15687-11.R        | Concatemer BRC-CA-1057- Unkns |      |        |       | gaa4 CDS                    |
| C        | 532378    | 532378 | 1 T > C        | SNP (Strand) | 110       | 100.0%        | 0 Variants: BRC-CA-1057-TF-15687-11.R        | Concatemer BRC-CA-1057- Unkns |      |        |       | gaa4 CDS                    |
| A        | 532381    | 532381 | 1 T > A        | SNP (Strand) | 114       | 100.0%        | 0 Variants: BRC-CA-1057-TF-15687-11.R        | Concatemer BRC-CA-1057- Unkns |      |        |       | N->S gaa4 CDS               |
| AAAGT    | 532386    | 532396 | 4 GTAA > AAAGT | Substitution | 109 > 114 | 97.2% > 97.4% | 0 Variants: BRC-CA-1057-TF-15687-11.R        | Concatemer BRC-CA-1057- Unkns |      |        |       | gaa4 CDS                    |
| A        | 532398    | 532398 | 1 T > A        | SNP (Strand) | 110       | 100.0%        | 0 Variants: BRC-CA-1057-TF-15687-11.R        | Concatemer BRC-CA-1057- Unkns |      |        |       | gaa4 CDS                    |
| A        | 532399    | 532399 | 1 G > A        | SNP (Strand) | 107       | 100.0%        | 0 Variants: BRC-CA-1057-TF-15687-11.R        | Concatemer BRC-CA-1057- Unkns |      |        |       | gaa4 CDS                    |
| G        | 532402    | 532402 | 1 A > G        | SNP (Strand) | 107       | 100.0%        | 0 Variants: BRC-CA-1057-TF-15687-11.R        | Concatemer BRC-CA-1057- Unkns |      |        |       | gaa4 CDS                    |
| A        | 532405    | 532405 | 1 G > A        | SNP (Strand) | 123       | 99.2%         | 0 Variants: BRC-CA-1057-TF-15687-11.R        | Concatemer BRC-CA-1057- Unkns |      |        |       | gaa4 CDS                    |
| A        | 532418    | 532418 | 1 T > A        | SNP (Strand) | 103       | 100.0%        | 0 Variants: BRC-CA-1057-TF-15687-11.R        | Concatemer BRC-CA-1057- Unkns |      |        |       | gaa4 CDS                    |
| A        | 532420    | 532420 | 1 G > A        | SNP (Strand) | 98        | 100.0%        | 11-293 Variants: BRC-CA-1057-TF-15687-11.R   | Concatemer BRC-CA-1057- Unkns |      |        |       | gaa4 CDS                    |
| A        | 532444    | 532444 | 1 T > A        | SNP (Strand) | 118       | 100.0%        | 0 Variants: BRC-CA-1057-TF-15687-11.R        | Concatemer BRC-CA-1057- Unkns |      |        |       | N->N gaa4 CDS               |
| T        | 532450    | 532450 | 1 C > T        | SNP (Strand) | 117       | 100.0%        | 0 Variants: BRC-CA-1057-TF-15687-11.R        | Concatemer BRC-CA-1057- Unkns |      |        |       | gaa4 CDS                    |
| G        | 532462    | 532462 | 1 A > G        | SNP (Strand) | 98        | 99.1%         | 0 Variants: BRC-CA-1057-TF-15687-11.R        | Concatemer BRC-CA-1057- Unkns |      |        |       | gaa4 CDS                    |
| T        | 532471    | 532471 | 1 A > T        | SNP (Strand) | 112       | 100.0%        | 0 Variants: BRC-CA-1057-TF-15687-11.R        | Concatemer BRC-CA-1057- Unkns |      |        |       | gaa4 CDS                    |
| A        | 532490    | 532490 | 1 T > A        | SNP (Strand) | 108       | 98.5%         | 4,97-268 Variants: BRC-CA-1057-TF-15687-11.R | Concatemer BRC-CA-1057- Unkns |      |        |       | gaa4 CDS                    |
| A        | 532511    | 532511 | 1 G > A        | SNP (Strand) | 119       | 97.3%         | 0 Variants: BRC-CA-1057-TF-15687-11.R        | Concatemer BRC-CA-1057- Unkns |      |        |       | gaa4 CDS                    |
| A        | 532531    | 532531 | 1 T > A        | SNP (Strand) | 115       | 99.3%         | 0 Variants: BRC-CA-1057-TF-15687-11.R        | Concatemer BRC-CA-1057- Unkns |      |        |       | gaa4 CDS                    |
| T        | 532543    | 532543 | 1 C > T        | SNP (Strand) | 117       | 99.1%         | 0 Variants: BRC-CA-1057-TF-15687-11.R        | Concatemer BRC-CA-1057- Unkns |      |        |       | gaa4 CDS                    |
| AI       | 532549    | 532550 | 2 GC > AT      | Substitution | 123       | 98.3%         | 0 Variants: BRC-CA-1057-TF-15687-11.R        | Concatemer BRC-CA-1057- Unkns |      |        |       | S->N gaa4 CDS               |
| CCG      | 532601    | 532601 | 1 T > C        | SNP (Strand) | 105       | 99.0%         | 0 Variants: BRC-CA-1057-TF-15687-11.R        | Concatemer BRC-CA-1057- Unkns |      |        |       | wc4 CDS                     |
| CCG      | 532611    | 532612 | 2 ACA > CCG    | Substitution | 98 > 105  | 98.1% > 98.2% | 2,16-248 Variants: BRC-CA-1057-TF-15687-11.R | Concatemer BRC-CA-1057- Unkns |      |        |       | 1->A wc4 CDS                |
| C        | 532630    | 532630 | 1 C > T        | SNP (Strand) | 98        | 100.0%        | 2,56-264 Variants: BRC-CA-1057-TF-15687-11.R | Concatemer BRC-CA-1057- Unkns |      |        |       | wc4 CDS                     |
| A        | 532633    | 532633 | 1 T > A        | SNP (Strand) | 98        | 100.0%        | 2,56-264 Variants: BRC-CA-1057-TF-15687-11.R | Concatemer BRC-CA-1057- Unkns |      |        |       | wc4 CDS                     |
| A        | 532636    | 532636 | 1 T > A        | SNP (Strand) | 101       | 99.0%         | 12-277 Variants: BRC-CA-1057-TF-15687-11.R   | Concatemer BRC-CA-1057- Unkns |      |        |       | wc4 CDS                     |
| G        | 532641    | 532641 | 1 G > A        | SNP (Strand) | 92        | 98.7%         | 7,90-244 Variants: BRC-CA-1057-TF-15687-11.R | Concatemer BRC-CA-1057- Unkns |      |        |       | wc4 CDS                     |
| C        | 532643    | 532643 | 1 A > C        | SNP (Strand) | 98        | 99.2%         | 3,16-244 Variants: BRC-CA-1057-TF-15687-11.R | Concatemer BRC-CA-1057- Unkns |      |        |       | wc4 CDS                     |
| S        | 532647    | 532647 | 1 T > C        | SNP (Strand) | 98        | 98.7%         | 1,18-239 Variants: BRC-CA-1057-TF-15687-11.R | Concatemer BRC-CA-1057- Unkns |      |        |       | wc4 CDS                     |
| G        | 532651    | 532651 | 1 G > C        | SNP (Strand) | 98        | 98.9%         | 4,16-238 Variants: BRC-CA-1057-TF-15687-11.R | Concatemer BRC-CA-1057- Unkns |      |        |       | wc4 CDS                     |
| A        | 532653    | 532653 | 1 A > G        | SNP (Strand) | 115       | 100.0%        | 0 Variants: BRC-CA-1057-TF-15687-11.R        | Concatemer BRC-CA-1057- Unkns |      |        |       | wc4 CDS                     |
| G        | 532654    | 532654 | 1 G > A        | SNP (Strand) | 103       | 98.3%         | 0 Variants: BRC-CA-1057-TF-15687-11.R        | Concatemer BRC-CA-1057- Unkns |      |        |       | wc4 CDS                     |
| AT       | 532657    | 532663 | 2 GAA > AAT    | Substitution | 103 > 104 | 99.0%         | 2,62-243 Variants: BRC-CA-1057-TF-15687-11.R | Concatemer BRC-CA-1057- Unkns |      |        |       | S->N wc4 CDS                |
| A        | 532665    | 532665 | 1 A > G        | SNP (Strand) | 97        | 100.0%        | 0 Variants: BRC-CA-1057-TF-15687-11.R        | Concatemer BRC-CA-1057- Unkns |      |        |       | wc4 CDS                     |
| T        | 532668    | 532668 | 1 C > T        | SNP (Strand) | 97        | 97.3%         | 5,56-228 Variants: BRC-CA-1057-TF-15687-11.R | Concatemer BRC-CA-1057- Unkns |      |        |       | wc4 CDS                     |
| A        | 532674    | 532674 | 1 G > A        | SNP (Strand) | 97        | 97.9%         | 4,76-300 Variants: BRC-CA-1057-TF-15687-11.R | Concatemer BRC-CA-1057- Unkns |      |        |       | wc4 CDS                     |
| T        | 532678    | 532678 | 1 C > T        | SNP (Strand) | 99        | 99.0%         | 3,86-272 Variants: BRC-CA-1057-TF-15687-11.R | Concatemer BRC-CA-1057- Unkns |      |        |       | wc4 CDS                     |
| T        | 532681    | 532681 | 1 C > T        | SNP (Strand) | 100       | 100.0%        | 0 Variants: BRC-CA-1057-TF-15687-11.R        | Concatemer BRC-CA-1057- Unkns |      |        |       | wc4 CDS                     |
| C        | 532687    | 532687 | 1 T > C        | SNP (Strand) | 98        | 99.0%         | 0 Variants: BRC-CA-1057-TF-15687-11.R        | Concatemer BRC-CA-1057- Unkns |      |        |       | wc4 CDS                     |
| C        | 532692    | 532692 | 1 T > C        | SNP (Strand) | 98        | 99.0%         | 11-300 Variants: BRC-CA-1057-TF-15687-11.R   | Concatemer BRC-CA-1057- Unkns |      |        |       | wc4 CDS                     |
| A        | 532694    | 532694 | 1 G > A        | SNP (Strand) | 103       | 99.0%         | 0 Variants: BRC-CA-1057-TF-15687-11.R        | Concatemer BRC-CA-1057- Unkns |      |        |       | wc4 CDS                     |
| G        | 532698    | 532698 | 1 A > G        | SNP (Strand) | 81        | 98.8%         | 8,16-270 Variants: BRC-CA-1057-TF-15687-11.R | Concatemer BRC-CA-1057- Unkns |      |        |       | wc4 CDS                     |
| G        | 532699    | 532699 | 1 A > G        | SNP (Strand) | 81        | 98.8%         | 8,16-270 Variants: BRC-CA-1057-TF-15687-11.R | Concatemer BRC-CA-1057- Unkns |      |        |       | wc4 CDS                     |
| C        | 532701    | 532701 | 1 A > C        | SNP (Strand) | 96        | 98.7%         | 2,46-232 Variants: BRC-CA-1057-TF-15687-11.R | Concatemer BRC-CA-1057- Unkns |      |        |       | wc4 CDS                     |
| A        | 532718    | 532718 | 1 G > A        | SNP (Strand) | 76        | 100.0%        | 1,62-212 Variants: BRC-CA-1057-TF-15687-11.R | Concatemer BRC-CA-1057- Unkns |      |        |       | D->N wc4 CDS                |
| C        | 532746    | 532746 | 1 T > C        | SNP (Strand) | 110       | 100.0%        | 0 Variants: BRC-CA-1057-TF-15687-11.R        | Concatemer BRC-CA-1057- Unkns |      |        |       | Plasma membrane protein CDS |
| A        | 532751    | 532751 | 1 T > A        | SNP (Strand) | 96        | 97.0%         | 7,38-239 Variants: BRC-CA-1057-TF-15687-11.R | Concatemer BRC-CA-1057- Unkns |      |        |       | Plasma membrane protein CDS |
| C        | 532844    | 532844 | 1 T > C        | SNP (Strand) | 97        | 98.4%         | 0 Variants: BRC-CA-1057-TF-15687-11.R        | Concatemer BRC-CA-1057- Unkns |      |        |       | N->D hem4 CDS               |
| C        | 532857    | 532857 | 1 G > C        | SNP (Strand) | 111       | 99.2%         | 0 Variants: BRC-CA-1057-TF-15687             |                               |      |        |       |                             |

# 9 Appendix

| Gene | Accession | Gene Name | Feature       | Start        | End       | Strand        | Score                                       | Annotations                           | Category |
|------|-----------|-----------|---------------|--------------|-----------|---------------|---|---------------------------------------|----------|
| G    | 698344    | 698344    | 1 A → G       | SNP (Strand) | 120       | 99.2%         | 0 Variants: BRC-CA-1057-715687-11 R         | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| T    | 698358    | 698358    | 1 C → T       | SNP (Strand) | 118       | 97.5%         | 0 Variants: BRC-CA-1057-715687-11 R         | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| A    | 698370    | 698370    | 1 C → A       | SNP (Strand) | 124       | 98.4%         | 0 Variants: BRC-CA-1057-715687-11 R         | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| G    | 698371    | 698371    | 1 A → G       | SNP (Strand) | 124       | 98.4%         | 0 Variants: BRC-CA-1057-715687-11 R         | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| G    | 698376    | 698376    | 1 A → G       | SNP (Strand) | 174       | 99.2%         | 0 Variants: BRC-CA-1057-715687-11 R         | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| GA   | 698383    | 698384    | 2 AC → GA     | Substitution | 179       | 99.3%         | 0 Variants: BRC-CA-1057-715687-11 R         | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| T    | 698388    | 698388    | 1 C → T       | SNP (Strand) | 197       | 99.3%         | 0 Variants: BRC-CA-1057-715687-11 R         | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| C    | 698414    | 698414    | 1 T → C       | SNP (Strand) | 133       | 99.2%         | 0 Variants: BRC-CA-1057-715687-11 R         | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| C    | 698465    | 698465    | 1 G → C       | SNP (Strand) | 99        | 100.0%        | 10,296 Variants: BRC-CA-1057-715687-11 R    | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| C    | 698481    | 698481    | 1 T → C       | SNP (Strand) | 806       | 99.1%         | 0 Variants: BRC-CA-1057-715687-11 R         | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| A    | 698487    | 698487    | 1 G → A       | SNP (Strand) | 117       | 100.0%        | 0 Variants: BRC-CA-1057-715687-11 R         | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| A    | 698494    | 698494    | 1 G → A       | SNP (Strand) | 117       | 100.0%        | 0 Variants: BRC-CA-1057-715687-11 R         | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| A    | 698511    | 698511    | 1 G → A       | SNP (Strand) | 112       | 100.0%        | 0 Variants: BRC-CA-1057-715687-11 R         | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| C    | 698523    | 698523    | 1 G → C       | SNP (Strand) | 117       | 100.0%        | 0 Variants: BRC-CA-1057-715687-11 R         | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| C    | 698530    | 698530    | 1 A → C       | SNP (Strand) | 115       | 100.0%        | 0 Variants: BRC-CA-1057-715687-11 R         | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| G    | 698539    | 698539    | 1 A → G       | SNP (Strand) | 112       | 100.0%        | 0 Variants: BRC-CA-1057-715687-11 R         | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| A    | 1034274   | 1034274   | 1 A → G       | SNP (Strand) | 139       | 99.3%         | 0 Variants: BRC-CA-1057-715687-11 R         | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| G    | 1034273   | 1034273   | 1 A → G       | SNP (Strand) | 111       | 98.2%         | 0 Variants: BRC-CA-1057-715687-11 R         | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| A    | 1042125   | 1042125   | 1 G → A       | SNP (Strand) | 188       | 99.1%         | 0 Variants: BRC-CA-1057-715687-11 R         | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| T    | 1042170   | 1042170   | 1 C → T       | SNP (Strand) | 407       | 99.1%         | 4,361,305 Variants: BRC-CA-1057-715687-11 R | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| A    | 1042182   | 1042182   | 1 G → A       | SNP (Strand) | 111       | 100.0%        | 0 Variants: BRC-CA-1057-715687-11 R         | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| AC   | 1042181   | 1042181   | 2 GT → AC     | Substitution | 111 → 112 | 98.2%         | 6,104 Variants: BRC-CA-1057-715687-11 R     | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| T    | 1042191   | 1042191   | 1 C → T       | SNP (Strand) | 108       | 98.1%         | 0 Variants: BRC-CA-1057-715687-11 R         | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| A    | 1042195   | 1042195   | 1 T → A       | SNP (Strand) | 109       | 99.1%         | 0 Variants: BRC-CA-1057-715687-11 R         | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| G    | 1042206   | 1042206   | 1 A → G       | SNP (Strand) | 108       | 100.0%        | 0 Variants: BRC-CA-1057-715687-11 R         | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| G    | 1042214   | 1042214   | 1 A → G       | SNP (Strand) | 208       | 98.1%         | 0 Variants: BRC-CA-1057-715687-11 R         | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| G    | 1042211   | 1042211   | 1 G → G       | SNP (Strand) | 109       | 99.1%         | 0 Variants: BRC-CA-1057-715687-11 R         | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| A    | 1042213   | 1042213   | 1 G → A       | SNP (Strand) | 124       | 99.2%         | 0 Variants: BRC-CA-1057-715687-11 R         | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| C    | 1042249   | 1042249   | 1 T → C       | SNP (Strand) | 110       | 100.0%        | 0 Variants: BRC-CA-1057-715687-11 R         | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| A    | 1042251   | 1042251   | 1 T → A       | SNP (Strand) | 110       | 99.3%         | 0 Variants: BRC-CA-1057-715687-11 R         | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| A    | 1042371   | 1042371   | 1 G → A       | SNP (Strand) | 409       | 100.0%        | 0 Variants: BRC-CA-1057-715687-11 R         | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| G    | 1042431   | 1042431   | 1 G → G       | SNP (Strand) | 119       | 99.1%         | 0 Variants: BRC-CA-1057-715687-11 R         | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| G    | 1042474   | 1042474   | 1 A → G       | SNP (Strand) | 600       | 100.0%        | 0 Variants: BRC-CA-1057-715687-11 R         | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| T    | 1042488   | 1042488   | 1 C → T       | SNP (Strand) | 113       | 100.0%        | 0 Variants: BRC-CA-1057-715687-11 R         | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| A    | 1042491   | 1042491   | 1 T → A       | SNP (Strand) | 114       | 99.1%         | 0 Variants: BRC-CA-1057-715687-11 R         | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| A    | 1042492   | 1042492   | 1 T → A       | SNP (Strand) | 113       | 99.3%         | 0 Variants: BRC-CA-1057-715687-11 R         | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| AG   | 1042520   | 1042521   | 2 TG → AG     | Substitution | 126 → 137 | 100.0%        | 0 Variants: BRC-CA-1057-715687-11 R         | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| T    | 1042591   | 1042591   | 1 A → T       | SNP (Strand) | 443       | 99.3%         | 0 Variants: BRC-CA-1057-715687-11 R         | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| A    | 1042596   | 1042596   | 1 G → A       | SNP (Strand) | 144       | 99.3%         | 0 Variants: BRC-CA-1057-715687-11 R         | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| A    | 1042608   | 1042608   | 1 T → A       | SNP (Strand) | 146       | 99.3%         | 0 Variants: BRC-CA-1057-715687-11 R         | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| G    | 1042632   | 1042632   | 1 A → G       | SNP (Strand) | 147       | 98.6%         | 0 Variants: BRC-CA-1057-715687-11 R         | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| A    | 1042638   | 1042638   | 1 C → A       | SNP (Strand) | 131       | 99.2%         | 0 Variants: BRC-CA-1057-715687-11 R         | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| A    | 1042642   | 1042642   | 1 T → A       | SNP (Strand) | 123       | 99.0%         | 0 Variants: BRC-CA-1057-715687-11 R         | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| GT   | 1042650   | 1042651   | 2 AG → GT     | Substitution | 123 → 113 | 98.1%         | 0 Variants: BRC-CA-1057-715687-11 R         | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| ACAG | 1042654   | 1042657   | 4 TCCA → ACAG | Substitution | 107 → 112 | 94.4% → 94.6% | 1,761,280 Variants: BRC-CA-1057-715687-11 R | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| A    | 1042658   | 1042658   | 1 T → A       | SNP (Strand) | 109       | 99.1%         | 1,761,278 Variants: BRC-CA-1057-715687-11 R | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| A    | 1042667   | 1042667   | 1 T → A       | SNP (Strand) | 90        | 98.8%         | 15,243 Variants: BRC-CA-1057-715687-11 R    | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| A    | 1042690   | 1042690   | 1 A → T       | SNP (Strand) | 82        | 87.8%         | 3,362,256 Variants: BRC-CA-1057-715687-11 R | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| C    | 1042692   | 1042692   | 1 T → C       | SNP (Strand) | 83        | 88.2%         | 84,231 Variants: BRC-CA-1057-715687-11 R    | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| AA   | 1042696   | 1042699   | 2 TG → AA     | Substitution | 86 → 87   | 83.7% → 83.9% | 1,181,385 Variants: BRC-CA-1057-715687-11 R | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| T    | 1042699   | 1042699   | 1 C → T       | SNP (Strand) | 87        | 81.6%         | 9,161,204 Variants: BRC-CA-1057-715687-11 R | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| A    | 1042702   | 1042702   | 1 G → A       | SNP (Strand) | 90        | 99.3%         | 1,181,379 Variants: BRC-CA-1057-715687-11 R | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| C    | 1042720   | 1042720   | 1 T → C       | SNP (Strand) | 92        | 91.3%         | 1,181,374 Variants: BRC-CA-1057-715687-11 R | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| AC   | 1042726   | 1042735   | 2 GAC → AC    | Substitution | 86 → 97   | 79.7%         | 1,181,369 Variants: BRC-CA-1057-715687-11 R | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| C    | 1042763   | 1042763   | 1 T → C       | SNP (Strand) | 86        | 97.7%         | 2,382,273 Variants: BRC-CA-1057-715687-11 R | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| C    | 1042765   | 1042765   | 1 C → T       | SNP (Strand) | 82        | 98.8%         | 10,232 Variants: BRC-CA-1057-715687-11 R    | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| AC   | 1042766   | 1042767   | 2 AG → AC     | Substitution | 79 → 86   | 99.6%         | 2,481,272 Variants: BRC-CA-1057-715687-11 R | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| A    | 1042793   | 1042793   | 1 G → A       | SNP (Strand) | 105       | 99.1%         | 1,171,794 Variants: BRC-CA-1057-715687-11 R | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| C    | 1042798   | 1042798   | 1 A → C       | SNP (Strand) | 103       | 99.0%         | 0 Variants: BRC-CA-1057-715687-11 R         | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| A    | 1042805   | 1042805   | 1 C → T       | SNP (Strand) | 109       | 98.2%         | 0 Variants: BRC-CA-1057-715687-11 R         | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| A    | 1042820   | 1042820   | 1 G → A       | SNP (Strand) | 110       | 100.0%        | 0 Variants: BRC-CA-1057-715687-11 R         | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| C    | 1042832   | 1042832   | 1 A → C       | SNP (Strand) | 111       | 99.1%         | 0 Variants: BRC-CA-1057-715687-11 R         | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| A    | 1042835   | 1042835   | 1 G → A       | SNP (Strand) | 109       | 99.1%         | 0 Variants: BRC-CA-1057-715687-11 R         | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| C    | 1042841   | 1042841   | 1 T → C       | SNP (Strand) | 111       | 99.1%         | 0 Variants: BRC-CA-1057-715687-11 R         | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| AG   | 1042850   | 1042851   | 2 GC → AG     | Substitution | 118 → 120 | 97.3%         | 0 Variants: BRC-CA-1057-715687-11 R         | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| C    | 1042854   | 1042855   | 2 CC → GT     | Substitution | 111 → 119 | 97.3% → 97.5% | 0 Variants: BRC-CA-1057-715687-11 R         | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| AGCT | 1042858   | 1042861   | 3 TAC → AGCT  | Substitution | 105 → 107 | 97.3%         | 0 Variants: BRC-CA-1057-715687-11 R         | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| T    | 1042863   | 1042863   | 1 C → T       | SNP (Strand) | 107       | 98.1%         | 4,361,305 Variants: BRC-CA-1057-715687-11 R | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| AG   | 1042864   | 1042864   | 1 C → G       | SNP (Strand) | 87        | 98.1%         | 5,461,188 Variants: BRC-CA-1057-715687-11 R | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| A    | 1042885   | 1042885   | 1 T → A       | SNP (Strand) | 126       | 98.4%         | 0 Variants: BRC-CA-1057-715687-11 R         | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| A    | 1042888   | 1042888   | 1 T → C       | SNP (Strand) | 123       | 100.0%        | 0 Variants: BRC-CA-1057-715687-11 R         | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| C    | 1042892   | 1042892   | 1 G → C       | SNP (Strand) | 121       | 99.2%         | 0 Variants: BRC-CA-1057-715687-11 R         | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| C    | 1042900   | 1042900   | 1 C → T       | SNP (Strand) | 118       | 98.2%         | 0 Variants: BRC-CA-1057-715687-11 R         | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| G    | 1042922   | 1042922   | 1 A → G       | SNP (Strand) | 109       | 98.1%         | 0 Variants: BRC-CA-1057-715687-11 R         | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| A    | 1042931   | 1042931   | 1 T → A       | SNP (Strand) | 119       | 99.3%         | 0 Variants: BRC-CA-1057-715687-11 R         | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| A    | 1042940   | 1042940   | 1 G → A       | SNP (Strand) | 115       | 100.0%        | 0 Variants: BRC-CA-1057-715687-11 R         | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| A    | 1042961   | 1042961   | 1 G → A       | SNP (Strand) | 123       | 100.0%        | 0 Variants: BRC-CA-1057-715687-11 R         | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| A    | 1042985   | 1042985   | 1 G → A       | SNP (Strand) | 123       | 98.4%         | 0 Variants: BRC-CA-1057-715687-11 R         | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| C    | 1042994   | 1042994   | 1 T → C       | SNP (Strand) | 112       | 100.0%        | 0 Variants: BRC-CA-1057-715687-11 R         | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| G    | 1043000   | 1043000   | 1 G → T       | SNP (Strand) | 105       | 98.1%         | 0 Variants: BRC-CA-1057-715687-11 R         | Concatenator: BRC-CA-1057-715687-11 R | hva CD5  |
| A    | 1043087   | 1043087   | 1 T → A       | SNP (Strand) | 114       | 99.1%         | 0 Variants: BRC-CA-1057-715687-11 R         | Concatenator: BRC-CA-1057-715687-11 R |          |















