Aus dem Bundesinstitut für Risikobewertung,
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Bacterial antimicrobial resistance (AMR) in human and different animal populations: A comparison of phenotypical data from German national surveillance and monitoring systems

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„…damit sie mit eigenen Augen sehen, wieviel Leiden das Schreiben den Schreibenden kostet, wie ich mich mein Leben lang abgemüht und geplagt habe, nur damit mein Stil schlicht und fließend und kristallklar wird, wie viel Wörter ich in jeder Zeile durchgestrichen, wie viele Entwürfe ich gemacht habe, zuweilen über eine halbes Dutzend, ehe ich etwas in Druck gab. Segen weilt nur dort, wo der Flügelschlag auf Schweiß und Plag beruht und die Inspiration auf Fleiß und Gründlichkeit fußt.“ – Amos Oz

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Abbreviations

AMP	Ampicillin
AMR	Antimicrobial Resistance
AMU	Antimicrobial Usage
AR	Antibiotic Resistance
ARMIN	*Antibiotika-Resistenz-Monitoring in Niedersachsen*
ARS	*Antibiotika-Resistenz-Surveillance*
AST	Antimicrobial Susceptibility Testing
BARDa	*Die Bayerische Antibiotikaresistenz-Datenbank*
BfR	*Bundesinstitut für Risikobewertung*
BfJ	*Bundesamt für Justiz*
BMEL	*Bundesministerium für Ernährung und Landwirtschaft*
BMG	*Bundesministerium für Gesundheit*
BMJ	*Bundesministerium für Justiz (und Verbrauchschutz)*
BVL	*Bundesamt für Verbrauchsschutz und Lebensmittelsicherheit*
CAESAR	Central Asian and European Surveillance of Antimicrobial Resistance
CIP	Ciprofloxacin
CLSI	Clinical & Laboratory Standard Institutes
CTX	Cefotaxime
DART	*Deutsche Antibiotika-Resistenzstrategie*
EARS-Net	European Antimicrobial Resistance Surveillance Network
ECDC	European Centre of Disease Control
EFSA	European Food Safety Authority
EHEC	Enterohemorrhagic *Escherichia coli*
EMA	European Medicine Agency
ESBL	Extended spectrum beta-lactamase
EU	European Union
EUCAST	European Committee of Antimicrobial Susceptibility Testing
ExPEC	Extraintestinal pathogenic *Escherichia coli*
FAO	The Food and Agricultural Organization
FLI	Friedrich-Löffler-Institut
GAP-AMR	Global Action Plan on Antimicrobial Resistance
GEN	Gentamicin
GE*RM*-Vet	German Resistance Monitoring for Veterinary Medicine (*Nationales Resistenzmonitoring tierpathogener Bakterien*)

GLASS	Global Antimicrobial Resistance and Use Surveillance System
GOHI	German One Health Initiative
HAI	Healthcare-associated infections
ICU	Intensive care unit
mcr	Mobile colistin resistance
MDR	Multidrug resistance
MIC	Minimal Inhibitory Concentration
MHK	*Minimale Hemm-Konzentration*
MRSA	Methicillin-resistant *Staphylococcus aureus*
NRC	National Reference Centers (NRZ)
NRL	National Reference Laboratories
NRL-AR	National Reference Laboratory for Antimicrobial Resistance
OIE	World Organization for Animal Health
PCA	Principal component analysis
PEI	Paul-Ehrlich-Institut
RKI	Robert Koch-Institut
SARI	*Surveillance der Antibiotika-Anwendung und Resistenzentwicklung auf Intensivstationen*
SIR	Susceptible, Intermediary, Resistance (*Sensibel, Intermediär, Resistent*)
STEC	Shiga toxin-producing *Escherichia coli*
UTI	Urinary Tract Infection
VTEC	Verotoxin-producing *Escherichia coli*
WAHIS	The World Animal Health Information System
WHO	World Health Organization

1. Introduction

1.1. Definition and mechanisms of antimicrobial resistance (AMR)

Antimicrobial resistance (AMR) is defined as the ability of bacteria, viruses, fungi and parasites to survive the medical treatments against them, which results in related infections that are more severe and difficult to treat (World Health Organization (WHO) 2021). Some have emphasised the importance of distinguishing between AMR and antibiotic resistance (AR) when describing differing types of resistance in bacteria. AR is described as resistance to exclusively antibiotic treatments for bacterial infections, while AMR applies to the broader resistance of microorganisms, such as parasites (e.g. malaria) or fungi (e.g. *Candida* spp.) to respective antimicrobial drugs (WHO Regional Office for Eastern Mediterranean 2021). For the purpose of this study, the term bacterial AMR is used to designate the resistance of bacteria, as this term is also frequently used in the literature.

Although resistance to antibiotics has always been present in nature (D'Costa et al. 2011), bacterial AMR has become increasingly problematic due to the use of antibiotics. The first indication of antibiotic resistance to penicillin was reported in 1940, twelve years after Alexander Fleming had discovered penicillin. Since then, similar events concerning the development of resistant bacteria after the invention of an antimicrobial drug were observed (Kupferschmidt 2016). There are two resistance mechanisms in bacteria: 1) intrinsic mechanisms – the resistance mechanisms that are always expressed naturally and independently after exposure to antibiotics, and 2) acquired resistance mechanisms – the mechanisms that are gained by previously susceptible bacteria through mutation or by receiving "additional" resistance genes from other bacteria through horizontal gene transfer, for example through the plasmid-mediated transmission of resistance genes or mobile genetic elements. Such acquired resistance mechanisms might also play a specific role in the evolution of bacterial AMR involving alteration of the bacterial genomes (Arnold et al. 2022). However, such resistance mechanisms in bacteria remain complex (Holmes et al. 2016). If the bacteria are resistant to at least one agent across three or more antimicrobial classes, these bacteria are categorised as multidrug-resistant (MDR) bacteria (Magiorakos et al. 2012).

1.2. Epidemiology of bacterial AMR

Bacterial AMR has been found in many bacterial infections, especially in immunocompromised patients due to the presence of frequently detected MDR bacteria (WHO 2020). In 2019, almost