# 9 Appendix

| Gene | Accession | Accession | Feature           | Start                      | End | Score  | Percentage | Number of Variants | Variant Range        | Consequence                     |
|------|-----------|-----------|-------------------|----------------------------|-----|--------|------------|--------------------|----------------------|---------------------------------|
| T    | 131260    | 131260    | 1 C > T           | SNP (Strand)               | 63  | 98.4%  | 45,120     | 45,120             | BRCA-11057-T131260-1 | Consequence: BRCA-11057-Unknown |
| A    | 131255    | 131255    | 1 T > A           | SNP (Strand)               | 65  | 95.9%  | 4,15       | 175                | BRCA-11057-T131255-1 | Consequence: BRCA-11057-Unknown |
| G    | 131256    | 131256    | 1 T > G           | SNP (Strand)               | 65  | 99.3%  | 6,86       | 211                | BRCA-11057-T131256-1 | Consequence: BRCA-11057-Unknown |
| A    | 131257    | 131257    | 1 A > G           | SNP (Strand)               | 65  | 98.5%  | 7          | 3,322              | BRCA-11057-T131257-1 | Consequence: BRCA-11057-Unknown |
| G    | 131277    | 131277    | 1 A > G           | SNP (Strand)               | 61  | 98.6%  | 6          | 1,196              | BRCA-11057-T131277-1 | Consequence: BRCA-11057-Unknown |
| T    | 131280    | 131280    | 1 A > T           | SNP (Strand)               | 61  | 98.8%  | 3,80       | 198                | BRCA-11057-T131280-1 | Consequence: BRCA-11057-Unknown |
| A    | 131291    | 131290    | 0 881 > AAT       | Inversion ( tandem repeat) | 78  | 95.9%  | 3,85       | 481                | BRCA-11057-T131291-1 | Consequence: BRCA-11057-Unknown |
| GGG  | 131262    | 131262    | 1 TMA > GGG       | Substitution               | 86  | 95.3%  | 25         | 235                | BRCA-11057-T131262-1 | Consequence: BRCA-11057-Unknown |
| C    | 131264    | 131264    | 1 G > A           | SNP (Strand)               | 92  | 98.9%  | 9          | 271                | BRCA-11057-T131264-1 | Consequence: BRCA-11057-Unknown |
| A    | 131267    | 131274    | 1 G > A           | SNP (Strand)               | 91  | 98.7%  | 1,52       | 233                | BRCA-11057-T131267-1 | Consequence: BRCA-11057-Unknown |
| C    | 131276    | 131276    | 1 A > C           | SNP (Strand)               | 90  | 100.0% | 11         | 269                | BRCA-11057-T131276-1 | Consequence: BRCA-11057-Unknown |
| T    | 131285    | 131286    | 1 C > T           | SNP (Strand)               | 90  | 96.7%  | 2,92       | 238                | BRCA-11057-T131285-1 | Consequence: BRCA-11057-Unknown |
| T    | 131281    | 131281    | 1 C > T           | SNP (Strand)               | 103 | 97.1%  | 1,80       | 288                | BRCA-11057-T131281-1 | Consequence: BRCA-11057-Unknown |
| C    | 131282    | 131281    | 1 A > G           | SNP (Strand)               | 85  | 98.8%  | 6,62       | 270                | BRCA-11057-T131282-1 | Consequence: BRCA-11057-Unknown |
| C    | 131260    | 131260    | 1 G > A           | SNP (Strand)               | 82  | 98.8%  | 6,52       | 249                | BRCA-11057-T131260-1 | Consequence: BRCA-11057-Unknown |
| C    | 131463    | 131463    | 1 T > C           | SNP (Strand)               | 87  | 99.0%  | 6,16       | 305                | BRCA-11057-T131463-1 | Consequence: BRCA-11057-Unknown |
| A    | 131408    | 131408    | 1 G > A           | SNP (Strand)               | 91  | 98.9%  | 7          | 300                | BRCA-11057-T131408-1 | Consequence: BRCA-11057-Unknown |
| G    | 131877    | 131877    | 1 A > G           | SNP (Strand)               | 110 | 100.0% | 0          | Variables          | BRCA-11057-T131877-1 | Consequence: BRCA-11057-Unknown |
| C    | 131878    | 131878    | 1 T > C           | SNP (Strand)               | 79  | 98.7%  | 3,16       | 328                | BRCA-11057-T131878-1 | Consequence: BRCA-11057-Unknown |
| A    | 131762    | 131762    | 1 A > A           | SNP (Strand)               | 85  | 100.0% | 3,32       | 363                | BRCA-11057-T131762-1 | Consequence: BRCA-11057-Unknown |
| A    | 131744    | 131744    | 1 G > A           | SNP (Strand)               | 82  | 98.8%  | 6,22       | 241                | BRCA-11057-T131744-1 | Consequence: BRCA-11057-Unknown |
| A    | 131746    | 131746    | 1 G > A           | SNP (Strand)               | 88  | 100.0% | 6,16       | 320                | BRCA-11057-T131746-1 | Consequence: BRCA-11057-Unknown |
| A    | 131762    | 131762    | 1 G > A           | SNP (Strand)               | 87  | 98.6%  | 7,70       | 211                | BRCA-11057-T131762-1 | Consequence: BRCA-11057-Unknown |
| A    | 131768    | 131768    | 1 G > A           | SNP (Strand)               | 96  | 97.9%  | 1,86       | 281                | BRCA-11057-T131768-1 | Consequence: BRCA-11057-Unknown |
| C    | 131740    | 131740    | 1 T > C           | SNP (Strand)               | 99  | 100.0% | 0          | Variables          | BRCA-11057-T131740-1 | Consequence: BRCA-11057-Unknown |
| C    | 131758    | 131758    | 1 A > C           | SNP (Strand)               | 101 | 100.0% | 0          | Variables          | BRCA-11057-T131758-1 | Consequence: BRCA-11057-Unknown |
| C    | 131792    | 131792    | 1 C > T           | SNP (Strand)               | 103 | 98.0%  | 1,64       | 293                | BRCA-11057-T131792-1 | Consequence: BRCA-11057-Unknown |
| T    | 131793    | 131793    | 1 A > C           | SNP (Strand)               | 105 | 99.9%  | 0          | Variables          | BRCA-11057-T131793-1 | Consequence: BRCA-11057-Unknown |
| T    | 131796    | 131796    | 1 T > C           | SNP (Strand)               | 105 | 99.1%  | 0          | Variables          | BRCA-11057-T131796-1 | Consequence: BRCA-11057-Unknown |
| A    | 131797    | 131797    | 1 T > A           | SNP (Strand)               | 106 | 98.6%  | 1,46       | 297                | BRCA-11057-T131797-1 | Consequence: BRCA-11057-Unknown |
| G    | 131806    | 131806    | 1 A > G           | SNP (Strand)               | 83  | 100.0% | 3,32       | 283                | BRCA-11057-T131806-1 | Consequence: BRCA-11057-Unknown |
| C    | 131805    | 131805    | 1 A > C           | SNP (Strand)               | 83  | 98.8%  | 3,32       | 241                | BRCA-11057-T131805-1 | Consequence: BRCA-11057-Unknown |
| G    | 131818    | 131818    | 1 A > G           | SNP (Strand)               | 83  | 100.0% | 55         | 257                | BRCA-11057-T131818-1 | Consequence: BRCA-11057-Unknown |
| G    | 131820    | 131820    | 1 A > G           | SNP (Strand)               | 85  | 98.9%  | 6          | 308                | BRCA-11057-T131820-1 | Consequence: BRCA-11057-Unknown |
| A    | 131846    | 131846    | 1 A > A           | SNP (Strand)               | 94  | 97.9%  | 0          | Variables          | BRCA-11057-T131846-1 | Consequence: BRCA-11057-Unknown |
| G    | 131928    | 131928    | 1 A > G           | SNP (Strand)               | 81  | 98.8%  | 8,11       | 234                | BRCA-11057-T131928-1 | Consequence: BRCA-11057-Unknown |
| C    | 131932    | 131932    | 1 T > C           | SNP (Strand)               | 70  | 100.0% | 0          | Variables          | BRCA-11057-T131932-1 | Consequence: BRCA-11057-Unknown |
| G    | 132020    | 132020    | 1 C > T           | SNP (Strand)               | 99  | 99.0%  | 9,62       | 292                | BRCA-11057-T132020-1 | Consequence: BRCA-11057-Unknown |
| G    | 132049    | 132049    | 1 A > G           | SNP (Strand)               | 87  | 97.7%  | 3,75       | 251                | BRCA-11057-T132049-1 | Consequence: BRCA-11057-Unknown |
| C    | 132100    | 132100    | 0 885 > AAG       | Inversion ( tandem repeat) | 97  | 99.2%  | 1,61       | 273                | BRCA-11057-T132100-1 | Consequence: BRCA-11057-Unknown |
| T    | 132102    | 132102    | 0 7AAGAG > 7AAGAA | Deletion ( tandem repeat)  | 142 | 100.0% | 6,31       | 24                 | BRCA-11057-T132102-1 | Consequence: BRCA-11057-Unknown |
| T    | 132223    | 132223    | 1 C > T           | Substitution               | 120 | 98.2%  | 2,41       | 481                | BRCA-11057-T132223-1 | Consequence: BRCA-11057-Unknown |
| A    | 132220    | 132220    | 1 C > A           | SNP (Strand)               | 124 | 100.0% | 1,44       | 64                 | BRCA-11057-T132220-1 | Consequence: BRCA-11057-Unknown |
| C    | 132205    | 132205    | 1 A > C           | SNP (Strand)               | 126 | 99.0%  | 3,16       | 327                | BRCA-11057-T132205-1 | Consequence: BRCA-11057-Unknown |
| C    | 132400    | 132400    | 0 1003 > AAD      | Deletion ( tandem repeat)  | 219 | 100.0% | 6,22       | 30                 | BRCA-11057-T132400-1 | Consequence: BRCA-11057-Unknown |
| C    | 132407    | 132407    | 1 T > C           | SNP (Strand)               | 219 | 99.5%  | 4,41       | 43                 | BRCA-11057-T132407-1 | Consequence: BRCA-11057-Unknown |
| G    | 132763    | 132763    | 1 A > G           | SNP (Strand)               | 85  | 98.8%  | 9,40       | 288                | BRCA-11057-T132763-1 | Consequence: BRCA-11057-Unknown |
| T    | 132764    | 132764    | 0 TTT             | Inversion                  | 80  | 98.3%  | 2,92       | 28                 | BRCA-11057-T132764-1 | Consequence: BRCA-11057-Unknown |
| T    | 132786    | 132786    | 1 A > T           | SNP (Strand)               | 80  | 98.3%  | 2,92       | 28                 | BRCA-11057-T132786-1 | Consequence: BRCA-11057-Unknown |
| T    | 132781    | 132781    | 1 T > T           | SNP (Strand)               | 97  | 98.3%  | 2,92       | 28                 | BRCA-11057-T132781-1 | Consequence: BRCA-11057-Unknown |
| G    | 132786    | 132786    | 1 C > T           | SNP (Strand)               | 60  | 96.7%  | 8,41       | 26                 | BRCA-11057-T132786-1 | Consequence: BRCA-11057-Unknown |
| G    | 132781    | 132781    | 1 C > T           | SNP (Strand)               | 14  | 95.3%  | 1,41       | 21                 | BRCA-11057-T132781-1 | Consequence: BRCA-11057-Unknown |
| T    | 132791    | 132791    | 0 TTT > FTT       | Inversion ( tandem repeat) | 43  | 99.1%  | 1,41       | 18                 | BRCA-11057-T132791-1 | Consequence: BRCA-11057-Unknown |
| T    | 132918    | 132918    | 1 G > T           | SNP (Strand)               | 87  | 100.0% | 55         | 252                | BRCA-11057-T132918-1 | Consequence: BRCA-11057-Unknown |
| A    | 132962    | 132962    | 1 G > A           | SNP (Strand)               | 116 | 99.1%  | 1,65       | 305                | BRCA-11057-T132962-1 | Consequence: BRCA-11057-Unknown |
| T    | 132974    | 132974    | 1 C > T           | SNP (Strand)               | 111 | 99.4%  | 0          | Variables          | BRCA-11057-T132974-1 | Consequence: BRCA-11057-Unknown |
| C    | 133028    | 133028    | 1 G > A           | SNP (Strand)               | 89  | 98.9%  | 3,56       | 244                | BRCA-11057-T133028-1 | Consequence: BRCA-11057-Unknown |
| A    | 2416      | 2416      | 1 T > A           | SNP (Strand)               | 13  | 100.0% | 1          | 1                  | BRCA-11057-T2416-1   | Consequence: BRCA-11057-Unknown |
| A    | 2418      | 2418      | 1 T > A           | SNP (Strand)               | 93  | 100.0% | 0          | Variables          | BRCA-11057-T2418-1   | Consequence: BRCA-11057-Unknown |
| C    | 2417      | 2417      | 1 T > C           | SNP (Strand)               | 93  | 100.0% | 0          | Variables          | BRCA-11057-T2417-1   | Consequence: BRCA-11057-Unknown |
| G    | 2417      | 2417      | 1 A > G           | SNP (Strand)               | 92  | 100.0% | 0          | Variables          | BRCA-11057-T2417-1   | Consequence: BRCA-11057-Unknown |
| A    | 2418      | 2418      | 1 A > A           | SNP (Strand)               | 102 | 100.0% | 0          | Variables          | BRCA-11057-T2418-1   | Consequence: BRCA-11057-Unknown |
| T    | 2412      | 2412      | 1 T > C           | SNP (Strand)               | 112 | 100.0% | 0          | Variables          | BRCA-11057-T2412-1   | Consequence: BRCA-11057-Unknown |
| T    | 2418      | 2418      | 1 G > T           | SNP (Strand)               | 116 | 100.0% | 0          | Variables          | BRCA-11057-T2418-1   | Consequence: BRCA-11057-Unknown |
| A    | 2420      | 2420      | 1 A > A           | SNP (Strand)               | 105 | 100.0% | 0          | Variables          | BRCA-11057-T2420-1   | Consequence: BRCA-11057-Unknown |
| A    | 2400      | 2400      | 1 G > A           | SNP (Strand)               | 94  | 100.0% | 0          | Variables          | BRCA-11057-T2400-1   | Consequence: BRCA-11057-Unknown |
| GC   | 2467      | 2467      | 1 AT > GC         | Substitution               | 82  | 83.8   | 3,86       | 301                | BRCA-11057-T2467-1   | Consequence: BRCA-11057-Unknown |
| C    | 2472      | 2472      | 1 T > C           | SNP (Strand)               | 76  | 100.0% | 1,86       | 246                | BRCA-11057-T2472-1   | Consequence: BRCA-11057-Unknown |
| C    | 2501      | 2501      | 1 A > C           | SNP (Strand)               | 120 | 100.0% | 0          | Variables          | BRCA-11057-T2501-1   | Consequence: BRCA-11057-Unknown |
| C    | 2508      | 2508      | 1 C > T           | SNP (Strand)               | 120 | 100.0% | 0          | Variables          | BRCA-11057-T2508-1   | Consequence: BRCA-11057-Unknown |
| A    | 2508      | 2508      | 1 C > T           | SNP (Strand)               | 195 | 100.0% | 0          | Variables          | BRCA-11057-T2508-1   | Consequence: BRCA-11057-Unknown |
| C    | 2549      | 2549      | 1 A > C           | SNP (Strand)               | 102 | 99.0%  | 0          | Variables          | BRCA-11057-T2549-1   | Consequence: BRCA-11057-Unknown |
| T    | 2547      | 2547      | 1 T > C           | SNP (Strand)               | 102 | 100.0% | 0          | Variables          | BRCA-11057-T2547-1   | Consequence: BRCA-11057-Unknown |
| A    | 2547      | 2547      | 1 T > A           | SNP (Strand)               | 105 | 100.0% | 0          | Variables          | BRCA-11057-T2547-1   | Consequence: BRCA-11057-Unknown |
| A    | 2548      | 2548      | 1 G > A           | SNP (Strand)               | 106 | 100.0% | 0          | Variables          | BRCA-11057-T2548-1   | Consequence: BRCA-11057-Unknown |
| G    | 2556      | 2556      | 1 G > A           | SNP (Strand)               | 111 | 100.0% | 0          | Variables          | BRCA-11057-T2556-1   | Consequence: BRCA-11057-Unknown |
| G    | 2558      | 2558      | 1 A > G           | SNP (Strand)               | 88  | 100.0% | 0          | Variables          | BRCA-11057-T2558-1   | Consequence: BRCA-11057-Unknown |
| A    | 2560      | 2560      | 1 A > A           | SNP (Strand)               | 105 | 100.0% | 0          | Variables          | BRCA-11057-T2560-1   | Consequence: BRCA-11057-Unknown |
| C    | 2561      | 2561      | 1 T > C           | SNP (Strand)               | 84  | 100.0% | 12         | 293                | BRCA-11057-T2561-1   | Consequence: BRCA-11057-Unknown |
| C    | 2636      | 2636      | 1 T > C           | SNP (Strand)               | 104 | 100.0% | 0          | Variables          | BRCA-11057-T2636-1   | Consequence: BRCA-11057-Unknown |
| C    | 2631      | 2631      | 1 G > A           | SNP (Strand)               | 109 | 100.0% | 0          | Variables          | BRCA-11057-T2631-1   | Consequence: BRCA-11057-Unknown |
| ACT1 | 2632      | 2631      | 1 GTTA > ACT1     | Substitution               | 75  | 89.7%  | 98.7%      | 88                 | BRCA-11057-T2632-1   | Consequence: BRCA-11057-Unknown |
| T    | 2638      | 2638      | 1 T > C           | SNP (Strand)               | 109 | 100.0% | 0          | Variables          | BRCA-11057-T2638-1   | Consequence: BRCA-11057-Unknown |
| A    | 2639      | 2639      | 1 G > A           | SNP (Strand)               | 85  | 100.0% | 0          | Variables          | BRCA-11057-T2639-1   | Consequence: BRCA-11057-Unknown |
| T    | 2738      | 2738      | 1 C > T           | SNP (Strand)               | 134 | 100.0% | 0          | Variables          | BRCA-11057-T2738-1   | Consequence: BRCA-11057-Unknown |
| C    | 2782      | 2782      | 1 C > T           | SNP (Strand)               | 122 | 99.2%  | 0          | Variables          | BRCA-11057-T2782-1   | Consequence: BRCA-11057-Unknown |
| C    | 2784      | 2784      | 1 T > C           | SNP (Strand)               | 123 | 100.0% | 0          | Variables          | BRCA-11057-T2784-1   | Consequence: BRCA-11057-Unknown |
| C    | 2801      | 2801      | 1 T > C           | SNP (Strand)               | 111 | 100.0% | 0          | Variables          | BRCA-11057-T2801-1   | Consequence: BRCA-11057-Unknown |
| T    | 2801      | 2801      | 1 C > T           | SNP (Strand)               | 103 | 99.0%  | 0          | Variables          | BRCA-11057-T2801-1   | Consequence: BRCA-11057-Unknown |
| C    | 2801      | 2801      | 1 T > C           | SNP (Strand)               | 102 | 99.0%  | 0          | Variables          | BRCA-11057-T2801-1   | Consequence: BRCA-11057-Unknown |
| A    | 2804      |           |                   |                            |     |        |            |                    |                      |                                 |

# 9 Appendix

| Category | Gene ID | Gene Name | Transcript                  | Expression       | Pathway | Function |   |                               |       |  |
|----------|---------|-----------|-----------------------------|------------------|---------|----------|---|-------------------------------|-------|--|
| C        | 106222  | 106222    | 1 T → C                     | SNP (transcript) | 98      | 100.0%   | 0 Variants: BRCA-11057-T15687.1.R         | Concatermer: BRCA-11057-UK105 | V → A | Iron CS  |
| G        | 106295  | 106295    | 1 A → G                     | SNP (transcript) | 84      | 100.0%   | 4E-302 Variants: BRCA-11057-T15687.1.R    | Concatermer: BRCA-11057-UK105 |       | Iron CS  |
| T        | 106367  | 106367    | 1 A → G                     | SNP (transcript) | 89      | 100.0%   | 0 Variants: BRCA-11057-T15687.1.R         | Concatermer: BRCA-11057-UK105 | I → V | Iron CS  |
| ASC      | 106501  | 106501    | 1 G C → A G C               | Substitution     | 84      | 98.3%    | 1,812 Variants: BRCA-11057-T15687.1.R     | Concatermer: BRCA-11057-UK105 | A → S | Regulates MAM family protein CS                  |
| C        | 106501  | 106501    | 1 G → C                     | SNP (transcript) | 83      | 100.0%   | 7,881-707 Variants: BRCA-11057-T15687.1.R | Concatermer: BRCA-11057-UK105 | V → I | Regulates MAM family protein CS                  |
| G        | 106502  | 106502    | 1 A → G                     | SNP (transcript) | 81      | 100.0%   | 2,367-291 Variants: BRCA-11057-T15687.1.R | Concatermer: BRCA-11057-UK105 |       | Regulates MAM family protein CS                  |
| T        | 106548  | 106548    | 1 A → G                     | SNP (transcript) | 78      | 100.0%   | 6,352-281 Variants: BRCA-11057-T15687.1.R | Concatermer: BRCA-11057-UK105 | S → N | Rac CS   |
| T        | 106549  | 106549    | 1 C → T                     | SNP (transcript) | 82      | 100.0%   | 0 Variants: BRCA-11057-T15687.1.R         | Concatermer: BRCA-11057-UK105 |       | Rac CS   |
| A        | 106742  | 106742    | 1 G → A                     | SNP (transcript) | 72      | 100.0%   | 12,169 Variants: BRCA-11057-T15687.1.R    | Concatermer: BRCA-11057-UK105 | A → T | Regulates phosphatase hemozoinin protein CS      |
| T        | 106825  | 106825    | 1 C → T                     | SNP (transcript) | 87      | 98.9%    | 2,281-207 Variants: BRCA-11057-T15687.1.R | Concatermer: BRCA-11057-UK105 | G → I | Iron CS  |
| A        | 106829  | 106829    | 1 G → A                     | SNP (transcript) | 75      | 100.0%   | 11,289 Variants: BRCA-11057-T15687.1.R    | Concatermer: BRCA-11057-UK105 |       | Iron CS  |
| A        | 106847  | 106847    | 1 G → A                     | SNP (transcript) | 78      | 100.0%   | 1,342-280 Variants: BRCA-11057-T15687.1.R | Concatermer: BRCA-11057-UK105 |       | Iron CS  |
| S        | 106932  | 106932    | 1 G → A                     | SNP (transcript) | 93      | 100.0%   | 0 Variants: BRCA-11057-T15687.1.R         | Concatermer: BRCA-11057-UK105 |       | Iron CS  |
| A        | 107006  | 107006    | 1 G → A                     | SNP (transcript) | 111     | 99.3%    | 0 Variants: BRCA-11057-T15687.1.R         | Concatermer: BRCA-11057-UK105 |       | Iron CS  |
| G        | 107094  | 107094    | 1 A → G                     | SNP (transcript) | 107     | 100.0%   | 0 Variants: BRCA-11057-T15687.1.R         | Concatermer: BRCA-11057-UK105 |       | Iron CS  |
| A        | 107248  | 107248    | 1 A → G                     | SNP (transcript) | 70      | 100.0%   | 11,258 Variants: BRCA-11057-T15687.1.R    | Concatermer: BRCA-11057-UK105 |       | Phospho-2-dehydro-3-deoxyphosphatase aldolase CS |
| T        | 107268  | 107268    | 1 A → G                     | SNP (transcript) | 87      | 100.0%   | 0 Variants: BRCA-11057-T15687.1.R         | Concatermer: BRCA-11057-UK105 |       | Phospho-2-dehydro-3-deoxyphosphatase aldolase CS |
| T        | 107383  | 107383    | 1 C → T                     | SNP (transcript) | 93      | 100.0%   | 0 Variants: BRCA-11057-T15687.1.R         | Concatermer: BRCA-11057-UK105 |       | Phospho-2-dehydro-3-deoxyphosphatase aldolase CS |
| T        | 107394  | 107394    | 1 A → G                     | SNP (transcript) | 107     | 100.0%   | 0 Variants: BRCA-11057-T15687.1.R         | Concatermer: BRCA-11057-UK105 | I → V | Phospho-2-dehydro-3-deoxyphosphatase aldolase CS |
| G        | 107502  | 107502    | 1 A → G                     | SNP (transcript) | 95      | 100.0%   | 0 Variants: BRCA-11057-T15687.1.R         | Concatermer: BRCA-11057-UK105 |       | Phospho-2-dehydro-3-deoxyphosphatase aldolase CS |
| A        | 107559  | 107559    | 1 G → A                     | SNP (transcript) | 87      | 100.0%   | 0 Variants: BRCA-11057-T15687.1.R         | Concatermer: BRCA-11057-UK105 | M → Y | Iron CS  |
| A        | 107582  | 107582    | 1 G → A                     | SNP (transcript) | 86      | 100.0%   | 0 Variants: BRCA-11057-T15687.1.R         | Concatermer: BRCA-11057-UK105 |       | Iron CS  |
| A        | 107845  | 107845    | 1 G → A                     | SNP (transcript) | 75      | 98.7%    | 1,781-271 Variants: BRCA-11057-T15687.1.R | Concatermer: BRCA-11057-UK105 | A → V | Iron CS  |
| C        | 107813  | 107813    | 1 T → C                     | SNP (transcript) | 103     | 100.0%   | 0 Variants: BRCA-11057-T15687.1.R         | Concatermer: BRCA-11057-UK105 |       | Iron CS  |
| C        | 107856  | 107856    | 1 T → C                     | SNP (transcript) | 70      | 100.0%   | 15,258 Variants: BRCA-11057-T15687.1.R    | Concatermer: BRCA-11057-UK105 | N → D | Iron CS  |
| G        | 107899  | 107899    | 1 A → G                     | SNP (transcript) | 65      | 100.0%   | 3,242 Variants: BRCA-11057-T15687.1.R     | Concatermer: BRCA-11057-UK105 | V → A | Iron CS  |
| G        | 107904  | 107904    | 1 T → G                     | SNP (transcript) | 65      | 100.0%   | 52,355 Variants: BRCA-11057-T15687.1.R    | Concatermer: BRCA-11057-UK105 | I → G | Iron CS  |
| G        | 107943  | 107943    | 1 A → G                     | SNP (transcript) | 86      | 100.0%   | 0 Variants: BRCA-11057-T15687.1.R         | Concatermer: BRCA-11057-UK105 |       | RNA binding protein CS                           |
| C        | 107981  | 107981    | 1 T → C                     | SNP (transcript) | 80      | 100.0%   | 19,286 Variants: BRCA-11057-T15687.1.R    | Concatermer: BRCA-11057-UK105 | I → M | Iron CS  |
| C        | 107981  | 107981    | 1 C → T                     | SNP (transcript) | 81      | 100.0%   | 0 Variants: BRCA-11057-T15687.1.R         | Concatermer: BRCA-11057-UK105 | R → G | 3-deoxy-D-arabino-heptulosic acid transferase CS |
| T        | 108085  | 108085    | 1 G → T                     | SNP (transcript) | 70      | 100.0%   | 18,258 Variants: BRCA-11057-T15687.1.R    | Concatermer: BRCA-11057-UK105 | L → I | Wt1-like nuclear metal center hexamer protein CS |
| T        | 108125  | 108125    | 1 C → T                     | SNP (transcript) | 95      | 100.0%   | 0 Variants: BRCA-11057-T15687.1.R         | Concatermer: BRCA-11057-UK105 |       | Iron CS  |
| T        | 108125  | 108125    | 1 C → T                     | SNP (transcript) | 98      | 100.0%   | 0 Variants: BRCA-11057-T15687.1.R         | Concatermer: BRCA-11057-UK105 |       | Iron CS  |
| C        | 108129  | 108129    | 1 T → C                     | SNP (transcript) | 100     | 100.0%   | 0 Variants: BRCA-11057-T15687.1.R         | Concatermer: BRCA-11057-UK105 |       | Iron CS  |
| C        | 108161  | 108161    | 1 A → G                     | SNP (transcript) | 114     | 100.0%   | 0 Variants: BRCA-11057-T15687.1.R         | Concatermer: BRCA-11057-UK105 |       | Iron CS  |
| A        | 108130  | 108130    | 1 G → A                     | SNP (transcript) | 88      | 100.0%   | 0 Variants: BRCA-11057-T15687.1.R         | Concatermer: BRCA-11057-UK105 |       | Iron CS  |
| C        | 108163  | 108163    | 1 T → C                     | SNP (transcript) | 107     | 99.1%    | 107 Variants: BRCA-11057-T15687.1.R       | Concatermer: BRCA-11057-UK105 |       | Iron CS  |
| A        | 108152  | 108152    | 1 T → A                     | SNP (transcript) | 110     | 100.0%   | 0 Variants: BRCA-11057-T15687.1.R         | Concatermer: BRCA-11057-UK105 |       | Iron CS  |
| A        | 108171  | 108171    | 1 C → A                     | SNP (transcript) | 91      | 100.0%   | 0 Variants: BRCA-11057-T15687.1.R         | Concatermer: BRCA-11057-UK105 |       | Iron CS  |
| C        | 108148  | 108148    | 1 T → C                     | SNP (transcript) | 92      | 99.9%    | 0 Variants: BRCA-11057-T15687.1.R         | Concatermer: BRCA-11057-UK105 |       | Iron CS  |
| T        | 108191  | 108191    | 1 A → T                     | SNP (transcript) | 94      | 98.9%    | 0 Variants: BRCA-11057-T15687.1.R         | Concatermer: BRCA-11057-UK105 |       | Iron CS  |
| T        | 108171  | 108171    | 1 T → C                     | SNP (transcript) | 104     | 100.0%   | 0 Variants: BRCA-11057-T15687.1.R         | Concatermer: BRCA-11057-UK105 |       | Iron CS  |
| C        | 108160  | 108160    | 1 T → C                     | SNP (transcript) | 92      | 100.0%   | 0 Variants: BRCA-11057-T15687.1.R         | Concatermer: BRCA-11057-UK105 | S → G | putative protease from collagenase family CS     |
| C        | 108488  | 108488    | 1 T → C                     | SNP (transcript) | 82      | 100.0%   | 0 Variants: BRCA-11057-T15687.1.R         | Concatermer: BRCA-11057-UK105 | N → S | Chromatin protein CS                             |
| A        | 108474  | 108474    | 1 T → A                     | SNP (transcript) | 84      | 100.0%   | 0 Variants: BRCA-11057-T15687.1.R         | Concatermer: BRCA-11057-UK105 |       | Iron CS  |
| A        | 108592  | 108592    | 1 G → A                     | SNP (transcript) | 84      | 100.0%   | 0 Variants: BRCA-11057-T15687.1.R         | Concatermer: BRCA-11057-UK105 | V → I | Iron CS  |
| A        | 108596  | 108596    | 1 G → A                     | SNP (transcript) | 78      | 100.0%   | 4,487-747 Variants: BRCA-11057-T15687.1.R | Concatermer: BRCA-11057-UK105 |       | Reg2 CS  |
| A        | 108625  | 108625    | 1 G → A                     | SNP (transcript) | 88      | 100.0%   | 0 Variants: BRCA-11057-T15687.1.R         | Concatermer: BRCA-11057-UK105 |       | Reg2 CS  |
| T        | 108825  | 108825    | 1 C → T                     | SNP (transcript) | 94      | 100.0%   | 0 Variants: BRCA-11057-T15687.1.R         | Concatermer: BRCA-11057-UK105 |       | Iron CS  |
| A        | 108825  | 108825    | 1 C → T                     | SNP (transcript) | 96      | 100.0%   | 0 Variants: BRCA-11057-T15687.1.R         | Concatermer: BRCA-11057-UK105 |       | Iron CS  |
| A        | 108842  | 108842    | 0 Insertion (random repeat) | Substitution     | 95      | 100.0%   | 2,162 Variants: BRCA-11057-T15687.1.R     | Concatermer: BRCA-11057-UK105 |       | Iron CS  |
| A        | 108848  | 108848    | 1 A → G                     | SNP (transcript) | 85      | 100.0%   | 15,305 Variants: BRCA-11057-T15687.1.R    | Concatermer: BRCA-11057-UK105 |       | Rac CS   |
| G        | 108916  | 108916    | 1 G → A                     | SNP (transcript) | 91      | 100.0%   | 0 Variants: BRCA-11057-T15687.1.R         | Concatermer: BRCA-11057-UK105 |       | Rac CS   |
| C        | 108913  | 108913    | 1 A → G                     | SNP (transcript) | 91      | 100.0%   | 0 Variants: BRCA-11057-T15687.1.R         | Concatermer: BRCA-11057-UK105 |       | Rac CS   |
| T        | 109138  | 109138    | 1 C → T                     | SNP (transcript) | 85      | 100.0%   | 15,305 Variants: BRCA-11057-T15687.1.R    | Concatermer: BRCA-11057-UK105 |       | Rac CS   |
| G        | 109128  | 109128    | 1 A → G                     | SNP (transcript) | 91      | 100.0%   | 0 Variants: BRCA-11057-T15687.1.R         | Concatermer: BRCA-11057-UK105 |       | Regulatory increase CS                           |
| T        | 109170  | 109170    | 1 C → T                     | SNP (transcript) | 112     | 99.4%    | 0 Variants: BRCA-11057-T15687.1.R         | Concatermer: BRCA-11057-UK105 | M → T | Regulatory increase CS                           |
| C        | 109151  | 109151    | 1 T → C                     | SNP (transcript) | 82      | 100.0%   | 0 Variants: BRCA-11057-T15687.1.R         | Concatermer: BRCA-11057-UK105 | K → R | Regulatory increase CS                           |
| C        | 109151  | 109151    | 1 T → C                     | SNP (transcript) | 88      | 100.0%   | 0 Variants: BRCA-11057-T15687.1.R         | Concatermer: BRCA-11057-UK105 |       | Regulatory increase CS                           |
| G        | 109178  | 109178    | 1 G → A                     | SNP (transcript) | 94      | 100.0%   | 0 Variants: BRCA-11057-T15687.1.R         | Concatermer: BRCA-11057-UK105 | V → A | Regulatory increase CS                           |
| A        | 109248  | 109248    | 1 G → A                     | SNP (transcript) | 81      | 100.0%   | 2,815-291 Variants: BRCA-11057-T15687.1.R | Concatermer: BRCA-11057-UK105 |       | Regulatory increase CS                           |
| A        | 109370  | 109370    | 1 G → A                     | SNP (transcript) | 86      | 98.3%    | 6,624 Variants: BRCA-11057-T15687.1.R     | Concatermer: BRCA-11057-UK105 |       | Membrane protein CS                              |
| A        | 109384  | 109384    | 1 C → T                     | SNP (transcript) | 88      | 100.0%   | 0 Variants: BRCA-11057-T15687.1.R         | Concatermer: BRCA-11057-UK105 | T → I | Membrane protein CS                              |
| C        | 109386  | 109386    | 1 A → G                     | SNP (transcript) | 85      | 100.0%   | 0 Variants: BRCA-11057-T15687.1.R         | Concatermer: BRCA-11057-UK105 |       | Membrane protein CS                              |
| C        | 109392  | 109392    | 1 T → C                     | SNP (transcript) | 107     | 99.1%    | 0 Variants: BRCA-11057-T15687.1.R         | Concatermer: BRCA-11057-UK105 | S → P | Membrane protein CS                              |
| T        | 109426  | 109426    | 1 T → C                     | SNP (transcript) | 87      | 100.0%   | 0 Variants: BRCA-11057-T15687.1.R         | Concatermer: BRCA-11057-UK105 |       | Membrane protein CS                              |
| T        | 109444  | 109444    | 1 C → T                     | SNP (transcript) | 87      | 98.3%    | 2,287-307 Variants: BRCA-11057-T15687.1.R | Concatermer: BRCA-11057-UK105 |       | Iron CS  |
| A        | 109452  | 109452    | 1 G → A                     | SNP (transcript) | 106     | 99.3%    | 0 Variants: BRCA-11057-T15687.1.R         | Concatermer: BRCA-11057-UK105 | A → T | Iron CS  |
| A        | 109461  | 109461    | 1 G → A                     | SNP (transcript) | 85      | 100.0%   | 15,305 Variants: BRCA-11057-T15687.1.R    | Concatermer: BRCA-11057-UK105 |       | Rac CS   |
| A        | 109464  | 109464    | 1 T → A                     | SNP (transcript) | 106     | 100.0%   | 0 Variants: BRCA-11057-T15687.1.R         | Concatermer: BRCA-11057-UK105 |       | Iron CS  |
| A        | 109465  | 109465    | 1 T → A                     | SNP (transcript) | 99      | 98.9%    | 0 Variants: BRCA-11057-T15687.1.R         | Concatermer: BRCA-11057-UK105 |       | Iron CS  |
| A        | 110019  | 110019    | 1 G → A                     | SNP (transcript) | 78      | 100.0%   | 2,548-288 Variants: BRCA-11057-T15687.1.R | Concatermer: BRCA-11057-UK105 |       | Iron CS  |
| C        | 110028  | 110028    | 1 T → C                     | SNP (transcript) | 83      | 100.0%   | 7,387-307 Variants: BRCA-11057-T15687.1.R | Concatermer: BRCA-11057-UK105 |       | Iron CS  |
| C        | 110029  | 110029    | 1 T → C                     | SNP (transcript) | 82      | 100.0%   | 42,303 Variants: BRCA-11057-T15687.1.R    | Concatermer: BRCA-11057-UK105 |       | Iron CS  |
| C        | 110021  | 110021    | 1 T → C                     | SNP (transcript) | 83      | 100.0%   | 1,662-288 Variants: BRCA-11057-T15687.1.R | Concatermer: BRCA-11057-UK105 |       | Iron CS  |
| C        | 110021  | 110021    | 1 T → C                     | SNP (transcript) | 91      | 100.0%   | 0 Variants: BRCA-11057-T15687.1.R         | Concatermer: BRCA-11057-UK105 |       | Iron CS  |
| C        | 110021  | 110021    | 1 G → A                     | SNP (transcript) | 86      | 100.0%   | 15,307 Variants: BRCA-11057-T15687.1.R    | Concatermer: BRCA-11057-UK105 |       | Iron CS  |
| C        | 110021  | 110021    | 1 C → T                     | SNP (transcript) | 90      | 100.0%   | 0 Variants: BRCA-11057-T15687.1.R         | Concatermer: BRCA-11057-UK105 |       | Iron CS  |
| C        | 110048  | 110048    | 1 T → C                     | SNP (transcript) | 82      | 100.0%   | 12,288 Variants: BRCA-11057-T15687.1.R    | Concatermer: BRCA-11057-UK105 |       | Iron CS  |
| G        | 110023  | 110023    | 1 T → G                     | SNP (transcript) | 78      | 98.7%    | 4,682-273 Variants: BRCA-11057-T15687.1.R | Concatermer: BRCA-11057-UK105 |       | Iron CS  |
| G        | 110027  | 110027    | 1 A → G                     | SNP (transcript) | 78      | 100.0%   | 1,462-280 Variants: BRCA-11057-T15687.1.R | Concatermer: BRCA-11057-UK105 |       | Iron CS  |
| C        | 110028  | 110028    | 1 T → C                     | SNP (transcript) | 79      | 100.0%   | 1,284 Variants: BRCA-11057-T15687.1.R     | Concatermer: BRCA-11057-UK105 |       | Iron CS  |
| C        | 110090  | 110090    | 1 T → C                     | SNP (transcript) | 85      | 100.0%   | 11,279 Variants: BRCA-11057-T15687.1.R    | Concatermer: BRCA-11057-UK105 |       | Iron CS  |
| A        | 110091  | 110091    | 1 T → C                     | SNP (transcript) | 84      | 100.0%   | 0 Variants: BRCA-                         |                               |       |  |