5 million deaths were associated with bacterial AMR globally, out of which 1.3 million deaths attributed to bacterial AMR alone (Murray et al. 2022). AMR has frequently been associated with healthcare-associated infections (HAI) in European countries. Between 2016 and 2017, 31% of the annual estimated 4.5 million HAIs in acute care hospitals in Europe were associated with bacterial AMR (Suetens et al. 2018). In 2015, HAIs associated with bacterial AMR were found to be the cause of more than 600,000 disability adjusted life years (DALYs) within European countries (Cassini et al. 2019). In these countries, HAI were most frequently associated with third-generation cephalosporin-resistant *Escherichia coli* (*E. coli*) (approx. 297,000 infections) followed by methicillin-resistant *Staphylococcus aureus* (MRSA) (approx. 148,000 infections), third-generation cephalosporin-resistant *Klebsiella pneumoniae* (*K. pneumoniae*) (approx. 68,000 infections) and carbapenem-resistant *Pseudomonas aeruginosa* (*P. aeruginosa*) (approx. 62,000 infections) (Cassini et al. 2019). Bacterial AMR has not only emerged in humans, but also in other animal species. In healthy animals, resistance genes to numerous antibiotics have been, for example, detected in fecal samples from broilers (Yang et al. 2019), pigs (Pollock et al. 2020), dairy cattle (Liu et al. 2019) and wildlife (Plaza-Rodríguez et al. 2021). Moreover, MRSA has been detected in dairy cattle (Hansen et al. 2019), pigs (Grøntvedt et al. 2016), poultry (Vossenkuhl et al. 2014) and companion animals (Kaspar et al. 2018). In food, extended-spectrum beta-lactamase (ESBL; e.g. AmpC) producing *E. coli* has been identified in pork, beef, broiler- and turkey-meat (EFSA and ECDC 2020). Amongst animals, researches have previously observed fluoroquinolone-resistant *E. coli* in diseased cattle, ceftiofur-resistant *E. coli* in diseased dogs and horses (RESAPATH 2019) and amoxicillin-resistant *E. coli* as a cause of enteritis in young bovine (<1 year) (BVL 2018). Moreover, bacterial AMR has also been detected in the environment, such as in urban sewage (Hendriksen et al. 2019, Pärnänen et al. 2019), surface waters (Falgenhauer et al. 2019) and wildlife (Swift et al. 2019). The presence of bacterial AMR in wildlife is also observed as a potential medium of AMR gene transmission into the environment (Baros Jorquera et al. 2021, Tinoco Torres et al. 2019) and livestock (Graham et al. 2019, Greig et al. 2015). Although the prevalence of bacterial AMR in wildlife remains relatively low (Plaza-Rodríguez et al. 2021), ongoing monitoring of bacterial AMR in the environment including wildlife as sentinels is important.

1.3. The ‘One Health’ approach to studying bacterial AMR

‘One Health’ is a collaborative multidisciplinary approach to the study of health in humans, animals and the environment that recognises the relationship between these three areas (One Health Commission 2022). It focuses on a diverse array of health issues, which include

bacterial AMR (One Health Initiative 2022). In relation to One Health and AMR, researches have focused on the association between antibiotic use and antibiotic resistance in both humans and food-producing animals, the transmission of bacterial AMR through direct and indirect contact, and environmental contamination through waste derived from humans and animals (ECDC et al. 2015). The use of antibiotics is frequently associated with the occurrence of bacterial AMR in humans and animals (Collignon et al. 2018, Holmes et al. 2016). Studies have previously corroborated the association between antibiotic use and antibiotic resistance in humans (Murray et al. 2022, Ricchizzi et al. 2018), livestock (He et al. 2020, Magouras et al. 2017) and aquaculture (Cabello et al. 2016, Schar et al. 2021). However, the association between the use of antibiotics and AMR in humans and livestock has changed over time in relation to different pathogens (ECDC et al. 2015, ECDC et al. 2017, ECDC et al. 2021).

Studies on similarities of bacterial AMR between humans and animals have previously been reported, such as between farmers and their farm animals (Aworh et al. 2021, Dorado-Garcia et al. 2018, van Hoek et al. 2020), pet owners and their companion animals (Belas et al. 2020, Kaspar et al. 2018) and veterinary healthcare workers and the animals that they have treated (Meijs et al. 2021, Post et al. 2017). This has demonstrated the possibility of transmission through direct contact between humans and animals. Within healthcare facilities, the transmission of bacterial AMR to patients most likely occurred through direct contact with health care professionals (Boone et al. 2021, Friedrich 2019, Steffen et al. 2019), surgeries (Worth et al. 2015) or the environmental contamination of medical devices or surfaces (D'Accolti et al. 2019), and in some cases through the consumption of contaminated food (Jans et al. 2018),. Additionally, indirect transmission by humans could play a role in transmitting bacterial AMR amongst animal populations as part of within- and between-herd dynamics (Crombé et al. 2013). Furthermore, environmental contamination may be compounded by fecal pollution from humans (Karkman et al. 2019), livestock and wildlife (Plaza-Rodríguez et al. 2021).

The common use of antibiotics and the common issue of bacterial AMR in humans and animals alongside the different possible transmission scenarios have highlighted the importance of a One Health approach to combating the development and the spreading of bacterial AMR. Therefore, it is important to continue monitoring bacterial AMR in human and veterinary medicine and in food by strengthening the joint surveillance and monitoring systems for bacterial AMR.

1.4. The surveillance and monitoring of bacterial AMR

Surveillance and monitoring systems are tools that are established to control the occurrence and spread of bacterial AMR. These systems rely on the continuous collection of specific data, such as that related to diseases or pathogens in relation to specific periods of time and defined places. In some definitions, a surveillance system is described for having a more specific goal than a monitoring system, which frequently aims to utilise and implement data for specific interventions and actions, such as disease control programmes (Christensen 2001, Lwanga 1978). Thus, the terms 'surveillance' and 'monitoring' have been used for various purposes.
The Global Antimicrobial Resistance and Use Surveillance System (GLASS) (WHO 2018), managed by the World Health Organization (WHO), was established as part of the Global Action Plan on Antimicrobial Resistance (GAP-AMR, Resolution WHA 68-7) (WHO 2015). GLASS provides a standardised approach to the surveillance of bacterial AMR, specifically on *Acinetobacter* spp., *E. coli*, *K. pneumoniae*, *Neisseria gonorrhoeae* (*N. gonorrhoeae*), *Salmonella* spp., *Shigella* spp., *Staphylococcus aureus* (*S. aureus*) and *Streptococcus pneumoniae* (*S. pneumoniae*) (WHO 2022). At the regional level, the European Antimicrobial Resistance Surveillance Network (EARS-Net) (ECDC 2022) and the Central Asian and European Surveillance of Antimicrobial Resistance (CAESAR) (WHO Regional Office for Europe 2022) focus on the following pathogens: *E. coli, K. pneumoniae, P. aeruginosa, Acinetobacter* spp*., S. pneumoniae, S. aureus*, *Enterococcus faecalis* (*E. faecalis*) and *Enterococcus faecium* (*E. faecium*). For animals, global data regarding bacterial AMR are currently being integrated into the World Animal Health Information System (WAHIS) (World Organisation for Animal Health (OIE) 2022). In European countries, bacterial AMR is monitored annually, as it relates to food safety (EFSA and ECDC 2018, EFSA and ECDC 2019, EFSA and ECDC 2020). Similarly, the EARS network for veterinary medicine (EARS-Vet) is being developed to provide data regarding bacterial AMR, particularly concentrating on pathogens in animals (European Union Joint Action Antimicrobial Resistance and Healthcare-Associated Infections (EU-JAMRAI) 2020, Mader et al. 2021). However, globally and within Europe, One Health surveillance and monitoring systems have been neglected. In 2010, the Food and Agricultural Organization (FAO), World Organization for Animal Health (OIE) and the WHO endorsed the work programs for the purpose of preventing, detecting, containing, eliminating, and responding to zoonotic pathogens, which are relevant to animal and public health. Subsequently, a tripartite concept was enacted (Food and Agriculture Organization of the United Nations (FAO) et al. 2010). The European Council has also promoted the One Health approach to combat AMR with the title "Council conclusions on the next steps under a One Health approach to combat antimicrobial resistance (2016/C 269/05)" (European Union 2016).