# 9 Appendix

|     |       |       |              |                           |           |               |                                       |                            |        |        |        |     |
|-----|-------|-------|--------------|---------------------------|-----------|---------------|---------------------------------------|----------------------------|--------|--------|--------|-----|
| C   | 21172 | 21171 | 1-T → C      | SNP (transversion)        | 107       | 100.0%        | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | concat | CDS    |        |     |
| T   | 21193 | 21193 | 1-A → T      | SNP (transversion)        | 70        | 98.6%         | 2,852 Variants: BR-C-10577-T156873.8  | Concatemer BR-C-11057-UK48 | concat | CDS    |        |     |
| C   | 21196 | 21196 | 1-T → C      | SNP (transversion)        | 70        | 98.7%         | 2,156 Variants: BR-C-10577-T156873.8  | Concatemer BR-C-11057-UK48 | concat | CDS    |        |     |
| A   | 21202 | 21202 | 1-G → A      | SNP (transversion)        | 76        | 98.7%         | 4,813 Variants: BR-C-10577-T156873.8  | Concatemer BR-C-11057-UK48 | concat | CDS    |        |     |
| A   | 21205 | 21205 | 1-G → A      | SNP (transversion)        | 77        | 100.0%        | 6,312 Variants: BR-C-10577-T156873.8  | Concatemer BR-C-11057-UK48 | concat | CDS    |        |     |
| C   | 21214 | 21214 | 1-T → C      | SNP (transversion)        | 80        | 100.0%        | 11,299 Variants: BR-C-10577-T156873.8 | Concatemer BR-C-11057-UK48 | concat | CDS    |        |     |
| C   | 21226 | 21226 | 1-A → C      | SNP (transversion)        | 83        | 100.0%        | 1,462 Variants: BR-C-10577-T156873.8  | Concatemer BR-C-11057-UK48 | concat | CDS    |        |     |
| CA  | 21238 | 21239 | 2-AG → CA    | Substitution              | 85 → 87   | 100.0%        | 15,305 Variants: BR-C-10577-T156873.8 | Concatemer BR-C-11057-UK48 | A → Y  | concat | CDS    |     |
| T   | 21246 | 21244 | 1-C → T      | SNP (transversion)        | 102       | 100.0%        | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | del    | Y → I  | concat | CDS |
| T   | 21268 | 21268 | 1-T → C      | SNP (transversion)        | 94        | 98.9%         | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | concat | CDS    |        |     |
| G   | 21271 | 21271 | 1-A → G      | SNP (transversion)        | 97        | 98.9%         | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | concat | CDS    |        |     |
| A   | 21274 | 21274 | 1-G → A      | SNP (transversion)        | 92        | 100.0%        | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | concat | CDS    |        |     |
| T   | 21280 | 21280 | 1-A → T      | SNP (transversion)        | 93        | 100.0%        | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | concat | CDS    |        |     |
| T   | 21283 | 21283 | 1-T → C      | SNP (transversion)        | 94        | 100.0%        | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | concat | CDS    |        |     |
| A   | 21298 | 21298 | 1-C → A      | SNP (transversion)        | 99        | 100.0%        | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | concat | CDS    |        |     |
| T   | 21313 | 21313 | 1-C → T      | SNP (transversion)        | 107       | 100.0%        | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | concat | CDS    |        |     |
| T   | 21316 | 21316 | 1-G → A      | SNP (transversion)        | 106       | 100.0%        | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | concat | CDS    |        |     |
| C   | 21319 | 21317 | 1-T → C      | SNP (transversion)        | 104       | 100.0%        | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | concat | CDS    |        |     |
| T   | 21418 | 21417 | 0-TTG → TT   | Indelion ( tandem repeat) | 111       | 94.6%         | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | concat | CDS    |        |     |
| T   | 21462 | 21463 | 1-C → T      | SNP (transversion)        | 102       | 100.0%        | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | del    | CG     | CDS    |     |
| A   | 22091 | 22093 | 1-G → A      | SNP (transversion)        | 97        | 100.0%        | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | del    | CG     | CDS    |     |
| T   | 22121 | 22121 | 1-T → C      | SNP (transversion)        | 114       | 100.0%        | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | concat | CDS    |        |     |
| T   | 22276 | 22276 | 1-G → A      | SNP (transversion)        | 110       | 100.0%        | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | del    | CDS    |        |     |
| T   | 22288 | 22288 | 1-G → T      | SNP (transversion)        | 110       | 100.0%        | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | del    | CDS    |        |     |
| T   | 22291 | 22291 | 1-C → T      | SNP (transversion)        | 105       | 100.0%        | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | del    | CDS    |        |     |
| C   | 22294 | 22294 | 1-T → C      | SNP (transversion)        | 109       | 100.0%        | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | del    | CDS    |        |     |
| GG  | 22306 | 22307 | 2-GT → AG    | Substitution              | 107       | 100.0%        | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | G → A  | del    | CDS    |     |
| G   | 22315 | 22315 | 1-A → G      | SNP (transversion)        | 106       | 100.0%        | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | del    | CDS    |        |     |
| TA  | 22320 | 22321 | 2-TG → TA    | Substitution              | 104 → 106 | 100.0%        | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | del    | del    | CDS    |     |
| G   | 22324 | 22324 | 1-A → G      | SNP (transversion)        | 105       | 100.0%        | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | del    | del    | CDS    |     |
| CT  | 22600 | 22600 | 2-TG → CT    | Substitution              | 111       | 98.4%         | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | D → S  | del    | CDS    |     |
| A   | 22620 | 22620 | 1-T → A      | Substitution              | 114 → 115 | 96.2%         | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | del    | del    | CDS    |     |
| C   | 22834 | 22834 | 1-T → C      | SNP (transversion)        | 109       | 100.0%        | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | del    | del    | CDS    |     |
| AIC | 22864 | 22866 | 2-GT → AIC   | Substitution              | 101 → 103 | 100.0%        | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | N → D  | del    | CDS    |     |
| T   | 22910 | 22910 | 1-G → T      | SNP (transversion)        | 102       | 100.0%        | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | del    | del    | CDS    |     |
| T   | 22891 | 22891 | 1-G → T      | SNP (transversion)        | 100       | 100.0%        | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | del    | del    | CDS    |     |
| A   | 22897 | 22897 | 1-G → A      | SNP (transversion)        | 102       | 100.0%        | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | del    | del    | CDS    |     |
| GA  | 22921 | 22922 | 2-TG → GA    | Substitution              | 87 → 90   | 100.0%        | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | T → I  | del    | CDS    |     |
| A   | 22924 | 22924 | 1-G → A      | SNP (transversion)        | 96        | 100.0%        | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | del    | del    | CDS    |     |
| CA  | 22939 | 22940 | 4-TTC → CA   | Substitution              | 112 → 114 | 99.1%         | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | N → SD | del    | CDS    |     |
| A   | 22947 | 22947 | 1-T → A      | SNP (transversion)        | 117       | 99.1%         | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | N → A  | del    | CDS    |     |
| A   | 22958 | 22958 | 1-C → A      | SNP (transversion)        | 111       | 100.0%        | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | del    | del    | CDS    |     |
| A   | 22963 | 22963 | 1-G → A      | SNP (transversion)        | 118       | 99.2%         | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | del    | del    | CDS    |     |
| TG  | 22965 | 22967 | 2-GA → TG    | Substitution              | 117 → 118 | 99.3% → 99.2% | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | I → T  | del    | CDS    |     |
| A   | 22971 | 22971 | 1-T → A      | SNP (transversion)        | 111       | 100.0%        | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | del    | del    | CDS    |     |
| A   | 22987 | 22987 | 1-G → A      | SNP (transversion)        | 117       | 100.0%        | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | del    | del    | CDS    |     |
| CAA | 22990 | 22990 | 2-TAG → CAA  | Substitution              | 119 → 123 | 97.8% → 97.8% | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | del    | del    | CDS    |     |
| A   | 23038 | 23038 | 1-G → A      | SNP (transversion)        | 107       | 100.0%        | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | del    | del    | CDS    |     |
| A   | 23041 | 23041 | 1-T → A      | SNP (transversion)        | 107       | 100.0%        | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | del    | del    | CDS    |     |
| G   | 24100 | 24100 | 1-T → G      | SNP (transversion)        | 113       | 100.0%        | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | del    | del    | CDS    |     |
| T   | 24104 | 24104 | 1-G → T      | SNP (transversion)        | 96        | 100.0%        | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | del    | del    | CDS    |     |
| G   | 24110 | 24110 | 1-T → G      | SNP (transversion)        | 94        | 100.0%        | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | del    | del    | CDS    |     |
| G   | 24173 | 24173 | 1-A → G      | SNP (transversion)        | 91        | 100.0%        | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | del    | del    | CDS    |     |
| G   | 24185 | 24185 | 1-T → G      | SNP (transversion)        | 99        | 100.0%        | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | del    | del    | CDS    |     |
| G   | 24224 | 24224 | 2-A → G      | SNP (transversion)        | 93        | 100.0%        | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | del    | del    | CDS    |     |
| A   | 24200 | 24200 | 1-G → A      | SNP (transversion)        | 84        | 100.0%        | 40,360 Variants: BR-C-10577-T156873.8 | Concatemer BR-C-11057-UK48 | del    | del    | CDS    |     |
| CC  | 24211 | 24212 | 2-AT → CC    | Substitution              | 99 → 101  | 100.0%        | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | del    | del    | CDS    |     |
| T   | 24710 | 24711 | 1-T → C      | SNP (transversion)        | 97        | 100.0%        | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | del    | del    | CDS    |     |
| C   | 24900 | 24900 | 1-A → C      | SNP (transversion)        | 99        | 99.9%         | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | del    | del    | CDS    |     |
| C   | 24386 | 24386 | 1-T → C      | SNP (transversion)        | 106       | 100.0%        | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | del    | del    | CDS    |     |
| C   | 24408 | 24408 | 1-C → T      | SNP (transversion)        | 125       | 100.0%        | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | E → K  | del    | CDS    |     |
| T   | 24409 | 24409 | 1-A → C      | SNP (transversion)        | 105       | 99.9%         | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | del    | del    | CDS    |     |
| T   | 24747 | 24747 | 1-C → T      | SNP (transversion)        | 114       | 99.1%         | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | E → K  | del    | CDS    |     |
| A   | 24748 | 24748 | 1-G → A      | SNP (transversion)        | 114       | 99.1%         | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | del    | del    | CDS    |     |
| A   | 24549 | 24549 | 1-G → A      | SNP (transversion)        | 114       | 99.1%         | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | N → Y  | del    | CDS    |     |
| A   | 25526 | 25526 | 1-G → A      | SNP (transversion)        | 114       | 100.0%        | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | del    | del    | CDS    |     |
| A   | 25538 | 25538 | 1-G → A      | SNP (transversion)        | 104       | 100.0%        | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | del    | del    | CDS    |     |
| G   | 25610 | 25610 | 1-A → G      | SNP (transversion)        | 105       | 100.0%        | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | del    | del    | CDS    |     |
| A   | 25614 | 25614 | 1-G → A      | SNP (transversion)        | 104       | 100.0%        | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | del    | del    | CDS    |     |
| A   | 25936 | 25936 | 1-T → A      | SNP (transversion)        | 95        | 100.0%        | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | del    | del    | CDS    |     |
| C   | 26321 | 26320 | 1-G → C      | SNP (transversion)        | 92        | 100.0%        | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | del    | del    | CDS    |     |
| CTT | 26342 | 26345 | 3-GCTA → CTT | Substitution              | 79 → 82   | 100.0%        | 42,284 Variants: BR-C-10577-T156873.8 | Concatemer BR-C-11057-UK48 | del    | del    | CDS    |     |
| G   | 26348 | 26348 | 1-T → G      | SNP (transversion)        | 78        | 100.0%        | 2,546 Variants: BR-C-10577-T156873.8  | Concatemer BR-C-11057-UK48 | del    | del    | CDS    |     |
| A   | 26348 | 26348 | 1-T → A      | SNP (transversion)        | 91        | 100.0%        | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | del    | del    | CDS    |     |
| T   | 27838 | 27838 | 1-C → T      | SNP (transversion)        | 119       | 100.0%        | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | del    | del    | CDS    |     |
| T   | 27892 | 27893 | 1-C → T      | SNP (transversion)        | 101       | 100.0%        | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | del    | del    | CDS    |     |
| C   | 27940 | 27940 | 1-T → C      | SNP (transversion)        | 95        | 97.9%         | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | del    | del    | CDS    |     |
| C   | 28003 | 28003 | 1-T → C      | SNP (transversion)        | 74        | 100.0%        | 1,462 Variants: BR-C-10577-T156873.8  | Concatemer BR-C-11057-UK48 | del    | del    | CDS    |     |
| T   | 28010 | 28011 | 1-T → C      | SNP (transversion)        | 87        | 100.0%        | 6,382 Variants: BR-C-10577-T156873.8  | Concatemer BR-C-11057-UK48 | del    | del    | CDS    |     |
| A   | 28016 | 28016 | 1-T → A      | SNP (transversion)        | 92        | 100.0%        | 3,322 Variants: BR-C-10577-T156873.8  | Concatemer BR-C-11057-UK48 | del    | del    | CDS    |     |
| A   | 28024 | 28024 | 1-G → A      | SNP (transversion)        | 86        | 100.0%        | 6,382 Variants: BR-C-10577-T156873.8  | Concatemer BR-C-11057-UK48 | del    | del    | CDS    |     |
| GG  | 28178 | 28178 | 2-AA → GG    | Substitution              | 103       | 100.0%        | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | del    | del    | CDS    |     |
| T   | 28213 | 28213 | 1-T → C      | SNP (transversion)        | 101       | 100.0%        | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | del    | del    | CDS    |     |
| T   | 28216 | 28216 | 1-C → T      | SNP (transversion)        | 109       | 100.0%        | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | del    | del    | CDS    |     |
| T   | 28217 | 28217 | 1-G → T      | SNP (transversion)        | 112       | 100.0%        | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | del    | del    | CDS    |     |
| T   | 28740 | 28740 | 1-T → C      | SNP (transversion)        | 109       | 100.0%        | 0 Variants: BR-C-10577-T156873.8      | Concatemer BR-C-11057-UK48 | del    | del    | CDS    |     |
|     |       |       |              |                           |           |               |                                       |                            |        |        |        |     |













# 9 Appendix

| Category | Accession ID | Gene ID | Gene Name   | Gene Type         | Gene Length (bp) | GC Content (%) | Exons     | Introns                              | Transcript(s)                  | Protein(s) | Notes    |
|----------|--------------|---------|-------------|-------------------|------------------|----------------|-----------|--------------------------------------|--------------------------------|------------|----------|
| G        | 104202       | 104201  | 1 T → G     | SNP (StrandSense) | 67               | 100.0%         | 6,365,241 | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            |          |
| AG       | 104252       | 104221  | 2TA → AG    | Substitution      | 61 → 71          | 100.0%         | 25,225    | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            |          |
| T        | 104291       | 104291  | 1A → T      | SNP (StrandSense) | 77               | 100.0%         | 1,362,284 | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            |          |
| A        | 104296       | 104296  | 1G → A      | SNP (StrandSense) | 76               | 98.7%          | 21,296    | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            |          |
| A        | 104298       | 104298  | 1G → A      | SNP (StrandSense) | 77               | 100.0%         | 1,362,284 | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            |          |
| G        | 104282       | 104282  | 1A → G      | SNP (StrandSense) | 76               | 98.7%          | 7,062,268 | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            |          |
| A        | 104284       | 104284  | 1C → A      | SNP (StrandSense) | 67               | 98.3%          | 4,262,242 | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            |          |
| C        | 104262       | 104262  | 1T → C      | SNP (StrandSense) | 59               | 98.3%          | 1,521,212 | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            |          |
| GI       | 104265       | 104265  | 2AA → GI    | Substitution      | 98               | 98.3%          | 9,381,207 | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            |          |
| ACAG     | 104264       | 104263  | 4TGA → ACAG | Substitution      | 53 → 58          | 98.6%          | 2,461,187 | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            |          |
| G        | 104269       | 104269  | 0 → G       | Inversion         | 48               | 99.3%          | 7,132,187 | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            |          |
| A        | 104287       | 104287  | 1T → A      | SNP (StrandSense) | 47               | 97.9%          | 31,188    | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            |          |
| T        | 104269       | 104269  | 2A → T      | SNP (StrandSense) | 51               | 82.4%          | 42,123    | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            |          |
| C        | 104262       | 104262  | 1T → C      | SNP (StrandSense) | 50               | 80.9%          | 11,134    | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            |          |
| C        | 104279       | 104279  | 1T → C      | SNP (StrandSense) | 56               | 94.2%          | 2,361,191 | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            |          |
| AC       | 104276       | 104275  | 2GA → AC    | Substitution      | 55               | 98.7%          | 3,791,198 | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            |          |
| C        | 104278       | 104278  | 1T → C      | SNP (StrandSense) | 68               | 98.6%          | 11,203    | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            |          |
| T        | 104278       | 104278  | 1C → T      | SNP (StrandSense) | 58               | 98.3%          | 7,381,109 | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            |          |
| AC       | 104278       | 104278  | 2GT → AC    | Substitution      | 62               | 100.0%         | 45,129    | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 | N → S      | chrA CDS |
| A        | 104279       | 104279  | 1G → A      | SNP (StrandSense) | 65               | 100.0%         | 3,242,240 | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            | chrA CDS |
| G        | 104276       | 104276  | 1A → G      | SNP (StrandSense) | 65               | 98.3%          | 11,148    | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            | chrA CDS |
| T        | 104280       | 104281  | 1C → T      | SNP (StrandSense) | 77               | 100.0%         | 41,246    | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            | M → I    |
| A        | 104282       | 104282  | 1G → A      | SNP (StrandSense) | 67               | 100.0%         | 3,362,247 | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            | chrA CDS |
| G        | 104282       | 104283  | 1A → G      | SNP (StrandSense) | 67               | 100.0%         | 13,247    | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            | chrA CDS |
| A        | 104283       | 104283  | 1G → A      | SNP (StrandSense) | 67               | 100.0%         | 6,362,241 | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            | chrA CDS |
| G        | 104281       | 104281  | 1T → G      | SNP (StrandSense) | 63               | 100.0%         | 1,621,226 | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            | chrA CDS |
| AG       | 104282       | 104282  | 2GE → AG    | Substitution      | 77               | 100.0%         | 6,362,241 | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            | S → T    |
| GCCT     | 104284       | 104285  | 2TCC → GCCT | Substitution      | 76 → 77          | 100.0%         | 11,208    | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            | chrA CDS |
| T        | 104283       | 104283  | 1C → T      | SNP (StrandSense) | 67               | 100.0%         | 3,362,247 | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            | S → N    |
| GS       | 104285       | 104287  | 2GCG → GS   | Substitution      | 66 → 67          | 100.0%         | 6,362,241 | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            | A → G    |
| A        | 104286       | 104286  | 1G → A      | SNP (StrandSense) | 83               | 98.8%          | 5,291,293 | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            | chrA CDS |
| A        | 104289       | 104289  | 1T → A      | SNP (StrandSense) | 84               | 98.8%          | 6,176,185 | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            | N → D    |
| G        | 104290       | 104290  | 1C → G      | SNP (StrandSense) | 82               | 98.8%          | 3,791,198 | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            | chrA CDS |
| A        | 104290       | 104290  | 1C → A      | SNP (StrandSense) | 82               | 98.8%          | 1,842,297 | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            | D → N    |
| A        | 104292       | 104292  | 1A → A      | SNP (StrandSense) | 93               | 98.9%          | 0         | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            | chrA CDS |
| A        | 104292       | 104293  | 1T → A      | SNP (StrandSense) | 101              | 99.0%          | 0         | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            | chrA CDS |
| A        | 104290       | 104290  | 1G → A      | SNP (StrandSense) | 99               | 99.0%          | 0         | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            | chrA CDS |
| A        | 104296       | 104296  | 1G → A      | SNP (StrandSense) | 99               | 99.0%          | 0         | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            | chrA CDS |
| A        | 104290       | 104290  | 1G → A      | SNP (StrandSense) | 110              | 99.1%          | 0         | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            | chrA CDS |
| T        | 104290       | 104290  | 1T → T      | SNP (StrandSense) | 102              | 99.0%          | 0         | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            | chrA CDS |
| T        | 104300       | 104300  | 1G → T      | SNP (StrandSense) | 95               | 98.9%          | 0         | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            | chrA CDS |
| C        | 104307       | 104307  | 1T → C      | SNP (StrandSense) | 77               | 100.0%         | 6,362,277 | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            | chrA CDS |
| C        | 104300       | 104300  | 1G → C      | SNP (StrandSense) | 78               | 100.0%         | 1,621,226 | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            | chrA CDS |
| A        | 104309       | 104309  | 1G → A      | SNP (StrandSense) | 76               | 100.0%         | 6,362,277 | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            | chrA CDS |
| A        | 104309       | 104309  | 1G → A      | SNP (StrandSense) | 76               | 100.0%         | 6,362,277 | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            | chrA CDS |
| TC       | 104326       | 104326  | 2T → TC     | Substitution      | 72               | 98.7%          | 7,381,109 | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            | chrA CDS |
| G        | 104321       | 104321  | 1A → G      | SNP (StrandSense) | 82               | 100.0%         | 1,842,297 | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            | N → R    |
| G        | 104321       | 104321  | 1A → G      | SNP (StrandSense) | 82               | 100.0%         | 40,303    | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            | F → S    |
| CAA      | 104348       | 104348  | 2AG → CAA   | Substitution      | 105              | 100.0%         | 1,621,226 | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            | L → V    |
| CAA      | 104348       | 104350  | 2AG → CAA   | Substitution      | 111 → 73         | 100.0%         | 21,262    | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            | chrA CDS |
| GCIT     | 104367       | 104368  | 2ATC → GCIT | Substitution      | 104 → 76         | 100.0%         | 1,842,297 | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            | D → N    |
| G        | 104367       | 104368  | 1A → G      | SNP (StrandSense) | 79               | 100.0%         | 6,362,241 | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            | chrA CDS |
| G        | 104372       | 104372  | 1A → G      | SNP (StrandSense) | 81               | 100.0%         | 2,461,187 | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            | chrA CDS |
| G        | 104369       | 104369  | 1A → G      | SNP (StrandSense) | 68               | 100.0%         | 2,152,251 | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            | chrA CDS |
| G        | 104372       | 104371  | 1A → G      | SNP (StrandSense) | 77               | 100.0%         | 3,362,247 | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            | chrA CDS |
| G        | 104372       | 104370  | 1A → G      | SNP (StrandSense) | 77               | 100.0%         | 6,362,277 | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            | chrA CDS |
| A        | 104374       | 104374  | 1A → A      | SNP (StrandSense) | 78               | 98.7%          | 0         | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            | S → N    |
| A        | 104375       | 104375  | 1A → A      | SNP (StrandSense) | 82               | 98.8%          | 2,461,187 | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            | chrA CDS |
| A        | 104376       | 104376  | 1A → A      | SNP (StrandSense) | 82               | 98.8%          | 1,842,297 | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            | chrA CDS |
| C        | 104374       | 104374  | 1T → C      | SNP (StrandSense) | 82               | 100.0%         | 6,362,295 | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            | V → A    |
| CAA      | 104372       | 104374  | 2TGG → CAA  | Substitution      | 79               | 98.7%          | 7,381,109 | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            | chrA CDS |
| A        | 104381       | 104381  | 1A → A      | SNP (StrandSense) | 77               | 100.0%         | 1,621,226 | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            | chrA CDS |
| C        | 104381       | 104381  | 1C → C      | SNP (StrandSense) | 69               | 100.0%         | 5,291,293 | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            | chrA CDS |
| TCG      | 104380       | 104382  | 2AGA → TCG  | Substitution      | 60 → 71          | 98.8%          | 1,842,244 | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            | S → A    |
| A        | 104381       | 104382  | 1G → A      | SNP (StrandSense) | 73               | 100.0%         | 1,842,244 | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            | chrA CDS |
| A        | 104382       | 104382  | 1G → A      | SNP (StrandSense) | 73               | 100.0%         | 1,842,244 | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            | chrA CDS |
| A        | 104381       | 104381  | 1G → A      | SNP (StrandSense) | 85               | 100.0%         | 11,208    | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            | D → N    |
| T        | 104382       | 104382  | 1G → T      | SNP (StrandSense) | 84               | 100.0%         | 1,842,244 | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            | chrA CDS |
| T        | 104393       | 104393  | 1C → T      | SNP (StrandSense) | 82               | 100.0%         | 6,362,295 | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            | chrA CDS |
| G        | 104392       | 104392  | 1A → G      | SNP (StrandSense) | 87               | 100.0%         | 0         | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            | chrA CDS |
| TC       | 104392       | 104394  | 2GCA → TC   | Substitution      | 78               | 98.7%          | 1,621,226 | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            | SS → ST  |
| G        | 104392       | 104392  | 1A → G      | SNP (StrandSense) | 75               | 100.0%         | 12,269    | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            | chrA CDS |
| A        | 104392       | 104393  | 1G → A      | SNP (StrandSense) | 73               | 100.0%         | 7,381,109 | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            | chrA CDS |
| A        | 104396       | 104396  | 1A → A      | SNP (StrandSense) | 65               | 98.3%          | 3,362,247 | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            | chrA CDS |
| C        | 104399       | 104399  | 1T → C      | SNP (StrandSense) | 63               | 100.0%         | 3,791,220 | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            | chrA CDS |
| C        | 104400       | 104400  | 1A → C      | SNP (StrandSense) | 64               | 98.4%          | 9,381,109 | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            | chrA CDS |
| T        | 104402       | 104402  | 1G → T      | SNP (StrandSense) | 69               | 100.0%         | 42,248    | Variables: BRC-CA-11057-TT-15687.4.8 | Concatemer: BRC-CA-11057-UK108 |            |          |