Moreover, in June 2017, the EU adopted the One Health approach in the EU Action Plan Against AMR (European Commission 2017), resulting in integrated analyses of antibiotic consumption and AMR in the human and animal sectors (ECDC et al. 2015, ECDC et al. 2017, ECDC et al. 2021). Such integrated analyses will promote an understanding of associations between bacterial AMR and antibiotic usage and support the early warning system regarding bacterial AMR development (WHO 2021). This will further support the establishment of joint One Health-based surveillance and monitoring systems for bacterial AMR in human and veterinary medicine, including in food safety.

1.4.1. The surveillance of bacterial AMR in humans in Germany

Several surveillance systems for bacterial AMR in humans currently exist in Germany: the unit based surveillance of antibiotic resistance as part of Central Reference Database for Nosocomial Infections (*Krankenhaus-Infektions-Surveillance-System* or KISS) (Nationales Referenzzentrum (NRZ) für Surveillance von nosokomialen Infektionen 2023) , the regional monitoring of antibiotic resistance in Lower Saxony (*Antibiotika-Resistenz-Monitoring in Niedersachsen* – ARMIN) (Niedersächsiches Landesgesundheitsamt (NGLA) 2020), the regional Bavarian antibiotic resistance database (*Die Bayerische Antibiotikaresistenz-Datenbank* – BARDa) (Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit (LGL) 2020), several national mandatory notifications for resistant bacteria (*§7 IfSG Abs. 1*) (BMJ 2001): resistance in *Mycobacterium tuberculosis*, MRSA since 2009, and carbapenem-resistant *Enterobacteriaceae* and *Acinetobacter* spp. since 2016, and the national Antibiotic Resistance Surveillance system (*Antibiotika-Resistenz-Surveillance* (ARS)) (Robert Koch-Institut 2021a). Additionally, Germany has established public health microbiological reference laboratories for different pathogens (*Nationales Referenzentrum*) (Beermann et al. 2015, Robert Koch-Institut 2021b) that fulfill the core functions of microbiological reference laboratories (ECDC 2010). These laboratories advise authorities about confirmatory testing, special investigations and the dissemination of standard operating procedures (SOPs) for laboratory analyses, and they provide guidance regarding infectious diseases surveillance for specified pathogens as well data on antibiotic susceptibilities in bacteria. These functions have exhibited substantial public health relevance (Beermann et al. 2015). Within these reference laboratories, antimicrobial susceptibility testing (AST) is conducted using broth microdilution (ISO 20776-1:2006) amongst other manual and automated methods (Beermann et al. 2015). Data concerning bacterial AMR, both phenotypical and genotypical, are collected for the purpose of research, diagnostics and frequently also for outbreak investigations (Robert Koch-Institut 2021b). These data focus on laboratory results with less consideration of epidemiological or clinical patient data. *Antibiotika-Resistenz-Surveillance* (ARS) is a

nationwide surveillance system for bacterial AMR in Germany (Robert Koch-Institut 2021a). Data on AMR of all diagnosed bacteria to various antibiotics alongside aggregated demographical information are routinely collected by laboratories that voluntarily participate in the ARS system. Due to the varying panels of tested antibiotics across participating laboratories, it is important to identify comparable minimum panels based on the specific pathogens. Taking *E. coli* as an example, different antibiotics were tested in three different health care settings, namely outpatient care, a general ward and an ICU from 2014 to 2017; these are compared in Figure 1. The detailed information regarding the ARS system is explained in the second publication included in this thesis (Suwono et al. 2021a).

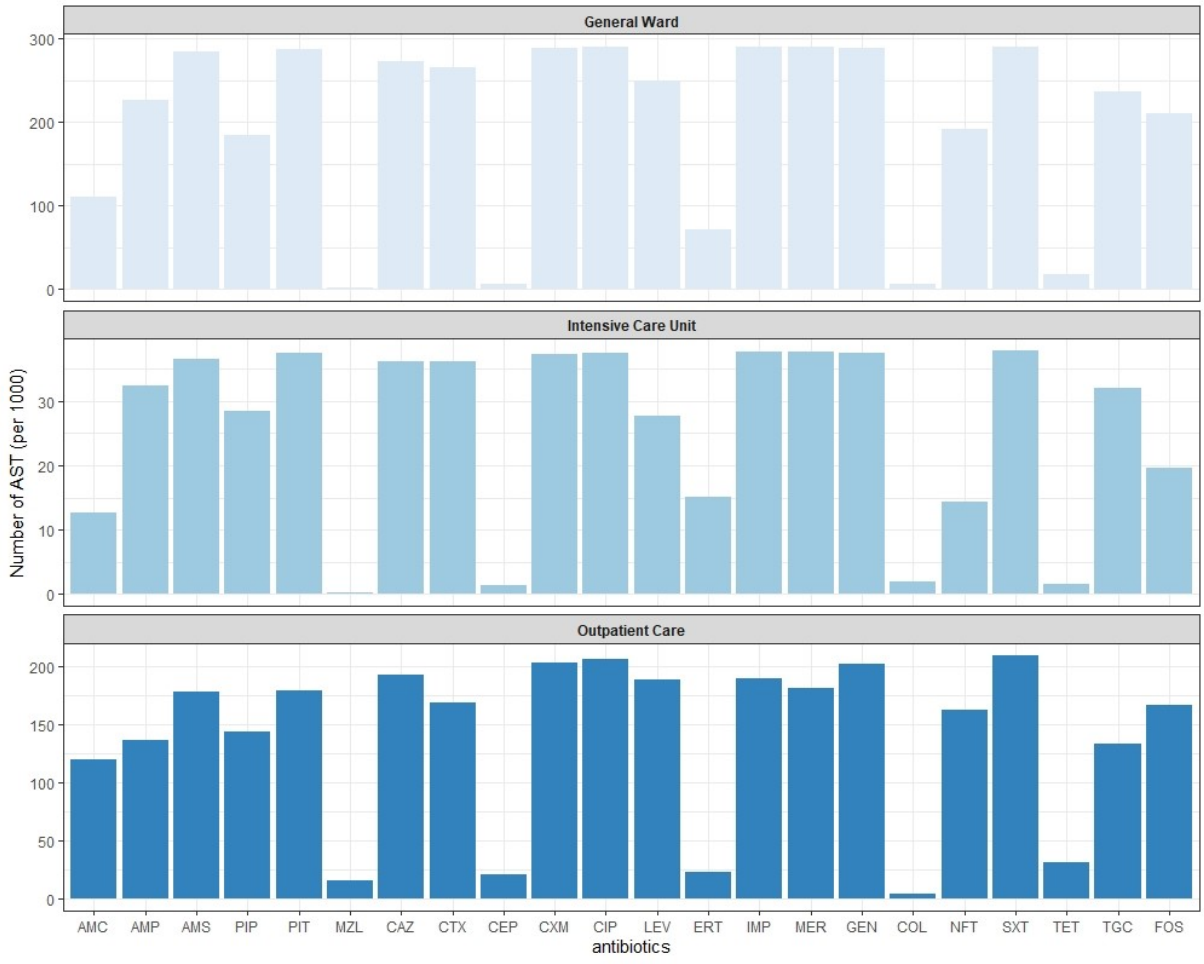

Figure 1. Number of Antimicrobial Susceptibility Testing (AST) for antibiotics (per 1,000 ASTs) that were tested for *E. coli* isolates from 2014 to 2017 in three health care settings. Y-axis describes the absolute number of AST for antibiotics (per 1,000 ASTs), while X-axis describes the antibiotics as follows: AMC: Amoxicillin / Clavulanic acid, AMP: Ampicillin, AMS: Amoxicillin / Sulbactam, PIP: Piperacillin, PIT: Piperacillin / Tazobactam, MZL: Mecillinam, CAZ: Ceftazidime CTX: Cefotaxime, CEP: Cefepime, CXM: Cefuroxime, CIP: Ciprofloxacin, LEV: Levofloxacin, ERT: Ertapenem, IMP: Imipenem, MER: Meropenem, GEN: Gentamicin, COL: Colistin, NFT: Nitrofurantoin, SXT: Co-trimoxazole, TET: Tetracycline, TGC: Tigecycline, FOS: Fosfomycin.