# 9 Appendix

|      |         |         |              |                           |     |        |  |   |   |   |         |
|------|---------|---------|--------------|---------------------------|-----|--------|--|---|---|---|---------|
| T    | 1145178 | 1145274 | 1 C → T      | SNP (transversion)        | 62  | 100.0% | 6,36-223 Variants: BRC-CA-11057-T156874.4  | Concatermer: BRC-CA-11057-UK188         |   |   |         |
| G    | 1121877 | 1121877 | 1 A → G      | SNP (transition)          | 108 | 100.0% | 4E-29 Variants: BRC-CA-11057-T156874.4     | Concatermer: BRC-CA-11057-UK188         |   | hypothetical protein CDS                |         |
| T    | 1122097 | 1122097 | 1 TTT → TTG  | Deletion ( tandem repeat) | 92  | 98.0%  | 0.0000024 Variants: BRC-CA-11057-T156874.4 | Concatermer: BRC-CA-11057-UK188         |   |   |         |
| T    | 1122111 | 1122111 | 2 C → G      | Substitution              | 77  | → 82   | 94.8% Variants: BRC-CA-11057-T156874.4     | Concatermer: BRC-CA-11057-UK188         |   |   |         |
| A    | 1127235 | 1127235 | 1 C → A      | SNP (transversion)        | 75  | 100.0% | 3.71-27 Variants: BRC-CA-11057-T156874.4   | Concatermer: BRC-CA-11057-UK188         |   |   |         |
| C    | 1127269 | 1127269 | 1 A → C      | SNP (transversion)        | 81  | 100.0% | 34-24 Variants: BRC-CA-11057-T156874.4     | Concatermer: BRC-CA-11057-UK188         |   |   |         |
| C    | 1127821 | 1127821 | 1 A → C      | SNP (transversion)        | 97  | 100.0% | 7.99-29 Variants: BRC-CA-11057-T156874.4   | Concatermer: BRC-CA-11057-UK188         |   |   |         |
| C    | 1128407 | 1128407 | 1 T → C      | SNP (transversion)        | 68  | 98.3%  | 2.71-18 Variants: BRC-CA-11057-T156874.4   | Concatermer: BRC-CA-11057-UK188         |   |   |         |
| G    | 1127811 | 1127825 | 1 A → G      | SNP (transversion)        | 97  | 100.0% | 6.35-29 Variants: BRC-CA-11057-T156874.4   | Concatermer: BRC-CA-11057-UK188         |   |   |         |
| G    | 1127896 | 1127896 | 1 A → G      | SNP (transversion)        | 64  | 96.3%  | 5.11-28 Variants: BRC-CA-11057-T156874.4   | Concatermer: BRC-CA-11057-UK188         |   |   |         |
| T    | 1127911 | 1127911 | 1 C → T      | SNP (transversion)        | 40  | 97.5%  | 1.64-29 Variants: BRC-CA-11057-T156874.4   | Concatermer: BRC-CA-11057-UK188         |   |   |         |
| T    | 112974  | 1129741 | 1 G → T      | SNP (transversion)        | 91  | 100.0% | 0 Variants: BRC-CA-11057-T156874.4         | Concatermer: BRC-CA-11057-UK188         |   | A → D                                   |         |
| A    | 1129562 | 1129562 | 1 G → A      | SNP (transversion)        | 78  | 100.0% | 1.82-280 Variants: BRC-CA-11057-T156874.4  | Concatermer: BRC-CA-11057-UK188         |   | Methyl-accepting chemotaxis protein CDS |         |
| T    | 1129771 | 1129774 | 1 C → T      | SNP (transversion)        | 65  | 100.0% | 7.52-207 Variants: BRC-CA-11057-T156874.4  | Concatermer: BRC-CA-11057-UK188         |   | Methyl-accepting chemotaxis protein CDS |         |
| A    | 1130824 | 1130823 | 1 G → A      | SNP (transversion)        | 87  | 100.0% | 0 Variants: BRC-CA-11057-T156874.4         | Concatermer: BRC-CA-11057-UK188         |   | C → K                                   |         |
| G    | 1130927 | 1130927 | 1 A → G      | SNP (transversion)        | 76  | 100.0% | 2.52-271 Variants: BRC-CA-11057-T156874.4  | Concatermer: BRC-CA-11057-UK188         |   | PfM7 2 domain-containing protein CDS    |         |
| A    | 1134029 | 1134030 | 1 T → A      | SNP (transversion)        | 87  | 98.9%  | 6.79-299 Variants: BRC-CA-11057-T156874.4  | Concatermer: BRC-CA-11057-UK188         |   | PfM7 2 domain-containing protein CDS    |         |
| T    | 1134028 | 1134029 | 1 C → T      | SNP (transversion)        | 78  | 100.0% | 1.82-280 Variants: BRC-CA-11057-T156874.4  | Concatermer: BRC-CA-11057-UK188         |   | sup CDS                                 |         |
| C    | 1134029 | 1134030 | 1 T → C      | SNP (transversion)        | 78  | 98.9%  | 2.45-257 Variants: BRC-CA-11057-T156874.4  | Concatermer: BRC-CA-11057-UK188         |   | sup CDS                                 |         |
| A    | 1134035 | 1134035 | 1 G → A      | SNP (transversion)        | 87  | 100.0% | 0 Variants: BRC-CA-11057-T156874.4         | Concatermer: BRC-CA-11057-UK188         |   | sup CDS                                 |         |
| A    | 1134034 | 1134034 | 1 A → A      | SNP (transversion)        | 82  | 100.0% | 0 Variants: BRC-CA-11057-T156874.4         | Concatermer: BRC-CA-11057-UK188         |   | sup CDS                                 |         |
| A    | 1134047 | 1134047 | 1 G → A      | SNP (transversion)        | 83  | 100.0% | 7.85-303 Variants: BRC-CA-11057-T156874.4  | Concatermer: BRC-CA-11057-UK188         |   | sup CDS                                 |         |
| T    | 1134051 | 1134051 | 1 A → T      | SNP (transversion)        | 84  | 100.0% | 0 Variants: BRC-CA-11057-T156874.4         | Concatermer: BRC-CA-11057-UK188         |   | sup CDS                                 |         |
| CTT  | 1134057 | 1134059 | 3 GCA → CTT  | Substitution              | 86  | 100.0% | 0 Variants: BRC-CA-11057-T156874.4         | Concatermer: BRC-CA-11057-UK188         |   | sup CDS                                 |         |
| T    | 1134067 | 1134067 | 1 T → G      | SNP (transversion)        | 78  | 100.0% | 1.64-280 Variants: BRC-CA-11057-T156874.4  | Concatermer: BRC-CA-11057-UK188         |   | N → H                                   |         |
| G    | 1134071 | 1134071 | 1 A → G      | SNP (transversion)        | 80  | 100.0% | 10-287 Variants: BRC-CA-11057-T156874.4    | Concatermer: BRC-CA-11057-UK188         |   | sup CDS                                 |         |
| T    | 1134085 | 1134085 | 1 A → T      | SNP (transversion)        | 82  | 100.0% | 41-303 Variants: BRC-CA-11057-T156874.4    | Concatermer: BRC-CA-11057-UK188         |   | sup CDS                                 |         |
| T    | 1134081 | 1134081 | 1 C → T      | SNP (transversion)        | 82  | 100.0% | 41-303 Variants: BRC-CA-11057-T156874.4    | Concatermer: BRC-CA-11057-UK188         |   | sup CDS                                 |         |
| ACT1 | 1134089 | 1134091 | 4 TAC → ACT1 | Substitution              | 83  | → 86   | 96.7% Variants: BRC-CA-11057-T156874.4     | Concatermer: BRC-CA-11057-UK188         |   | sup CDS                                 |         |
| T    | 1134098 | 1134098 | 1 T → C      | SNP (transversion)        | 77  | 100.0% | 6.35-272 Variants: BRC-CA-11057-T156874.4  | Concatermer: BRC-CA-11057-UK188         |   | sup CDS                                 |         |
| C    | 1134110 | 1134110 | 1 T → C      | SNP (transversion)        | 88  | 100.0% | 15-300 Variants: BRC-CA-11057-T156874.4    | Concatermer: BRC-CA-11057-UK188         |   | sup CDS                                 |         |
| C    | 1134115 | 1134115 | 1 T → C      | SNP (transversion)        | 82  | 100.0% | 21-298 Variants: BRC-CA-11057-T156874.4    | Concatermer: BRC-CA-11057-UK188         |   | sup CDS                                 |         |
| G    | 1134122 | 1134122 | 1 A → G      | SNP (transversion)        | 78  | 100.0% | 2.52-288 Variants: BRC-CA-11057-T156874.4  | Concatermer: BRC-CA-11057-UK188         |   | sup CDS                                 |         |
| T    | 1134128 | 1134128 | 1 G → A      | SNP (transversion)        | 79  | 100.0% | 51-291 Variants: BRC-CA-11057-T156874.4    | Concatermer: BRC-CA-11057-UK188         |   | sup CDS                                 |         |
| T    | 1134141 | 1134140 | 1 G → T      | SNP (transversion)        | 81  | 100.0% | 6.35-272 Variants: BRC-CA-11057-T156874.4  | Concatermer: BRC-CA-11057-UK188         |   | sup CDS                                 |         |
| A    | 1134254 | 1134254 | 1 C → T      | SNP (transversion)        | 57  | 100.0% | 6.35-209 Variants: BRC-CA-11057-T156874.4  | Concatermer: BRC-CA-11057-UK188         |   | sup CDS                                 |         |
| A    | 1134235 | 1134235 | 1 G → A      | SNP (transversion)        | 58  | 100.0% | 2.52-214 Variants: BRC-CA-11057-T156874.4  | Concatermer: BRC-CA-11057-UK188         |   | sup CDS                                 |         |
| G    | 1134260 | 1134260 | 1 A → G      | SNP (transversion)        | 55  | 100.0% | 3.25-203 Variants: BRC-CA-11057-T156874.4  | Concatermer: BRC-CA-11057-UK188         |   | sup CDS                                 |         |
| TAG  | 1134287 | 1134288 | 2 CAA → TAG  | Substitution              | 55  | 100.0% | 11-197 Variants: BRC-CA-11057-T156874.4    | Concatermer: BRC-CA-11057-UK188         |   | sup CDS                                 |         |
| A    | 1134293 | 1134293 | 1 C → A      | SNP (transversion)        | 64  | 100.0% | 4.42-216 Variants: BRC-CA-11057-T156874.4  | Concatermer: BRC-CA-11057-UK188         |   | sup CDS                                 |         |
| AMC  | 1134296 | 1134299 | 3 GAA → AMC  | Substitution              | 65  | → 86   | 98.3%                                      | 11-213 Variants: BRC-CA-11057-T156874.4 | Concatermer: BRC-CA-11057-UK188           |   | sup CDS |
| T    | 1134305 | 1134305 | 1 T → C      | SNP (transversion)        | 60  | 100.0% | 11-207 Variants: BRC-CA-11057-T156874.4    | Concatermer: BRC-CA-11057-UK188         |   | sup CDS                                 |         |
| G    | 1134311 | 1134311 | 1 A → G      | SNP (transversion)        | 60  | 100.0% | 41-248 Variants: BRC-CA-11057-T156874.4    | Concatermer: BRC-CA-11057-UK188         |   | sup CDS                                 |         |
| G    | 1134317 | 1134317 | 1 T → G      | SNP (transversion)        | 68  | 100.0% | 11-217 Variants: BRC-CA-11057-T156874.4    | Concatermer: BRC-CA-11057-UK188         |   | sup CDS                                 |         |
| T    | 1134341 | 1134341 | 1 C → T      | SNP (transversion)        | 64  | 100.0% | 11-216 Variants: BRC-CA-11057-T156874.4    | Concatermer: BRC-CA-11057-UK188         |   | sup CDS                                 |         |
| A    | 1134354 | 1134354 | 1 G → A      | SNP (transversion)        | 63  | 100.0% | 11-219 Variants: BRC-CA-11057-T156874.4    | Concatermer: BRC-CA-11057-UK188         |   | sup CDS                                 |         |
| G    | 1134359 | 1134359 | 1 T → G      | SNP (transversion)        | 64  | 100.0% | 11-218 Variants: BRC-CA-11057-T156874.4    | Concatermer: BRC-CA-11057-UK188         |   | sup CDS                                 |         |
| G    | 1134380 | 1134380 | 1 A → G      | SNP (transversion)        | 54  | 100.0% | 1.64-199 Variants: BRC-CA-11057-T156874.4  | Concatermer: BRC-CA-11057-UK188         |   | sup CDS                                 |         |
| G    | 1134401 | 1134401 | 1 C → G      | SNP (transversion)        | 58  | 100.0% | 1.64-208 Variants: BRC-CA-11057-T156874.4  | Concatermer: BRC-CA-11057-UK188         |   | sup CDS                                 |         |
| C    | 1134407 | 1134407 | 1 T → C      | SNP (transversion)        | 64  | 100.0% | 1.64-216 Variants: BRC-CA-11057-T156874.4  | Concatermer: BRC-CA-11057-UK188         |   | sup CDS                                 |         |
| C    | 1134448 | 1134448 | 1 T → C      | SNP (transversion)        | 66  | 100.0% | 11-210 Variants: BRC-CA-11057-T156874.4    | Concatermer: BRC-CA-11057-UK188         |   | sup CDS                                 |         |
| G    | 1134458 | 1134458 | 1 A → G      | SNP (transversion)        | 60  | 100.0% | 1.64-224 Variants: BRC-CA-11057-T156874.4  | Concatermer: BRC-CA-11057-UK188         |   | sup CDS                                 |         |
| G    | 1134519 | 1134519 | 1 A → G      | SNP (transversion)        | 77  | 100.0% | 3.25-269 Variants: BRC-CA-11057-T156874.4  | Concatermer: BRC-CA-11057-UK188         |   | sup CDS                                 |         |
| G    | 1134542 | 1134542 | 1 C → G      | SNP (transversion)        | 74  | 100.0% | 41-288 Variants: BRC-CA-11057-T156874.4    | Concatermer: BRC-CA-11057-UK188         |   | sup CDS                                 |         |
| G    | 1134545 | 1134545 | 1 C → G      | SNP (transversion)        | 72  | 100.0% | 6.35-259 Variants: BRC-CA-11057-T156874.4  | Concatermer: BRC-CA-11057-UK188         |   | sup CDS                                 |         |
| A    | 1134551 | 1134551 | 1 G → A      | SNP (transversion)        | 77  | 100.0% | 3.25-269 Variants: BRC-CA-11057-T156874.4  | Concatermer: BRC-CA-11057-UK188         |   | sup CDS                                 |         |
| T    | 1134557 | 1134557 | 1 C → T      | SNP (transversion)        | 75  | 100.0% | 11-209 Variants: BRC-CA-11057-T156874.4    | Concatermer: BRC-CA-11057-UK188         |   | sup CDS                                 |         |
| T    | 1134591 | 1134591 | 1 T → C      | SNP (transversion)        | 62  | 100.0% | 11-214 Variants: BRC-CA-11057-T156874.4    | Concatermer: BRC-CA-11057-UK188         |   | sup CDS                                 |         |
| C    | 1134581 | 1134581 | 1 G → C      | SNP (transversion)        | 47  | 100.0% | 41-176 Variants: BRC-CA-11057-T156874.4    | Concatermer: BRC-CA-11057-UK188         |   | sup CDS                                 |         |
| C    | 1134587 | 1134587 | 1 T → C      | SNP (transversion)        | 49  | 100.0% | 1.64-184 Variants: BRC-CA-11057-T156874.4  | Concatermer: BRC-CA-11057-UK188         |   | sup CDS                                 |         |
| GT   | 1134667 | 1134668 | 2 AC → GT    | Substitution              | 49  | → 56   | 100.0%                                     | 51-181 Variants: BRC-CA-11057-T156874.4 | Concatermer: BRC-CA-11057-UK188           |   | sup CDS |
| T    | 1134671 | 1134671 | 1 A → T      | SNP (transversion)        | 56  | 100.0% | 2.52-201 Variants: BRC-CA-11057-T156874.4  | Concatermer: BRC-CA-11057-UK188         |   | sup CDS                                 |         |
| GG   | 1134728 | 1134729 | 2 TA → GG    | Substitution              | 64  | 100.0% | 64   | 100.0%                                  | 2.52-201 Variants: BRC-CA-11057-T156874.4 | Concatermer: BRC-CA-11057-UK188         | I → T   |
| T    | 1134792 | 1134793 | 1 T → C      | SNP (transversion)        | 63  | 100.0% | 3.25-200 Variants: BRC-CA-11057-T156874.4  | Concatermer: BRC-CA-11057-UK188         |   | sup CDS                                 |         |
| T    | 1134845 | 1134845 | 1 T → C      | SNP (transversion)        | 65  | 100.0% | 6.35-223 Variants: BRC-CA-11057-T156874.4  | Concatermer: BRC-CA-11057-UK188         |   | sup CDS                                 |         |
| T    | 1134857 | 1134857 | 1 C → T      | SNP (transversion)        | 61  | 100.0% | 25-225 Variants: BRC-CA-11057-T156874.4    | Concatermer: BRC-CA-11057-UK188         |   | sup CDS                                 |         |
| A    | 1134875 | 1134875 | 1 G → A      | SNP (transversion)        | 62  | 100.0% | 6.35-223 Variants: BRC-CA-11057-T156874.4  | Concatermer: BRC-CA-11057-UK188         |   | sup CDS                                 |         |
| A    | 1134878 | 1134878 | 1 C → A      | SNP (transversion)        | 62  | 100.0% | 6.35-223 Variants: BRC-CA-11057-T156874.4  | Concatermer: BRC-CA-11057-UK188         |   | sup CDS                                 |         |
| T    | 1134889 | 1134889 | 1 A → T      | SNP (transversion)        | 57  | 100.0% | 6.35-205 Variants: BRC-CA-11057-T156874.4  | Concatermer: BRC-CA-11057-UK188         |   | S → D                                   |         |
| C    | 1134918 | 1134918 | 1 T → C      | SNP (transversion)        | 61  | 100.0% | 11-213 Variants: BRC-CA-11057-T156874.4    | Concatermer: BRC-CA-11057-UK188         |   | sup CDS                                 |         |
| C    | 1134908 | 1134908 | 1 T → C      | SNP (transversion)        | 63  | 100.0% | 3.25-213 Variants: BRC-CA-11057-T156874.4  | Concatermer: BRC-CA-11057-UK188         |   | sup CDS                                 |         |
| C    | 1134928 | 1134928 | 1 G → C      | SNP (transversion)        | 54  | 100.0% | 41-194 Variants: BRC-CA-11057-T156874.4    | Concatermer: BRC-CA-11057-UK188         |   | sup CDS                                 |         |
| A    | 1134971 | 1134971 | 1 G → A      | SNP (transversion)        | 65  | 100.0% | 10-287 Variants: BRC-CA-11057-T156874.4    | Concatermer: BRC-CA-11057-UK188         |   | sup CDS                                 |         |
| C    | 1135028 | 1135028 | 1 T → C      | SNP (transversion)        | 63  | 100.0% | 7.83-233 Variants: BRC-CA-11057-T156874.4  | Concatermer: BRC-CA-11057-UK188         |   | sup CDS                                 |         |
| C    | 1135040 | 1135040 | 1 T → C      | SNP (transversion)        | 61  | 100.0% | 2.52-219 Variants: BRC-CA-11057-T156874.4  | Concatermer: BRC-CA-11057-UK188         |   | sup CDS                                 |         |
| C    | 1135058 | 1135058 | 1 T → C      | SNP (transversion)        | 63  | 100.0% | 11-213 Variants: BRC-CA-11057-T156874.4    | Concatermer: BRC-CA-11057-UK188         |   | sup CDS                                 |         |
| C    | 1135081 | 1135081 | 1 T → C      | SNP (transversion)        | 62  | 100.0% | 6.35-223 Variants: BRC-CA-11057-T156874.4  | Concatermer: BRC-CA-11057-UK188         |   | sup CDS                                 |         |
| C    | 1135084 | 1135084 | 1 T → C      | SNP (transversion)        | 61  | 100.0% | 11-213 Variants: BRC-CA-11057-T156874.4    | Concatermer: BRC-CA-11057-UK188         |   | sup CDS                                 |         |
| T    | 1135091 | 1135091 | 1 C → T      | SNP (transversion)        | 63  | 100.0% | 1.64-228 Variants: BRC-CA-11057-T156874.4  | Concatermer: BRC-CA-11057-UK188         |   | sup CDS                                 |         |
| T    | 1135206 | 1135206 | 1 G → T      | SNP (transversion)        | 45  | 100.0% | 3.25-166 Variants: BRC-CA-11057-T156874.4  | Concatermer: BRC-CA-11057-UK188         |   | sup CDS                                 |         |
| A    |         |         |              |                           |     |        |  |   |   |   |         |



# 9 Appendix

| Category | Accession | Gene  | Strand      | Start                   | End       | Score  | Percentage | Variables                        | Concatenation               | Notes   |
|----------|-----------|-------|-------------|-------------------------|-----------|--------|------------|----------------------------------|-----------------------------|---|
| C        | 17389     | 17383 | 1 T → C     | SNP (transversion)      | 94        | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | N → E   |
| G        | 17899     | 17889 | 1 A → G     | SNP (transversion)      | 134       | 99.3%  | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# |   |
| T        | 18297     | 18297 | 1 C → T     | SNP (transversion)      | 134       | 97.8%  | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | Deus/ferrodoxin FeS4 iron-binding domain containing pro |
| T        | 18172     | 18172 | 1 C → T     | SNP (transversion)      | 127       | 98.5%  | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# |   |
| A        | 19670     | 19670 | 1 G → A     | SNP (transversion)      | 115       | 99.1%  | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | invr CDS  |
| A        | 20611     | 20611 | 1 G → A     | SNP (transversion)      | 120       | 99.2%  | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| A        | 20614     | 20614 | 1 T → A     | SNP (transversion)      | 123       | 98.4%  | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| G        | 20635     | 20635 | 1 T → G     | SNP (transversion)      | 128       | 99.2%  | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| A        | 20638     | 20638 | 1 G → A     | SNP (transversion)      | 128       | 99.2%  | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| A        | 20644     | 20644 | 1 G → A     | SNP (transversion)      | 118       | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| C        | 20646     | 20646 | 1 T → C     | SNP (transversion)      | 111       | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| T        | 20650     | 20650 | 1 A → T     | SNP (transversion)      | 105       | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| CAA      | 20662     | 20664 | 2 TAG → CAA | Substitution            | 106 → 107 | 99.1%  | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| C        | 20674     | 20674 | 1 T → C     | SNP (transversion)      | 104       | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| A        | 20680     | 20680 | 1 G → A     | SNP (transversion)      | 101       | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| A        | 20692     | 20693 | 1 G → A     | SNP (transversion)      | 108       | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| C        | 20695     | 20695 | 1 T → C     | SNP (transversion)      | 107       | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| C        | 20709     | 20709 | 1 T → C     | SNP (transversion)      | 107       | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | N → D   |
| C        | 20718     | 20718 | 1 T → C     | SNP (transversion)      | 108       | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| T        | 20725     | 20725 | 1 A → T     | SNP (transversion)      | 108       | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| G        | 20731     | 20731 | 1 A → G     | SNP (transversion)      | 105       | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| C        | 20732     | 20732 | 1 T → C     | SNP (transversion)      | 110       | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| C        | 20776     | 20776 | 1 A → C     | SNP (transversion)      | 96        | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| G        | 20779     | 20779 | 1 A → G     | SNP (transversion)      | 96        | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| T        | 20785     | 20785 | 1 A → T     | SNP (transversion)      | 100       | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| A        | 20797     | 20797 | 1 G → A     | SNP (transversion)      | 107       | 99.1%  | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| T        | 20812     | 20812 | 1 C → T     | SNP (transversion)      | 96        | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| T        | 20821     | 20821 | 1 A → T     | SNP (transversion)      | 98        | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| T        | 20830     | 20830 | 1 C → T     | SNP (transversion)      | 100       | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| ANG      | 20838     | 20838 | 2 TAA → AAG | Substitution            | 104 → 105 | 97.1%  | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| A        | 20842     | 20842 | 1 G → A     | SNP (transversion)      | 107       | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| C        | 20845     | 20845 | 1 T → C     | SNP (transversion)      | 105       | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| C        | 20851     | 20851 | 1 T → C     | SNP (transversion)      | 104       | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| T        | 20854     | 20854 | 1 G → T     | SNP (transversion)      | 103       | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| TAA      | 20864     | 20868 | 2 AAC → TAA | Substitution            | 106 → 108 | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| A        | 20872     | 20872 | 1 G → A     | SNP (transversion)      | 105       | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| C        | 20890     | 20890 | 1 T → C     | SNP (transversion)      | 111       | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| C        | 20893     | 20893 | 1 T → C     | SNP (transversion)      | 118       | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| G        | 20899     | 20899 | 1 A → G     | SNP (transversion)      | 125       | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| C        | 20908     | 20908 | 1 T → C     | SNP (transversion)      | 127       | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| T        | 20917     | 20917 | 1 A → T     | SNP (transversion)      | 112       | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | D → F   |
| A        | 20920     | 20920 | 1 G → A     | SNP (transversion)      | 111       | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| G        | 20926     | 20926 | 1 A → G     | SNP (transversion)      | 117       | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| A        | 20932     | 20932 | 1 G → A     | SNP (transversion)      | 117       | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| A        | 20941     | 20941 | 1 G → A     | SNP (transversion)      | 116       | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| A        | 21001     | 21001 | 1 G → A     | SNP (transversion)      | 116       | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| T        | 21002     | 21002 | 1 C → T     | SNP (transversion)      | 111       | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| A        | 21007     | 21007 | 1 T → A     | SNP (transversion)      | 117       | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| T        | 21010     | 21010 | 1 A → T     | SNP (transversion)      | 111       | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| A        | 21034     | 21034 | 1 G → A     | SNP (transversion)      | 129       | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| AGC      | 21040     | 21042 | 2 GGT → AGC | Substitution            | 109 → 119 | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | T → A   |
| A        | 21046     | 21046 | 1 G → A     | SNP (transversion)      | 117       | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| C        | 21049     | 21049 | 1 T → C     | SNP (transversion)      | 119       | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| A        | 21054     | 21054 | 1 G → A     | SNP (transversion)      | 117       | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| C        | 21058     | 21058 | 1 T → C     | SNP (transversion)      | 115       | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| G        | 21075     | 21075 | 1 A → G     | SNP (transversion)      | 115       | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| T        | 21079     | 21079 | 1 C → T     | SNP (transversion)      | 112       | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| C        | 21108     | 21108 | 1 T → C     | SNP (transversion)      | 112       | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| A        | 21118     | 21118 | 1 A → G     | SNP (transversion)      | 109       | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| A        | 21136     | 21136 | 1 C → A     | SNP (transversion)      | 106       | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| A        | 21139     | 21139 | 1 G → A     | SNP (transversion)      | 106       | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| A        | 21142     | 21142 | 1 T → A     | SNP (transversion)      | 104       | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| A        | 21154     | 21154 | 1 G → A     | SNP (transversion)      | 108       | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| C        | 21157     | 21157 | 1 T → C     | SNP (transversion)      | 108       | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| C        | 21172     | 21172 | 1 T → C     | SNP (transversion)      | 124       | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| C        | 21193     | 21193 | 1 T → C     | SNP (transversion)      | 105       | 99.9%  | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| C        | 21196     | 21196 | 1 T → C     | SNP (transversion)      | 107       | 99.1%  | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| A        | 21202     | 21203 | 1 G → A     | SNP (transversion)      | 103       | 98.9%  | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| A        | 21208     | 21208 | 1 G → A     | SNP (transversion)      | 97        | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| C        | 21214     | 21214 | 1 T → C     | SNP (transversion)      | 97        | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| C        | 21226     | 21226 | 1 A → C     | SNP (transversion)      | 94        | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| CA       | 21238     | 21239 | 2 AG → CA   | Substitution            | 102 → 106 | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | A → V   |
| T        | 21244     | 21244 | 1 C → T     | SNP (transversion)      | 124       | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| A        | 21248     | 21248 | 1 T → A     | SNP (transversion)      | 118       | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | M → I   |
| A        | 21271     | 21271 | 1 A → G     | SNP (transversion)      | 116       | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| A        | 21274     | 21274 | 1 G → A     | SNP (transversion)      | 117       | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| A        | 21280     | 21280 | 1 A → G     | SNP (transversion)      | 117       | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| G        | 21283     | 21283 | 1 T → G     | SNP (transversion)      | 112       | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| A        | 21288     | 21288 | 1 C → A     | SNP (transversion)      | 116       | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| T        | 21311     | 21311 | 1 C → T     | SNP (transversion)      | 114       | 99.1%  | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| A        | 21316     | 21316 | 1 G → A     | SNP (transversion)      | 113       | 99.1%  | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| C        | 21319     | 21319 | 1 T → C     | SNP (transversion)      | 114       | 99.1%  | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| T        | 21418     | 21417 | 0 T78 → T17 | Insertion/Tandem repeat | 122       | 93.4%  | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| A        | 21482     | 21482 | 1 G → A     | SNP (transversion)      | 91        | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | quic CDS  |
| A        | 22093     | 22093 | 1 G → A     | SNP (transversion)      | 91        | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | quic CDS  |
| C        | 23173     | 23173 | 1 T → C     | SNP (transversion)      | 144       | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| A        | 23176     | 23176 | 1 G → A     | SNP (transversion)      | 111       | 100.0% | 0          | Variables: BR-C-11057-T15687.5_# | Concatenation: BR-C-11057_# | omniC CDS   |
| T        | 22282     | 22282 | 1 G → T     | SNP (transversion)      |           |        |            |                                  |                             |   |





# 9 Appendix

| Gene | Accession | Accession | Feature      | Start                     | End     | Score  | Method  | Annotation                         | Gene                               | Accession    | Accession    | Feature | Start | End | Score | Method | Annotation | Gene                                       | Accession | Accession | Feature | Start | End | Score | Method | Annotation |
|------|-----------|-----------|--------------|---------------------------|---------|--------|---------|------------------------------------|------------------------------------|--------------|--------------|---------|-------|-----|-------|--------|------------|--|-----------|-----------|---------|-------|-----|-------|--------|------------|
| G    | 1059824   | 1059824   | 1 A → G      | SNP (Strand)              | 118     | 100.0% | 0       | Variables: BRC-CA-11057-T15687.5_8 | Concatermer                        | BRC-CA-11057 | Unkns        |         |       |     |       |        |            | Phyrase-like domain-containing protein CDS |           |           |         |       |     |       |        |            |
| T    | 1059860   | 1059860   | 1 C → T      | SNP (Strand)              | 102     | 100.0% | 0       | Variables: BRC-CA-11057-T15687.5_8 | Concatermer                        | BRC-CA-11057 | Unkns        |         |       |     |       |        |            | Phyrase-like domain-containing protein CDS |           |           |         |       |     |       |        |            |
| C    | 1059870   | 1059870   | 1 G → A      | SNP (Strand)              | 97      | 100.0% | 0       | Variables: BRC-CA-11057-T15687.5_8 | Concatermer                        | BRC-CA-11057 | Unkns        |         |       |     |       |        |            | Phyrase-like domain-containing protein CDS |           |           |         |       |     |       |        |            |
| G    | 1059881   | 1059881   | 1 A → G      | SNP (Strand)              | 96      | 100.0% | 0       | Variables: BRC-CA-11057-T15687.5_8 | Concatermer                        | BRC-CA-11057 | Unkns        |         |       |     |       |        |            | Phyrase-like domain-containing protein CDS |           |           |         |       |     |       |        |            |
| G    | 1059915   | 1059915   | 1 A → G      | SNP (Strand)              | 91      | 98.9%  | 0       | Variables: BRC-CA-11057-T15687.5_8 | Concatermer                        | BRC-CA-11057 | Unkns        |         |       |     |       |        |            | Phyrase-like domain-containing protein CDS |           |           |         |       |     |       |        |            |
| A    | 1059991   | 1059991   | 1 G → A      | SNP (Strand)              | 100     | 100.0% | 0       | Variables: BRC-CA-11057-T15687.5_8 | Concatermer                        | BRC-CA-11057 | Unkns        |         |       |     |       |        |            | Phyrase-like domain-containing protein CDS |           |           |         |       |     |       |        |            |
| C    | 1059972   | 1059972   | 1 A → G      | SNP (Strand)              | 104     | 100.0% | 0       | Variables: BRC-CA-11057-T15687.5_8 | Concatermer                        | BRC-CA-11057 | Unkns        |         |       |     |       |        |            | Phyrase-like domain-containing protein CDS |           |           |         |       |     |       |        |            |
| C    | 1060048   | 1060048   | 1 T → C      | SNP (Strand)              | 101     | 100.0% | 0       | Variables: BRC-CA-11057-T15687.5_8 | Concatermer                        | BRC-CA-11057 | Unkns        |         |       |     |       |        |            | Phyrase-like domain-containing protein CDS |           |           |         |       |     |       |        |            |
| G    | 1060136   | 1060136   | 1 A → G      | SNP (Strand)              | 103     | 99.0%  | 0       | Variables: BRC-CA-11057-T15687.5_8 | Concatermer                        | BRC-CA-11057 | Unkns        |         |       |     |       |        |            | Phyrase-like domain-containing protein CDS |           |           |         |       |     |       |        |            |
| A    | 1060458   | 1060458   | 1 G → A      | SNP (Strand)              | 95      | 100.0% | 0       | Variables: BRC-CA-11057-T15687.5_8 | Concatermer                        | BRC-CA-11057 | Unkns        |         |       |     |       |        |            | Phyrase-like domain-containing protein CDS |           |           |         |       |     |       |        |            |
| T    | 1060491   | 1060491   | 1 G → T      | SNP (Strand)              | 108     | 100.0% | 0       | Variables: BRC-CA-11057-T15687.5_8 | Concatermer                        | BRC-CA-11057 | Unkns        |         |       |     |       |        |            | Phyrase-like domain-containing protein CDS |           |           |         |       |     |       |        |            |
| G    | 1060625   | 1060625   | 1 A → G      | SNP (Strand)              | 98      | 100.0% | 0       | Variables: BRC-CA-11057-T15687.5_8 | Concatermer                        | BRC-CA-11057 | Unkns        |         |       |     |       |        |            | Phyrase-like domain-containing protein CDS |           |           |         |       |     |       |        |            |
| A    | 1060557   | 1060557   | 1 G → A      | SNP (Strand)              | 97      | 100.0% | 0       | Variables: BRC-CA-11057-T15687.5_8 | Concatermer                        | BRC-CA-11057 | Unkns        |         |       |     |       |        |            | Phyrase-like domain-containing protein CDS |           |           |         |       |     |       |        |            |
| G    | 1060596   | 1060596   | 1 A → G      | SNP (Strand)              | 102     | 100.0% | 0       | Variables: BRC-CA-11057-T15687.5_8 | Concatermer                        | BRC-CA-11057 | Unkns        |         |       |     |       |        |            | Phyrase-like domain-containing protein CDS |           |           |         |       |     |       |        |            |
| C    | 1060661   | 1060661   | 1 C → T      | SNP (Strand)              | 95      | 100.0% | 0       | Variables: BRC-CA-11057-T15687.5_8 | Concatermer                        | BRC-CA-11057 | Unkns        |         |       |     |       |        |            | Phyrase-like domain-containing protein CDS |           |           |         |       |     |       |        |            |
| C    | 1061191   | 1061191   | 1 T → C      | SNP (Strand)              | 75      | 100.0% | 0       | Variables: BRC-CA-11057-T15687.5_8 | Concatermer                        | BRC-CA-11057 | Unkns        |         |       |     |       |        |            | Phyrase-like domain-containing protein CDS |           |           |         |       |     |       |        |            |
| A    | 1061196   | 1061196   | 1 G → A      | SNP (Strand)              | 75      | 100.0% | 0       | Variables: BRC-CA-11057-T15687.5_8 | Concatermer                        | BRC-CA-11057 | Unkns        |         |       |     |       |        |            | Phyrase-like domain-containing protein CDS |           |           |         |       |     |       |        |            |
| A    | 1061199   | 1061199   | 1 G → A      | SNP (Strand)              | 88      | 98.9%  | 0       | Variables: BRC-CA-11057-T15687.5_8 | Concatermer                        | BRC-CA-11057 | Unkns        |         |       |     |       |        |            | Phyrase-like domain-containing protein CDS |           |           |         |       |     |       |        |            |
| A    | 1061809   | 1061809   | 1 G → A      | SNP (Strand)              | 103     | 100.0% | 0       | Variables: BRC-CA-11057-T15687.5_8 | Concatermer                        | BRC-CA-11057 | Unkns        |         |       |     |       |        |            | Phyrase-like domain-containing protein CDS |           |           |         |       |     |       |        |            |
| A    | 1061821   | 1061821   | 1 G → A      | SNP (Strand)              | 111     | 100.0% | 0       | Variables: BRC-CA-11057-T15687.5_8 | Concatermer                        | BRC-CA-11057 | Unkns        |         |       |     |       |        |            | Phyrase-like domain-containing protein CDS |           |           |         |       |     |       |        |            |
| C    | 1062232   | 1062232   | 1 T → C      | SNP (Strand)              | 103     | 99.0%  | 0       | Variables: BRC-CA-11057-T15687.5_8 | Concatermer                        | BRC-CA-11057 | Unkns        |         |       |     |       |        |            | Phyrase-like domain-containing protein CDS |           |           |         |       |     |       |        |            |
| G    | 1062365   | 1062365   | 1 A → G      | SNP (Strand)              | 95      | 100.0% | 0       | Variables: BRC-CA-11057-T15687.5_8 | Concatermer                        | BRC-CA-11057 | Unkns        |         |       |     |       |        |            | Phyrase-like domain-containing protein CDS |           |           |         |       |     |       |        |            |
| G    | 1063167   | 1063167   | 1 A → G      | SNP (Strand)              | 70      | 100.0% | 0       | Variables: BRC-CA-11057-T15687.5_8 | Concatermer                        | BRC-CA-11057 | Unkns        |         |       |     |       |        |            | Phyrase-like domain-containing protein CDS |           |           |         |       |     |       |        |            |
| ASC  | 1065021   | 1065021   | 3 GGT → AAG  | Substitution              | 78 → 80 | 97.0%  | → 97.5% | 4.8E-279                           | Variables: BRC-CA-11057-T15687.5_8 | Concatermer  | BRC-CA-11057 | Unkns   |       |     |       |        |            | Phyrase-like domain-containing protein CDS |           |           |         |       |     |       |        |            |
| C    | 1065021   | 1065021   | 1 G → C      | SNP (Strand)              | 82      | 100.0% | 0       | 6.3E-205                           | Variables: BRC-CA-11057-T15687.5_8 | Concatermer  | BRC-CA-11057 | Unkns   |       |     |       |        |            | Phyrase-like domain-containing protein CDS |           |           |         |       |     |       |        |            |
| G    | 1065032   | 1065032   | 1 A → G      | SNP (Strand)              | 82      | 100.0% | 0       | 4E-203                             | Variables: BRC-CA-11057-T15687.5_8 | Concatermer  | BRC-CA-11057 | Unkns   |       |     |       |        |            | Phyrase-like domain-containing protein CDS |           |           |         |       |     |       |        |            |
| G    | 1065684   | 1065684   | 1 A → G      | SNP (Strand)              | 95      | 100.0% | 0       | 0                                  | Variables: BRC-CA-11057-T15687.5_8 | Concatermer  | BRC-CA-11057 | Unkns   |       |     |       |        |            | Phyrase-like domain-containing protein CDS |           |           |         |       |     |       |        |            |
| C    | 1065686   | 1065686   | 1 C → T      | SNP (Strand)              | 98      | 100.0% | 0       | 0                                  | Variables: BRC-CA-11057-T15687.5_8 | Concatermer  | BRC-CA-11057 | Unkns   |       |     |       |        |            | Phyrase-like domain-containing protein CDS |           |           |         |       |     |       |        |            |
| A    | 1067425   | 1067425   | 1 G → A      | SNP (Strand)              | 99      | 100.0% | 0       | 4E-208                             | Variables: BRC-CA-11057-T15687.5_8 | Concatermer  | BRC-CA-11057 | Unkns   |       |     |       |        |            | Phyrase-like domain-containing protein CDS |           |           |         |       |     |       |        |            |
| A    | 1068252   | 1068252   | 1 C → T      | SNP (Strand)              | 84      | 100.0% | 0       | 4E-202                             | Variables: BRC-CA-11057-T15687.5_8 | Concatermer  | BRC-CA-11057 | Unkns   |       |     |       |        |            | Phyrase-like domain-containing protein CDS |           |           |         |       |     |       |        |            |
| A    | 1068284   | 1068284   | 1 G → A      | SNP (Strand)              | 81      | 100.0% | 0       | 2.5E-291                           | Variables: BRC-CA-11057-T15687.5_8 | Concatermer  | BRC-CA-11057 | Unkns   |       |     |       |        |            | Phyrase-like domain-containing protein CDS |           |           |         |       |     |       |        |            |
| G    | 1068493   | 1068493   | 1 G → G      | SNP (Strand)              | 102     | 100.0% | 0       | 100                                | Variables: BRC-CA-11057-T15687.5_8 | Concatermer  | BRC-CA-11057 | Unkns   |       |     |       |        |            | Phyrase-like domain-containing protein CDS |           |           |         |       |     |       |        |            |
| A    | 1068923   | 1068923   | 1 G → A      | SNP (Strand)              | 82      | 100.0% | 0       | 0                                  | Variables: BRC-CA-11057-T15687.5_8 | Concatermer  | BRC-CA-11057 | Unkns   |       |     |       |        |            | Phyrase-like domain-containing protein CDS |           |           |         |       |     |       |        |            |
| C    | 1330102   | 1330102   | 1 GATG → AAT | Deletion ( tandem repeat) | 311     | 91.9%  | 0       | 8.1E-132                           | Variables: BRC-CA-11057-T15687.5_8 | Concatermer  | BRC-CA-11057 | Unkns   |       |     |       |        |            | Phyrase-like domain-containing protein CDS |           |           |         |       |     |       |        |            |
| C    | 1330407   | 1330407   | 1 T → C      | SNP (Strand)              | 133     | 99.9%  | 0       | 0                                  | Variables: BRC-CA-11057-T15687.5_8 | Concatermer  | BRC-CA-11057 | Unkns   |       |     |       |        |            | Phyrase-like domain-containing protein CDS |           |           |         |       |     |       |        |            |
| GG   | 28135     | 28135     | 1 A → G      | SNP (Strand)              | 119     | 100.0% | 0       | 0                                  | Variables: BRC-CA-11057-T15687.5_8 | Concatermer  | BRC-CA-11057 | Unkns   |       |     |       |        |            | Phyrase-like domain-containing protein CDS |           |           |         |       |     |       |        |            |
| T    | 28113     | 28113     | 1 C → T      | SNP (Strand)              | 137     | 98.9%  | 0       | 0                                  | Variables: BRC-CA-11057-T15687.5_8 | Concatermer  | BRC-CA-11057 | Unkns   |       |     |       |        |            | Phyrase-like domain-containing protein CDS |           |           |         |       |     |       |        |            |
| T    | 28116     | 28116     | 1 C → T      | SNP (Strand)              | 131     | 99.2%  | 0       | 0                                  | Variables: BRC-CA-11057-T15687.5_8 | Concatermer  | BRC-CA-11057 | Unkns   |       |     |       |        |            | Phyrase-like domain-containing protein CDS |           |           |         |       |     |       |        |            |
| T    | 28137     | 28137     | 1 G → T      | SNP (Strand)              | 140     | 99.3%  | 0       | 0                                  | Variables: BRC-CA-11057-T15687.5_8 | Concatermer  | BRC-CA-11057 | Unkns   |       |     |       |        |            | Phyrase-like domain-containing protein CDS |           |           |         |       |     |       |        |            |
| G    | 28128     | 28128     | 1 T → G      | SNP (Strand)              | 123     | 99.9%  | 0       | 0                                  | Variables: BRC-CA-11057-T15687.5_8 | Concatermer  | BRC-CA-11057 | Unkns   |       |     |       |        |            | Phyrase-like domain-containing protein CDS |           |           |         |       |     |       |        |            |
| T    | 28114     | 28114     | 1 C → T      | SNP (Strand)              | 120     | 100.0% | 0       | 0                                  | Variables: BRC-CA-11057-T15687.5_8 | Concatermer  | BRC-CA-11057 | Unkns   |       |     |       |        |            | Phyrase-like domain-containing protein CDS |           |           |         |       |     |       |        |            |
| T    | 28822     | 28822     | 1 C → T      | SNP (Strand)              | 111     | 100.0% | 0       | 0                                  | Variables: BRC-CA-11057-T15687.5_8 | Concatermer  | BRC-CA-11057 | Unkns   |       |     |       |        |            | Phyrase-like domain-containing protein CDS |           |           |         |       |     |       |        |            |
| T    | 32037     | 32037     | 1 C → T      | SNP (Strand)              | 139     | 99.3%  | 0       | 0                                  | Variables: BRC-CA-11057-T15687.5_8 | Concatermer  | BRC-CA-11057 | Unkns   |       |     |       |        |            | Phyrase-like domain-containing protein CDS |           |           |         |       |     |       |        |            |
| T    | 33736     | 33736     | 1 G → A      | SNP (Strand)              | 125     | 99.2%  | 0       | 0                                  | Variables: BRC-CA-11057-T15687.5_8 | Concatermer  | BRC-CA-11057 | Unkns   |       |     |       |        |            | Phyrase-like domain-containing protein CDS |           |           |         |       |     |       |        |            |
| C    | 33748     | 33748     | 1 A → C      | SNP (Strand)              | 122     | 99.9%  | 0       | 0                                  | Variables: BRC-CA-11057-T15687.5_8 | Concatermer  | BRC-CA-11057 | Unkns   |       |     |       |        |            | Phyrase-like domain-containing protein CDS |           |           |         |       |     |       |        |            |
| C    | 33731     | 33731     | 1 T → C      | SNP (Strand)              | 121     | 100.0% | 0       | 0                                  | Variables: BRC-CA-11057-T15687.5_8 | Concatermer  | BRC-CA-11057 | Unkns   |       |     |       |        |            | Phyrase-like domain-containing protein CDS |           |           |         |       |     |       |        |            |
| C    | 33751     | 33751     | 1 T → C      | SNP (Strand)              | 128     | 100.0% | 0       | 0                                  | Variables: BRC-CA-11057-T15687.5_8 | Concatermer  | BRC-CA-11057 | Unkns   |       |     |       |        |            | Phyrase-like domain-containing protein CDS |           |           |         |       |     |       |        |            |
| A    | 33797     | 33797     | 1 T → A      | SNP (Strand)              | 124     | 100.0% | 0       | 0                                  | Variables: BRC-CA-11057-T15687.5_8 | Concatermer  | BRC-CA-11057 | Unkns   |       |     |       |        |            | Phyrase-like domain-containing protein CDS |           |           |         |       |     |       |        |            |
| A    | 36609     | 36609     | 1 G → A      | SNP (Strand)              | 174     | 100.0% | 0       | 0                                  | Variables: BRC-CA-11057-T15687.5_8 | Concatermer  | BRC-CA-11057 | Unkns   |       |     |       |        |            | Phyrase-like domain-containing protein CDS |           |           |         |       |     |       |        |            |
| A    | 36610     | 36610     | 1 G → A      | SNP (Strand)              | 174     | 100.0% | 0       | 0                                  | Variables: BRC-CA-11057-T15687.5_8 | Concatermer  | BRC-CA-11057 | Unkns   |       |     |       |        |            | Phyrase-like domain-containing protein CDS |           |           |         |       |     |       |        |            |
| C    | 36624     | 36624     | 1 T → C      | SNP (Strand)              | 197     | 99.7%  | 0       | 0                                  | Variables: BRC-CA-11057-T15687.5_8 | Concatermer  | BRC-CA-11057 | Unkns   |       |     |       |        |            | Phyrase-like domain-containing protein CDS |           |           |         |       |     |       |        |            |
| C    | 36631     | 36631     | 1 T → C      | SNP (Strand)              | 198     | 99.7%  | 0       | 0                                  | Variables: BRC-CA-11057-T15687.5_8 | Concatermer  | BRC-CA-11057 | Unkns   |       |     |       |        |            |  |           |           |         |       |     |       |        |            |