1.4.2. Monitoring of bacterial AMR in animals in Germany

There are various regulations related to maintaining and monitoring animal health and welfare in Germany (BMJ and BfJ 2011a, BMJ and BfJ 2011b, BMJ and BfJ 2018). This thesis focuses on the monitoring of zoonoses and zoonotic agents (Zoonosis Monitoring), including their resistance characterisation in healthy food-producing animals. The mandate of this Zoonosis Monitoring is described in selected German and European directives including the *Allgemeine Verwaltungsvorschrift über die Erfassung, Auswertung und Veröffentlichung von Daten über das Auftreten von Zoonosen und Zoonoseerregern entlang der Lebensmittelkette (AVV Zoonosen Lebensmittelkette)* (Die Bundesregierung 2012), the Commission Implementing Decision (CID) 2013/652/EU (European Commission 2013) and the EU directive order 2003/99/EC (European Commission 2020). As mandated in *AVV Zoonosen Lebensmittelkette*, this system monitors indicator commensal bacteria *E. coli*, vero- and shiga-toxin producing *E. coli* (STEC/VTEC), *Campylobacter* spp., *Salmonella spp*., *Listeria spp*., MRSA and other antibiotic-resistant bacteria based on an annual sampling plan in food-producing animals and foods. Moreover, Germany has also monitored animal pathogens within the German Resistance Monitoring for Veterinary Medicine (GE*RM*-Vet) system since 2001 (BMJ and BfJ 2005b, Wallmann et al. 2003). GE*RM*-Vet is coordinated by BVL as mandated in The German Medicinal Products Act (*Arzneimittelgesetz, AMG*) *9. Abschnitt (section 56 to 61) and 15. Abschnitt §77 (3)* (BMJ and BfJ 2005a). This monitoring was also warranted by new regulations for veterinary medical products that have recently been introduced in European countries (EU 2019/6, (European Commission 2018)) and in Germany (*Tierarzneimittelgesetz* – TAMG §61 (BMJ and BfJ 2021)). GE*RM*-Vet monitoring is performed annually and involves the collection of animals' bacterial pathogens according to an annual sampling plan. As this study used data from Zoonosis Monitoring for AMR and GE*RM*-Vet, these two systems are described in more detail in the second publication included in this thesis (Suwono et al. 2021a).

1.4.3. Bacterial AMR management strategies in Germany

Germany initiated the German Antimicrobial Resistance Strategy (DART) in 2008 (BMG et al. 2011). Developed by the German Federal Ministry of Health (BMG), the German Federal Ministry of Food and Agriculture (BMEL) and the German Federal Ministry of Education and Research (BMBF), the strategy outlined ten goals concerning the reduction of antimicrobial usage (AMU) and bacterial AMR in human medicine. The surveillance of bacterial AMR and AMU comprises a component of these strategies. In 2015, DART was updated to DART 2020 *(DART 2020 – Fighting antibiotic resistance for the good of both humans and animals)* (BMG

et al. 2015), where the One Health approach for bacterial AMR was integrated into one of the primary goals.

Germany does not yet have routine One Health-based surveillance and monitoring systems for bacterial AMR. The 'GERMAP' project was initiated in 2008 to describe and report the current status concerning bacterial AMR and the consumption of antimicrobials in human and veterinary medicine. GERMAP was published every two to four years (BVL and Paul-Ehrlich-Gesellschaft für Chemotheraphie e.V. (PEG) 2016). However, the GERMAP data are limited, as no integrated database of surveillance and monitoring systems is included.

As part of DART 2020, the German One Health Initiative (GOHI) was established in 2016 connecting four national research institutions: Robert Koch Institute (RKI), the German Federal Institute for Risk Assessment (BfR), the Paul Ehrlich Institute (PEI) and the Friedrich Loeffler Institute (FLI). The GOHI aims to create coordinated strategies related to zoonosis and One Health (German One Health Initiative (GOHI) 2017). One of GOHI frameworks focuses on bacterial AMR with the following title: 'Comparison of data on bacterial antibiotic resistance from various surveillance and monitoring systems in veterinary and human medicine in Germany' (*Vergleich von Resistenzdaten von Bakterien aus der medizinischen Surveillance und Monitoring und Surveillance in der Tiermedizin und Lebensmittelproduktion*), which is specifically studied in this thesis.

1.5. Comparative investigations of bacterial AMR in humans and animals

1.5.1. *E. coli* as a model organism

E. coli is regarded as part of the healthy intestinal microbiota in humans and animals, serving as a commensal and occasionally acting as a zoonotic pathogen inside and outside the intestine (extraintestinal pathogenic *E. coli* (ExPEC)). Inside the intestine, enteropathogenic *E. coli* predominantly causes gastroenteritis/gastrointestinal infections (GI), while outside the intestine or ExPEC causes urinary tract infections (UTI) and bloodstream infections (BSI) (Poirel et al. 2018). Moreover, in humans, pathogenic *E. coli* causes various types of food-borne outbreaks (Aurass et al. 2011, Bottichio et al. 2020, Buchholz et al. 2011, Mulchandani et al. 2021) and in some cases, severe complications such as haemolytic uremic syndrome (HUS) (Joseph et al. 2020, Launders et al. 2016, Scheutz et al. 2012). As for infections in animals, *E. coli* was frequently reported to be major cause of mastitis in dairy cattle (Boireau et al. 2018, Thomas et al. 2015) and colibacillosis in poultry (Johnson et al. 2022, Monson and Lamont 2021).

Resistance mechanisms in *E. coli* isolates, particularly in the case of ESBL-producing *E. coli*, have been frequently studied in different human and animal populations. The presence of ESBL genes in different human and animal populations has indicated the transmission of bacterial AMR through direct contact between these populations (Dorado-Garcia et al. 2018, Mughini-Gras et al. 2019, van Hoek et al. 2020). Moreover, *E. coli* can receive or transfer resistance genes to other bacteria via mobile genetic elements such as plasmids or transposons; for example, this applies to the mobile colistin resistance (*mcr*) gene, which causes colistin resistance (Poirel et al. 2018, Wang et al. 2020b). As *E. coli* acts as an exchange platform for resistance genes, commensal *E. coli* is frequently used as indicator bacterium to measure resistance in host species (Poirel et al. 2018). Thus, *E. coli* has been routinely monitored due to its importance in the human and animal health sectors. The abundant availability and reliability of *E. coli* data provides an effective subset of data for this comparison study and was used as a model organism in this thesis.

1.5.2. Thesis objective and research questions

To date, routine surveillance and monitoring systems of bacterial AMR in human and veterinary medicine including in food safety have relied on phenotypical data. The integrated analyses of such data from the available surveillance and monitoring systems are important to describe the current epidemiological situation of zoonotic bacterial AMR in these different sectors. Understanding resistance patterns in zoonotic bacteria has been also mentioned as an important objective of integrated surveillance systems (EFSA et al. 2019). Using *E. coli* as a model organism, this thesis aims to investigate comparability of phenotypical data of *E. coli* isolates collected from ARS, Zoonosis Monitoring, and GE*RM*-Vet in order to optimise the possibilities for the integration of bacterial AMR data in humans and different animal populations. Based on this objective, several research questions are addressed as follows:

1. How comparable are the bacterial AMR data from surveillance systems for humans and monitoring systems for animals and food safety in Germany?
2. What are the characteristics of the surveillance and monitoring systems for bacterial AMR in relation to humans, animals and food safety in Germany? What kind of variables are collected via ARS, Zoonosis Monitoring and GE*RM*-Vet? Are the collected variables similar across these three systems?
3. Based on the existing variables related to the available surveillance and monitoring systems, what types of analyses could be used to compare data between the human and animal sectors?

4. How will the analyses outlined in question three contribute to understanding bacterial AMR situations in the human and animal sectors? Will these analyses be capable of identifying the similarities in resistance patterns between humans and different animal populations, thereby improving the understanding of transmission between populations?
5. Is there any further demographical stratification, such as region-based stratification, that affects the comparative analyses?

1.5.2.1. First publication: Comparison of minimum inhibitory concentrations (MICs) in *Escherichia coli* isolates from human health surveillance with MICs obtained for the same isolates by broth microdilution

This study aimed to analyse the comparability of AST methods for *E. coli* isolates from ARS (humans) and Zoonosis Monitoring (food-producing animals and food safety). Broth microdilution is used for food safety monitoring systems in Germany and Europe, while automated AST based on kinetic growth curves is used primarily to support treatment decisions and human health surveillance. Thus, before comparing bacterial AMR data from humans and animals, it is important to define harmonised AST standards between human medical laboratories and veterinary diagnostics including for food safety monitoring. In this study, the *E. coli* isolates routinely collected and tested using automated AST in human health surveillance were retested using broth microdilution to compare the results. By studying the comparability of the MICs from two different AST methods, this publication supports the comparative analysis of AMR data from ARS, Zoonosis Monitoring and GE*RM*-Vet (second publication in this thesis) and addresses the first research question of this thesis.

1.5.2.2. Second publication: Cluster analysis of resistance combinations in *Escherichia coli* from different human and animal populations in Germany 2014-2017

The aim of this study was to use cluster analysis to examine similarities in resistance patterns to four antibiotics in *E. coli* isolates from different human and animal populations. This comparison study used data from ARS, Zoonosis Monitoring and GE*RM*-Vet. Prior to this study, a literature review was performed to summarise and compare the existing surveillance and monitoring systems for bacterial AMR in human and veterinary medicine in Germany

(Suwono et al. 2021a). This review expanded the summary described in the previous GERMAP report (BVL and Paul-Ehrlich-Gesellschaft für Chemotheraphie e.V. (PEG) 2016) by comparing the collected variables in greater detail to outline the overlapping elements of the ARS, Zoonosis Monitoring and GE*RM*-Vet systems. Based on the findings of the literature review, the methods for the comparative cluster analysis *E. coli* resistance data from the three surveillance and monitoring systems were determined.

The analysis focused on the resistance combinations to four antibiotics, namely ampicillin (AMP), cefotaxime (CTX), ciprofloxacin (CIP) and gentamicin (GEN), which were included in the testing panels of all three systems. Using an integrated dataset from these three surveillance and monitoring systems, hierarchical clustering of these resistance combinations was performed. As similar antibiotics are routinely used in humans and animals, similar antibiotic selection pressure and resistance patterns should be illustrated in the case of *E. coli* across the different populations. Therefore, the second publication addresses the second, third and fourth research question.

1.5.2.3. Third publication: A joint regional analysis of resistance combinations in *Escherichia coli* in humans and different food-producing animal populations in Germany between 2014-2017

This study aimed to further analyse the resistance combination patterns identified in the second publication through an in-depth examination of three regions within Germany, namely North West, South West and East, as defined by the population structures of different animal populations. In the second publication, similarities in human populations from the different healthcare units were observed. This third publication analyses the resistance patterns in the studied regions to ascertain whether the close similarities in human populations might also be observed in the different structures of animal populations. Similar statistical methods were applied relative to this thesis's second publication. This third publication addresses the fifth research question of this thesis.

2. First Publication

Beneditta Suwono, Jens André Hammerl, Tim Eckmanns, Roswitha Merle, Ulrich Eigner Michaela Lümen, Sven Lauter, Rüdiger Stock, Ines Fenner, Eva Boemke, Bernd-Alois Tenhagen

Comparison of minimum inhibitory concentrations (MICs) in *Escherichia coli* isolates from human health surveillance with MICs obtained for the same isolates by broth microdilution

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Doi: 10.1093/jacamr/dlab145

JAC Antimicrob Resist
doi:10.1093/jacamr/dlab145

JAC-Antimicrobial Resistance

Comparison of MICs in *Escherichia coli* isolates from human health surveillance with MICs obtained for the same isolates by broth microdilution

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Objectives: Human health surveillance and food safety monitoring systems use different antimicrobial susceptibility testing (AST) methods. In this study, we compared the MICs of *Escherichia coli* isolates provided by these methods.

Methods: *E. coli* isolates ($n = 120$) from human urine samples and their MICs were collected from six medical laboratories that used automated AST methods based on bacterial growth kinetic analyses. These isolates were retested using broth microdilution, which is used by the food safety monitoring system. The essential and categorical agreements (EA and CA), very major errors (VME), major errors (ME) and minor errors (mE) for these two methods were calculated for 11 antibiotics using broth microdilution as a reference. For statistical analysis, clinical breakpoints provided by EUCAST were used.

Results: Five study laboratories used VITEK®2 and one MicroScan (Walkaway Combo Panel). Out of 120 isolates, 118 isolates (98.3%) were confirmed as *E. coli*. The 99 *E. coli* isolates from five study laboratories that used VITEK®2 showed high proportions of EA and CA with full agreements for gentamicin, meropenem, imipenem and ertapenem. Additionally, 100% CA was also observed in cefepime. Few VME (0.5%), ME (1.9%) and mE (1.5%) were observed across all antibiotics. One VME for ceftazidime (7.1%) and 12 MEs for ampicillin (29.4%), cefotaxime (2.4%), ciprofloxacin (3.2%), tigecycline (1.5%) and trimethoprim (22.2%) were detected.

Conclusions: MICs from *E. coli* isolates produced by VITEK®2 were similar to those determined by broth microdilution. These results will be valuable for comparative analyses of resistance data from human health surveillance and food safety monitoring systems.