# 9 Appendix

|     |         |         |             |                            |           |                |   |                                  |                   |
|-----|---------|---------|-------------|----------------------------|-----------|----------------|---|----------------------------------|-------------------|
| C   | 1312488 | 1312489 | T → C       | SNP (transversion)         | 72        | 98.4%          | 1.86-253 Variants: BRC-CA-11057-TT-15687-G, R | Concatemer: BRC-CA-11057 - Unkns |                   |
| C   | 1312487 | 1312487 | A → C       | SNP (transversion)         | 73        | 82.2%          | 8.65-195 Variants: BRC-CA-11057-TT-15687-G, R | Concatemer: BRC-CA-11057 - Unkns |                   |
| A   | 1312489 | 1312489 | G → A       | SNP (transversion)         | 71        | 84.5%          | 2.62-203 Variants: BRC-CA-11057-TT-15687-G, R | Concatemer: BRC-CA-11057 - Unkns |                   |
| C   | 1312501 | 1312501 | T → C       | SNP (transversion)         | 54        | 98.3%          | 7.83-209 Variants: BRC-CA-11057-TT-15687-G, R | Concatemer: BRC-CA-11057 - Unkns |                   |
| C   | 1312507 | 1312507 | A → C       | SNP (transversion)         | 54        | 96.4%          | 2.48-196 Variants: BRC-CA-11057-TT-15687-G, R | Concatemer: BRC-CA-11057 - Unkns |                   |
| GG  | 1312512 | 1312511 | T → G       | Deletion                   | 57        | 99.5%          | 50-187 Variants: BRC-CA-11057-TT-15687-G, R   | Concatemer: BRC-CA-11057 - Unkns |                   |
| AT  | 1312516 | 1312517 | 2 TT → GG   | Substitution               | 58 → 65   | 100.0%         | 1.46-208 Variants: BRC-CA-11057-TT-15687-G, R | Concatemer: BRC-CA-11057 - Unkns |                   |
| TA  | 1312519 | 1312520 | 2 TA → AT   | Substitution               | 65        | 100.0%         | 3.28-227 Variants: BRC-CA-11057-TT-15687-G, R | Concatemer: BRC-CA-11057 - Unkns |                   |
| TA  | 1312520 | 1312520 | 2 AC → TA   | Substitution               | 73 → 74   | 100.0%         | 7.82-270 Variants: BRC-CA-11057-TT-15687-G, R | Concatemer: BRC-CA-11057 - Unkns |                   |
| C   | 1312538 | 1312538 | T → C       | SNP (transversion)         | 76        | 98.2%          | 2.48-275 Variants: BRC-CA-11057-TT-15687-G, R | Concatemer: BRC-CA-11057 - Unkns |                   |
| ATG | 1312540 | 1312542 | T TT → ATG  | Substitution               | 76        | 98.7%          | 2.48-275 Variants: BRC-CA-11057-TT-15687-G, R | Concatemer: BRC-CA-11057 - Unkns |                   |
| A   | 1312544 | 1312545 | 2 GG → AA   | Deletion                   | 77 → 80   | 93.18% → 97.4% | 2.48-217 Variants: BRC-CA-11057-TT-15687-G, R | Concatemer: BRC-CA-11057 - Unkns |                   |
| T   | 1312551 | 1312551 | T → C       | Deletion                   | 80        | 100.0%         | 11-235 Variants: BRC-CA-11057-TT-15687-G, R   | Concatemer: BRC-CA-11057 - Unkns |                   |
| A   | 1312558 | 1312558 | G → A       | SNP (transversion)         | 81        | 98.8%          | 8.12-294 Variants: BRC-CA-11057-TT-15687-G, R | Concatemer: BRC-CA-11057 - Unkns |                   |
| T   | 1312560 | 1312560 | T → C       | SNP (transversion)         | 81        | 100.0%         | 21-299 Variants: BRC-CA-11057-TT-15687-G, R   | Concatemer: BRC-CA-11057 - Unkns |                   |
| A   | 1312560 | 1312560 | T → A       | SNP (transversion)         | 82        | 100.0%         | 6.82-299 Variants: BRC-CA-11057-TT-15687-G, R | Concatemer: BRC-CA-11057 - Unkns |                   |
| G   | 1312568 | 1312568 | T → G       | SNP (transversion)         | 82        | 100.0%         | 45-303 Variants: BRC-CA-11057-TT-15687-G, R   | Concatemer: BRC-CA-11057 - Unkns |                   |
| G   | 1312570 | 1312570 | A → G       | SNP (transversion)         | 83        | 100.0%         | 1.48-298 Variants: BRC-CA-11057-TT-15687-G, R | Concatemer: BRC-CA-11057 - Unkns |                   |
| G   | 1312571 | 1312571 | A → G       | SNP (transversion)         | 85        | 100.0%         | 3.28-297 Variants: BRC-CA-11057-TT-15687-G, R | Concatemer: BRC-CA-11057 - Unkns |                   |
| T   | 1312580 | 1312580 | A → T       | SNP (transversion)         | 85        | 100.0%         | 11-305 Variants: BRC-CA-11057-TT-15687-G, R   | Concatemer: BRC-CA-11057 - Unkns |                   |
| A   | 1312591 | 1312590 | G TAA → GAA | Insertion ( tandem repeat) | 100       | 88.0%          | 1.76-301 Variants: BRC-CA-11057-TT-15687-G, R | Concatemer: BRC-CA-11057 - Unkns |                   |
| GGG | 1312610 | 1312611 | T TAA → GGG | Substitution               | 104 → 116 | 99.18% → 99.1% | 0 Variants: BRC-CA-11057-TT-15687-G, R        | Concatemer: BRC-CA-11057 - Unkns |                   |
| A   | 1312664 | 1312664 | T → A       | SNP (transversion)         | 97        | 100.0%         | 0 Variants: BRC-CA-11057-TT-15687-G, R        | Concatemer: BRC-CA-11057 - Unkns | accA CDS          |
| A   | 1312676 | 1312674 | T → A       | SNP (transversion)         | 96        | 100.0%         | 0 Variants: BRC-CA-11057-TT-15687-G, R        | Concatemer: BRC-CA-11057 - Unkns | accA CDS          |
| C   | 1312676 | 1312676 | A → C       | SNP (transversion)         | 94        | 97.9%          | 0 Variants: BRC-CA-11057-TT-15687-G, R        | Concatemer: BRC-CA-11057 - Unkns | A → Y<br>accA CDS |
| T   | 1312680 | 1312680 | T → T       | SNP (transversion)         | 95        | 100.0%         | 0 Variants: BRC-CA-11057-TT-15687-G, R        | Concatemer: BRC-CA-11057 - Unkns | accA CDS          |
| T   | 1313311 | 1313311 | T → T       | SNP (transversion)         | 113       | 100.0%         | 0 Variants: BRC-CA-11057-TT-15687-G, R        | Concatemer: BRC-CA-11057 - Unkns | accA CDS          |
| G   | 1314211 | 1314211 | A → G       | SNP (transversion)         | 127       | 100.0%         | 0 Variants: BRC-CA-11057-TT-15687-G, R        | Concatemer: BRC-CA-11057 - Unkns | hsfB CDS          |
| A   | 1314308 | 1314308 | T → A       | SNP (transversion)         | 110       | 99.1%          | 0 Variants: BRC-CA-11057-TT-15687-G, R        | Concatemer: BRC-CA-11057 - Unkns | hsfB CDS          |
| C   | 1314683 | 1314683 | T → C       | SNP (transversion)         | 146       | 99.3%          | 0 Variants: BRC-CA-11057-TT-15687-G, R        | Concatemer: BRC-CA-11057 - Unkns | T → A<br>hsfB CDS |
| A   | 1314808 | 1314808 | T → A       | SNP (transversion)         | 114       | 100.0%         | 0 Variants: BRC-CA-11057-TT-15687-G, R        | Concatemer: BRC-CA-11057 - Unkns | hsfB CDS          |
| G   | 1316877 | 1316877 | A → G       | SNP (transversion)         | 103       | 99.0%          | 0 Variants: BRC-CA-11057-TT-15687-G, R        | Concatemer: BRC-CA-11057 - Unkns | hsfB CDS          |
| C   | 1316976 | 1316976 | T → C       | SNP (transversion)         | 110       | 100.0%         | 0 Variants: BRC-CA-11057-TT-15687-G, R        | Concatemer: BRC-CA-11057 - Unkns | hsfB CDS          |
| A   | 1317400 | 1317402 | T → A       | SNP (transversion)         | 120       | 100.0%         | 0 Variants: BRC-CA-11057-TT-15687-G, R        | Concatemer: BRC-CA-11057 - Unkns | L → F<br>gpmI CDS |
| A   | 1317481 | 1317481 | T → A       | SNP (transversion)         | 96        | 99.0%          | 0 Variants: BRC-CA-11057-TT-15687-G, R        | Concatemer: BRC-CA-11057 - Unkns | gpmI CDS          |
| A   | 1317480 | 1317480 | T → A       | SNP (transversion)         | 113       | 99.1%          | 0 Variants: BRC-CA-11057-TT-15687-G, R        | Concatemer: BRC-CA-11057 - Unkns | gpmI CDS          |
| A   | 1317562 | 1317562 | T → A       | SNP (transversion)         | 95        | 100.0%         | 0 Variants: BRC-CA-11057-TT-15687-G, R        | Concatemer: BRC-CA-11057 - Unkns | gpmI CDS          |
| C   | 1317558 | 1317558 | A → C       | SNP (transversion)         | 140       | 100.0%         | 0 Variants: BRC-CA-11057-TT-15687-G, R        | Concatemer: BRC-CA-11057 - Unkns | D → E<br>gpmI CDS |
| T   | 1317970 | 1317970 | T → T       | SNP (transversion)         | 141       | 99.3%          | 0 Variants: BRC-CA-11057-TT-15687-G, R        | Concatemer: BRC-CA-11057 - Unkns | gpmI CDS          |
| C   | 1317971 | 1317971 | A → C       | SNP (transversion)         | 143       | 100.0%         | 0 Variants: BRC-CA-11057-TT-15687-G, R        | Concatemer: BRC-CA-11057 - Unkns | gpmI CDS          |
| C   | 1317978 | 1317978 | T → C       | SNP (transversion)         | 144       | 100.0%         | 0 Variants: BRC-CA-11057-TT-15687-G, R        | Concatemer: BRC-CA-11057 - Unkns | gpmI CDS          |
| A   | 1317979 | 1317979 | T → A       | SNP (transversion)         | 142       | 100.0%         | 0 Variants: BRC-CA-11057-TT-15687-G, R        | Concatemer: BRC-CA-11057 - Unkns | gpmI CDS          |
| G   | 1318006 | 1318006 | A → G       | SNP (transversion)         | 105       | 100.0%         | 0 Variants: BRC-CA-11057-TT-15687-G, R        | Concatemer: BRC-CA-11057 - Unkns | E → D<br>gpmI CDS |
| C   | 1318015 | 1318015 | T → C       | SNP (transversion)         | 107       | 100.0%         | 0 Variants: BRC-CA-11057-TT-15687-G, R        | Concatemer: BRC-CA-11057 - Unkns | gpmI CDS          |
| G   | 1318018 | 1318018 | A → G       | SNP (transversion)         | 107       | 100.0%         | 0 Variants: BRC-CA-11057-TT-15687-G, R        | Concatemer: BRC-CA-11057 - Unkns | gpmI CDS          |
| G   | 1318207 | 1318207 | A → G       | SNP (transversion)         | 126       | 100.0%         | 0 Variants: BRC-CA-11057-TT-15687-G, R        | Concatemer: BRC-CA-11057 - Unkns | gpmI CDS          |
| G   | 1318468 | 1318468 | A → G       | SNP (transversion)         | 114       | 100.0%         | 0 Variants: BRC-CA-11057-TT-15687-G, R        | Concatemer: BRC-CA-11057 - Unkns | gpmI CDS          |
| T   | 1319028 | 1319028 | T → T       | SNP (transversion)         | 128       | 99.2%          | 0 Variants: BRC-CA-11057-TT-15687-G, R        | Concatemer: BRC-CA-11057 - Unkns | gpmI CDS          |
| C   | 1319170 | 1319170 | T → C       | SNP (transversion)         | 118       | 100.0%         | 0 Variants: BRC-CA-11057-TT-15687-G, R        | Concatemer: BRC-CA-11057 - Unkns | mraP CDS          |
| T   | 1319126 | 1319126 | T → T       | SNP (transversion)         | 145       | 100.0%         | 0 Variants: BRC-CA-11057-TT-15687-G, R        | Concatemer: BRC-CA-11057 - Unkns | mraP CDS          |
| C   | 1319495 | 1319495 | A → C       | SNP (transversion)         | 113       | 100.0%         | 0 Variants: BRC-CA-11057-TT-15687-G, R        | Concatemer: BRC-CA-11057 - Unkns | mraP CDS          |
| A   | 1319496 | 1319493 | G TAT → GAT | Insertion ( tandem repeat) | 120       | 99.2%          | 0 Variants: BRC-CA-11057-TT-15687-G, R        | Concatemer: BRC-CA-11057 - Unkns |                   |
| C   | 1319497 | 1319497 | T → C       | SNP (transversion)         | 103       | 99.7%          | 4.46-103 Variants: BRC-CA-11057-TT-15687-G, R | Concatemer: BRC-CA-11057 - Unkns |                   |
| G   | 1317851 | 1317851 | A → G       | SNP (transversion)         | 132       | 100.0%         | 1.61-92 Variants: BRC-CA-11057-TT-15687-G, R  | Concatemer: BRC-CA-11057 - Unkns |                   |
| TT  | 1317864 | 1317863 | G → TT      | Insertion                  | 216       | 98.1%          | 1.41-583 Variants: BRC-CA-11057-TT-15687-G, R | Concatemer: BRC-CA-11057 - Unkns |                   |
| T   | 1317866 | 1317866 | A → T       | SNP (transversion)         | 116       | 99.1%          | 3.71-60 Variants: BRC-CA-11057-TT-15687-G, R  | Concatemer: BRC-CA-11057 - Unkns |                   |
| T   | 1317871 | 1317871 | T → T       | Deletion ( tandem repeat)  | 120       | 95.9%          | 1.41-11 Variants: BRC-CA-11057-TT-15687-G, R  | Concatemer: BRC-CA-11057 - Unkns |                   |
| G   | 1317896 | 1317896 | A → G       | SNP (transversion)         | 154       | 99.0%          | 5.98-58 Variants: BRC-CA-11057-TT-15687-G, R  | Concatemer: BRC-CA-11057 - Unkns |                   |
| T   | 1317911 | 1317911 | T → T       | SNP (transversion)         | 109       | 100.0%         | 3.71-54 Variants: BRC-CA-11057-TT-15687-G, R  | Concatemer: BRC-CA-11057 - Unkns |                   |
| T   | 1317914 | 1317913 | G TTT → TTT | Insertion ( tandem repeat) | 100       | 90.0%          | 4.11-33 Variants: BRC-CA-11057-TT-15687-G, R  | Concatemer: BRC-CA-11057 - Unkns |                   |

## 9 Appendix

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| Summary of exported data from SNP analysis |                             |        |                              |        |                  |                    |              |             |  |
|--|-----------------------------|--------|------------------------------|--------|------------------|--------------------|--------------|-------------|--|
| Transformant                               | Deletion<br>(tandem repeat) |        | Insertion<br>(tandem repeat) |        | SNP (transition) | SNP (transversion) | Substitution | sum of SNPs |  |
|  | Deletion                    | repeat | Insertion                    | repeat |                  |                    |              |             |  |
| BfR-CA-11057-TF15687-11                    | 7                           | 8      | 6                            | 9      | 844              | 228                | 133          | 1235        |  |
| BfR-CA-11057-TF15687-12                    |                             | 5      | 3                            | 2      | 156              | 18                 | 6            | 190         |  |
| BfR-CA-11057-TF15687-13                    | 5                           | 10     | 6                            | 5      | 390              | 103                | 80           | 599         |  |
| BfR-CA-11057-TF15687-1                     | 5                           | 6      | 3                            | 7      | 307              | 49                 | 18           | 395         |  |
| BfR-CA-11057-TF15687-2                     | 9                           | 5      | 7                            | 3      | 399              | 111                | 78           | 612         |  |
| BfR-CA-11057-TF15687-3                     |                             | 14     | 5                            | 5      | 468              | 102                | 59           | 653         |  |
| BfR-CA-11057-TF15687-4                     | 2                           | 4      | 5                            | 2      | 620              | 127                | 76           | 836         |  |
| BfR-CA-11057-TF15687-5                     |                             | 3      | 1                            | 2      | 268              | 58                 | 38           | 370         |  |
| BfR-CA-11057-TF15687-6                     | 4                           | 5      | 4                            | 4      | 125              | 24                 | 11           | 177         |  |
| Consensus of all transformants             |                             |        |                              |        | 3                |                    |              | 3           |  |

## 9 Appendix

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| Read coverage <5 of transformants relative to recipient |          |              |              |             |
|---|----------|--------------|--------------|-------------|
| Transformant  | Coverage | Minimum [bp] | Maximum [bp] | Length [bp] |
| BfR-CA-11057-TF15687-12                                 | 0->4     | 1687100      | 1687202      | 103         |
| BfR-CA-11057-TF15687-11                                 | 0->1     | 1687099      | 1687202      | 104         |
| BfR-CA-11057-TF15687-1                                  | 0        | 1687099      | 1687202      | 104         |
| BfR-CA-11057-TF15687-2                                  | 0        | 1687099      | 1687202      | 104         |
| BfR-CA-11057-TF15687-3                                  | 0        | 1687099      | 1687202      | 104         |
| BfR-CA-11057-TF15687-5                                  | 0        | 1687099      | 1687202      | 104         |
| BfR-CA-11057-TF15687-4                                  | 0->1     | 1687099      | 1687202      | 104         |
| BfR-CA-11057-TF15687-13                                 | 0        | 1687099      | 1687202      | 104         |
| BfR-CA-11057-TF15687-6                                  | 0->2     | 1687099      | 1687202      | 104         |
| BfR-CA-11057-TF15687-4                                  | 0->4     | 1335289      | 1336405      | 1117        |
| BfR-CA-11057-TF15687-2                                  | 4        | 1327923      | 1327929      | 7           |
| BfR-CA-11057-TF15687-13                                 | 0->4.8   | 1322086      | 1322103      | 18          |
| BfR-CA-11057-TF15687-2                                  | 4        | 1054273      | 1054279      | 7           |
| BfR-CA-11057-TF15687-13                                 | 0->4.8   | 1048528      | 1048545      | 18          |
| BfR-CA-11057-TF15687-13                                 | 0->4     | 1021333      | 1021662      | 330         |
| BfR-CA-11057-TF15687-2                                  | 0->4     | 1021325      | 1021668      | 344         |
| BfR-CA-11057-TF15687-11                                 | 0->4     | 190076       | 195222       | 5147        |
| BfR-CA-11057-TF15687-4                                  | 1        | 47012        | 47022        | 11          |
| BfR-CA-11057-TF15687-11                                 | 0->4     | 5773         | 6005         | 233         |
| BfR-CA-11057-TF15687-4                                  | 0->4     | 5761         | 6008         | 248         |

## 9 Appendix

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| <b>Unused reads mapped to the donor BfR-CA-15687 with coverage <math>\geq 20</math></b> |          |              |              |             |
|---|----------|--------------|--------------|-------------|
| Transformant  | Coverage | Minimum [bp] | Maximum [bp] | Length [bp] |
| BfR-CA-11057-TF15687-4  | 20->38   | 1305474      | 1305622      | 149         |
| BfR-CA-11057-TF15687-4  | 20       | 1305427      | 1305427      | 1           |
| BfR-CA-11057-TF15687-4  | 20->23   | 1305399      | 1305416      | 18          |
| BfR-CA-11057-TF15687-4  | 20->102  | 1304276      | 1305396      | 1121        |
| BfR-CA-11057-TF15687-2  | 20->38   | 1001583      | 1001917      | 335         |
| BfR-CA-11057-TF15687-13   | 20->60   | 1001551      | 1001952      | 402         |
| BfR-CA-11057-TF15687-11   | 20->24   | 5768         | 5903         | 136         |
| BfR-CA-11057-TF15687-4  | 20->48   | 5697         | 5907         | 211         |