Introduction

Few efforts have been made to compare the results of routinely performed antimicrobial susceptibility testing (AST) in medical laboratories with broth microdilution as used for food safety monitoring in Germany and Europe. The direct comparison of MICs will facilitate reliable comparative analyses that are also robust when changes are made in the evaluation criteria or breakpoints over time.[1] The comparison needs to consider that MICs in the human, animal and food sectors are determined by different AST methods.[2–7] Better harmonization of surveillance and monitoring for antibiotic resistance in the human and animal sector is demanded by the German national antibiotic resistance strategy DART.[8] Therefore, comparison of AST results generated by different methods is crucial. The main objective was to study the comparability of

the MICs of *Escherichia coli* isolates determined by two different methods: automated AST systems used in German human health surveillance and the broth microdilution method used in German food safety monitoring. The agreement of the results from these two methods was calculated.

Materials and methods

One hundred and twenty randomly chosen *E. coli* isolates from urine samples were collected from six medical laboratories between March and May 2019 (20 isolates per participating laboratory). The medical laboratories participated regularly in the German *Antibiotika Resistenz Surveillance* (ARS) system[9] from 2014 to 2017 and provided their results as MICs. *E. coli* isolates were sent to the National Reference Laboratory for Antimicrobial Resistance (NRL-AR) using transport swabs (Amies Agar Gel Transport Swab, Thermo Scientific Oxoid TS0001A). They were non-selectively cultured on Columbia blood agar (Oxoid, Wesel, Germany). Following incubation at 37 ± 2°C for 16–22 h, the purity of the isolates was assessed. Bacterial species were confirmed as *E. coli* using a MALDI-MS Biotyper (Bruker, Bremen, Germany). If the colony morphologies of an isolate differed after initial cultivation on blood agar, PFGE (XbaI, PulsNet) was conducted. AST was performed by lyophilized broth microdilution according to the CLSI guidelines (ISO 20776-1:2006 or CLSI M31-A3) using a standardized antibiotic panel [EUVSEC and EUVSEC2 scheme, TREK Diagnostic Systems/Thermo Fisher Scientific (lyophilized), Schwerte, Germany]. Essential agreement (EA) was stated if MICs determined by the automated AST systems and by broth microdilution showed no discrepancies. A discrepancy was observed if the MICs differed by more than one dilution step (Table S1, available as Supplementary data at *JAC-AMR* Online). For the measurement of categorical agreement (CA) and errors, MICs were interpreted using clinical breakpoints published by EUCAST (Version 9.0).[10] CA was the agreement between the two measurements concerning the resulting evaluation as susceptible, intermediate or resistant. A very major error (VME) was stated if the reference test result was 'resistant' while the result from automated AST systems was 'susceptible'. A major error (ME) was defined as reference test result 'susceptible' while the automated AST systems resulted in 'resistant'. A minor error (mE) was determined if the results of one method was 'intermediate' and in the other method it was either 'susceptible' or 'resistant'. All analyses were run in R (R 3.5.1; Rstudio 1.1.442).

Results

Five participating laboratories used VITEK®2 (bioMérieux, Nürtingen, Germany). One laboratory used the MicroScan (Walkaway Combo Panel, Beckmann Coulter, Germany). The use of three different AST cards for the VITEK®2 system was reported (GN AST N387, GN AST-N371 and GN AST N263). Since the data were coming mostly from VITEK®2, this study will focus on the results of VITEK®2 system. The results and analyses of MicroScan are documented separately in the Supplementary data (Table S2). One hundred presumptive *E. coli* isolates were obtained from the five participating medical laboratories (20 isolates/participating laboratory). Out of these, 99 isolates (99%) were confirmed as *E. coli*. One isolate was identified as *Klebsiella pneumoniae* and excluded from the analyses. Of the 99 *E. coli* isolates, 7 isolates exhibited two different colony morphologies with similar PFGE patterns (Figure S1). Both of the seven pairs of isolates were included in the analyses to study this potential source of variation (Table S3). In total, 106 isolates were included in the analysis. Table 1 highlights the results of agreements and errors. Full EA and CA (100%) were observed for gentamicin, meropenem, imipenem and ertapenem. Additionally, 100% CA was detected in cefepime. One VME was detected for ceftazidime (1 VME/14 ceftazidime-resistant isolates, 7.1% and 1/199 all resistant isolates, 0.5%). Twelve MEs (12 MEs/623 all susceptible isolates, 1.9%) were detected for ampicillin (5/17 susceptible isolates, 29.4%), cefotaxime (2/83 susceptible isolates, 2.4%), ciprofloxacin (2/63 susceptible isolates, 3.2%), tigecycline (1/65 susceptible isolates, 1.5%) and trimethoprim (2/9 susceptible isolates, 22.2%). Eight mEs (8 mEs/530 tested isolates, 1.5%) were detected in cefotaxime (1/106 tested isolates, 0.9%), and ciprofloxacin (7/106 tested isolates, 6.6%). All mEs were observed with a difference of one dilution step.

Discussion

Good agreement was observed between the result of the automated AST systems and broth microdilution (Table 1). Our study results are in line with earlier studies that reported a high level of agreement between VITEK®2 test results and broth microdilution as the reference method for AST *E. coli* isolates.[11,12] Both studies found fewer VMEs and MEs than our study (Tables 1 and S4). In these studies, testing with the automated system was repeated if discrepancies occurred. Bobenchik *et al.* (2015)[12] reported the correction of 12 VMEs out of 13 VMEs from the initial testing for their study antibiotics and 9 of 24 MEs after repeated measurements. Only if the errors still occurred after repeating the measurements were these errors included in the analyses.[11,12] This repeated testing was not foreseen in our study as we wanted to compare routine results rather than results optimized by repeated testing. As part of routine diagnostics, AST will probably only be repeated if the results are contradictory (e.g. *E. coli* resistant to cefotaxime but susceptible to ampicillin). Therefore, surveillance data are not optimized as in the cited studies. The comparative interpretation of MICs was limited by different antibiotics included in the AST in the five participating laboratories (Table S5). Different concentration ranges of antibiotics were tested in the participating laboratories and NRL-AR (Tables S6 and S7). In the medical laboratories, the variability of antibiotic substances and their range of MICs is the consequence of the use of three different AST cards manufactured for slightly different purposes[13] that contain slightly different antibiotics[7] (Table S8). Two cards were manufactured for all Gram-negative bacteria. Another card is specifically manufactured for Gram-negative bacteria from urinary samples. In food safety monitoring, fixed EUVSEC panels established by the European Commission and harmonized across Europe are used for AST of *E. coli* and *Salmonella*.[6] These panels include antimicrobial agents that are relevant to human and veterinary medicine and are considered representative of the different antimicrobial families. Some of the frequently tested antibiotics for *E. coli* in the participating laboratories, e.g. piperacillin/tazobactam, are not included in the EUVSEC panels (Table S5).[14] A broader range of concentrations than in medical laboratories is tested in the monitoring of food safety to allow for further epidemiological analyses. This is however not the purpose of routine medical laboratories that primarily aim to guide therapy decisions. The difference of the ranges results in a limited comparability of the individual MICs with respect to EA. However, as all ranges included the clinical breakpoints provided by EUCAST, the CA could be fully analysed.

Table 1. EA, CA, VMEs, MEs and mEs for each antibiotic that was tested with VITEK®2 and included in the food safety resistance monitoring panel (EUVSEC)

			Ref based on EUCAST[10]						
Antibiotics	No. of tested isolates (*n*)	EA (%)	S	I	R	CA (%)	VME (%)	ME (%)	mE (%)
Ampicillin	106	101 (95.3)	17	ND[a]	89	101 (95.3)	0 (0)	5 (29.4)	ND[a]
Cefotaxime	106	102 (96.2)	83	0	23	103 (97.2)	0 (0)	2 (2.4)	1 (0.9)
Ceftazidime	84	82 (97.6)	70	ND[a]	14	83 (98.8)	1 (7.1)	0 (0)	ND[a]
Cefepime	41	40 (97.6)	30	0	11	41 (100)	0 (0)	0 (0)	0 (0)
Ciprofloxacin	106	104 (98.1)	63	4	39	97 (91.5)	0 (0)	2 (3.2)	7 (6.6)
Gentamicin	85	85 (100)	74	0	11	85 (100)	0 (0)	0 (0)	0 (0)
Meropenem	106	106 (100)	106	0	0	106 (100)	0 (NA)	0 (0)	0
Imipenem	65	65 (100)	65	0	0	65 (100)	0 (NA)	0 (0)	0 (0)
Ertapenem	41	41 (100)	41	ND[a]	0	41 (100)	0 (NA)	0 (0)	ND[a]
Tigecycline	65	64 (98.5)	65	ND[a]	0	64 (98.5)	0 (NA)	1 (1.5)	ND[a]
Trimethoprim	21	19 (90.5)	9	0	12	19(90.5)	0(NA)	2 (22.2)	0

Ref, reference AST (lyophilized broth microdilution); S, susceptible; I, intermediate; R, resistant.
[a]Not determined (ND): no breakpoints for 'intermediate' AST results.

Our study has a few limitations. The measurements for errors could not be repeated since VITEK®2 and broth microdilution were performed in different laboratories. Moreover, this study does not cover the complete current situation of AST testing in medical laboratories in Germany because of the limited number of participating laboratories ($n = 6$) and the exclusive testing of *E. coli*. *E. coli* was chosen because it represents a substantial part of the AST data in the ARS system[9] (21.6% out of all collected pathogens in 2018) and is likewise routinely tested in food safety monitoring where it is considered as an indicator of the antimicrobial resistance situation in the population.[15] We only wanted to include laboratories that routinely provide MIC values to the ARS system together with SIR results. One laboratory used the MicroScan for automated AST and was finally excluded from the analysis. However, we observed no obvious difference between the results for this laboratory and the other laboratories (Table S2). Further comparisons of routine results of other automated AST methods with broth microdilution also using a wider range of bacteria are therefore necessary.

Conclusions

To the best of our knowledge, this is the first study that compares MIC data, which are routinely generated by automated AST systems in medical laboratories, with the results of broth microdilution used in food chain monitoring. The study findings underline the overall comparability of the AST results from medical laboratories that are part of human health surveillance with the AST results from food safety monitoring.

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Transparency declarations

None to declare.

Supplementary data

Figure S1 and Tables S1 to S8 are available as Supplementary data at *JAC-AMR* Online.

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2.1. Role of the authors for the first publication

Name	Author's Role	Author's contribution
Beneditta Suwono	First author	Conceptualisation, data curation, methodology, data validation, visualization, literature researches, statistical analysis, and preparation the manuscript, incl. writing, reviewing and editing the manuscript.
Jens André Hammerl	Co-author	Data curation and validation, methodology (laboratory supervision at NRL), reviewing and editing the manuscript.
Tim Eckmanns	Co-author	Conceptualisation, data validation, methodology (supervision), reviewing and editing the manuscript.
Roswitha Merle	Co-author	Statistical analysis (supervision), reviewing and editing the manuscript.
Ulrich Eigner	Co-author	Study participants (resources for the isolates), reviewing and editing the manuscript.
Michaela Lümen	Co-author	Study participants (resources for the isolates), reviewing and editing the manuscript.
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Ines Fenner	Co-author	Study participants (resources for the isolates), reviewing and editing the manuscript.
Eva Boemke	Co-author	Study participants (resources for the isolates), reviewing and editing the manuscript.
Bernd-Alois Tenhagen	Corresponding author	Conceptualisation, data validation, study supervision, reviewing and editing the manuscript.

3. Second Publication

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Cluster analysis of resistance combinations in *Escherichia coli* from different human and animal populations in Germany 2014-2017

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RESEARCH ARTICLE

Cluster analysis of resistance combinations in *Escherichia coli* from different human and animal populations in Germany 2014-2017

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Abstract

Recent findings on Antibiotic Resistance (AR) have brought renewed attention to the comparison of data on AR from human and animal sectors. This is however a major challenge since the data is not harmonized. This study performs a comparative analysis of data on resistance combinations in *Escherichia coli* (*E. coli*) from different routine surveillance and monitoring systems for human and different animal populations in Germany. Data on *E. coli* isolates were collected between 2014 and 2017 from human clinical isolates, non-clinical animal isolates from food-producing animals and food, and clinical animal isolates from food-producing and companion animals from national routine surveillance and monitoring for AR in Germany. Sixteen possible resistance combinations to four antibiotics—ampicillin, cefotaxime, ciprofloxacin and gentamicin–for these populations were used for hierarchical clustering (Euclidian and average distance). All analyses were performed with the software R 3.5.1 (Rstudio 1.1.442). Data of 333,496 *E. coli* isolates and forty-one different human and animal populations were included in the cluster analysis. Three main clusters were detected. Within these three clusters, all human populations (intensive care unit (ICU), general ward and outpatient care) showed similar relative frequencies of the resistance combinations and clustered together. They demonstrated similarities with clinical isolates from different animal populations and most isolates from pigs from both non-clinical and clinical isolates. Isolates from healthy poultry demonstrated similarities in relative frequencies of resistance combinations and clustered together. However, they clustered separately from the human isolates. All isolates from different animal populations with low relative frequencies of resistance combinations clustered together. They also clustered separately from the human populations. Cluster analysis has been able to demonstrate the linkage among human isolates and isolates from various animal populations based on the resistance combinations. Further analyses based on these findings might support a better one-health approach for AR in Germany.

Competing interests: The authors have declared that no competing interests exist.

Introduction

Antibiotic resistance (AR) poses a global threat to humans, animals, and the environment [1]. AR in humans and different animal populations has increased in recent years. As noted in a recent report [2], European countries have dealt with 670,000 human infections with resistant bacteria in the year 2015. Third-generation cephalosphorin-resistant *Escherichia coli* (*E. coli*) were the major cause with 290,000 infections. In animals, *E. coli* has also been studied intensively in recent years since Extended-Spectrum-Beta-Lactamase/AmpC producing *E. coli* (ESBL/AmpC *E. coli*) have been detected in food-producing animals [3, 4]. *E. coli* have, however, not only been seen as very important pathogenic bacteria in humans and animals, but also as indicator bacteria or commensal bacteria in animals that may play a specific role in the transmission of AR genes from animals to humans [5, 6]. In order to tackle the increase in AR coming from numerous sectors, a multi-disciplinary approach is necessary, as humans, animals and the environment share similar resistance genes [7–12]. A "One Health" approach combines human, animal and environmental sectors in order to study, for example, transmission within and between the different reservoirs. "One Health"-based initiatives have been launched on national, European and global levels to act on the spread of AR [13–17]. In Germany, the National Action Plan on Antimicrobial Resistance (DART 2020, [18]) prioritizes adaption of this approach both nationally and internationally. One major challenge in adapting the "One Health" approach in Germany is the harmonization of data coming from various surveillance and monitoring systems on AR. First, in concordance with DART 2020, this study addresses the comparison of the various surveillance and monitoring systems on AR in human and veterinary medicine in Germany. Second, we describe resistance combinations in each population using phenotypic AR-data of non-clinical *E. coli* isolates from various food-producing animal populations including foods, clinical *E. coli* isolates from food-producing and companion animal populations and clinical *E. coli* isolates from different human populations collected through these surveillance and monitoring systems. *E. coli* is used as a model organism because of its prevalence in animals and humans, as well as the availability of respective data in Germany. In this study, non-clinical *E. coli* data from different food-producing animal populations and food defined the commensal *E. coli*. Finally, cluster analysis based on the relative frequencies of resistance combinations was used to study similarities in resistance combinations of *E. coli* isolates from the investigated populations.

Materials and methods

Ethic statements

For human datasets, this study has solely included anonymised routine surveillance data. Ethical approval for analysis of such surveillance data is not required according to the Medical Association's professional code of conduct. Data on antimicrobial resistance of *E. coli* from animals and food were collected in the framework of national monitoring projects and have been published in aggregated form in the National reports as provided in the reference list. The data basis of this analysis is presented in S4 Table.

Surveillance and monitoring of Antibiotic Resistance (AR) in Germany

Antibiotika-Resistenz-Surveillance (ARS) is the German national surveillance system for AR in humans. It is coordinated by the Robert Koch Institute (RKI) since 2007. The system collects routine laboratory data on AR in different bacterial pathogens that originate from clinical samples of patients in health care facilities (in- and outpatient care). It stores information on demographics (e.g. age and gender of the patients), type and region of health care facility as well type of hospital ward. Aggregated ARS datasets are sent to the European Antimicrobial

Resistance Surveillance Network (EARS-Net) in the European Centre of Disease Prevention and Control (ECDC) and published annually. The participation of the laboratories in ARS is voluntary [19]. Seventeen commercial diagnostic laboratories covering 187 hospitals and 3,436 general practices have participated continuously in ARS from the year 2014 to 2017 (Status: May 2020). Antimicrobial susceptibility testing (AST) is conducted in the laboratories with routine diagnostic procedures, such as automated broth-microdilution (ISO standard 20776–1) [20] or agar disk diffusion [21]. Results are presented as susceptible (S), intermediate (I) and resistant (R) (SIR) based on internationally harmonized evaluation criteria such as clinical breakpoints provided by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the Clinical Laboratory Standards Institute (CLSI).

Zoonosis-Monitoring (ZoMo) is the German monitoring system for AR in healthy food producing animals and food. It is a collaboration between federal institutions (German Federal Institute for Risk Assessment (BfR) and Federal Office of Consumer Protection and Food Safety (BVL)), regional veterinary and food safety authorities and regional public laboratories. *Zoonosis-Monitoring* has been implemented as national regulation according to Directive 2003/99/EC [22]. Details on mandatory bacteria-commodity combinations, antimicrobials used in the testing, laboratory methods and evaluation criteria for the determined minimum inhibitory concentrations (MIC) are fixed in Commission Implementing Decision (CID) 2013/652/EU [23]. In Germany, the federal states' food safety authorities annually decide on a sampling plan. They collect representative samples at different levels (farm, slaughter, retail) of different food chains according to this sampling plan. Regional laboratories run by the federal states isolate the bacteria from the samples and submit them to the National Reference Laboratory for Antimicrobial Resistance (NRL-AR). AST at the NRL-AR is done according to CID 2013/652/EU using broth-microdilution. For *E. coli* there is a fixed panel of 14 antibiotics used in the testing (S1 Table). The MIC values are interpreted using Epidemiological Cut-Off (ECOFF) values published by EUCAST and laid down in the CID. Results are reported to the European Food Safety Authority (EFSA) and included in the annual "European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food" [6]. At the national level they are reported annually by the BVL [24–27].

The *German Resistance Monitoring* (GE*RM*-Vet) on AR in animal pathogens is coordinated and conducted by the BVL. Based on §77 [3] of the German Medicinal Products Act (AMG), the BVL must report these data to the Federal Ministry of Food and Agriculture (BMEL) annually. Thirty-two participating public, private, and university laboratories submit voluntarily bacterial pathogens from diseased animals based on an annual sampling plan for different animal populations and indications. This annual sampling plan is established together with participating laboratories based on the experience from the previous years. Background information on the animals that has been sampled (e.g. age, disease) is also stored in the system. A customized BVL fixed panel of 24 antibiotics is used for AST in *E. coli* using broth microdilution (S1 Table). MIC values with CLSI breakpoints for animal pathogens are routinely reported [28–30]. Table 1 summarizes the comparison between the three German surveillance and monitoring systems.

Description of data and study design

We included *E. coli* data available in ARS, ZoMo and GE*RM*-Vet from January 2014 to December 2017. From ARS we took only data from laboratories and health care facilities in Germany, which participated in the system continuously from January 2014 to December 2017. The first isolate per patient per type of clinical specimen per year was used for the analysis. Screening samples, duplicate isolates (same type of clinical specimen from the same patient) and isolates with

Table 1. Comparison of surveillance and monitoring systems for AR in humans and animals in Germany.

Variable	ARS	ZoMo	GE*RM*-Vet
Type of bacteria	Human clinical isolates	Animal non-clinical isolates (commensal and food)	Animal clinical isolates
Participation	Voluntary	Mandatory	Voluntary
Population	Humans	Animal species and food	Animal species
AST panel	Not harmonized	Harmonized Panel	Harmonized Panel
		14 substances	24 substances
AST methods	Broth-Microdilution	Broth—Microdilution	Broth—Microdilution
	(kinetic growth curves)		
AST results	'susceptible', 'intermediary', 'resistant' (SIR) or MIC	Minimum Inhibitory Concentration (MIC)	Minimum Inhibitory Concentration (MIC)
Evaluation criteria	EUCAST / CLSI clinical breakpoints	EUCAST-ECOFFs	CLSI clinical breakpoints for animals
Accreditation	All laboratories	All laboratories	All laboratories

*AST: antimicrobial susceptibility testing.

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incomplete information were excluded. All used types of specimen are listed in Tables 2 and S3. This study focused on qualitative interpretation of AST (SIR) according to EUCAST clinical breakpoints. Further, ARS-data were classified by type of health care facility, i.e. human isolates from intensive care units (ICU), general wards and from outpatient care. We included all *E. coli* isolates from the annual sampling plans in ZoMo between 2014 and 2017. A summary of these data has been previously published in annual national reports [24–27]. ZoMo data include food-producing animals' isolates from farms, slaughterhouses and from food at retail from all German federal states (Table 2). Distribution of the samples across the federal states was proportionate to the number of animals of the targeted animal population in the federal state for samples taken on farms. For slaughterhouse samples, the distribution was proportionate to the slaughter capacity within the federal state for the targeted animal population. Numbers of samples at retail were based on the distribution of the human population. All materials are listed in Table 2.

The GE*RM*-Vet study year lasts from April to March from each observation year. In this study we included all *E. coli*-isolates, which had been collected from January 2014 to December 2017 (study years 2013 to 2017). A summary of the data has been published previously in annual reports [28–30]. The isolates originated from diseased animals, which had not been treated with antibiotics in the month prior to sampling. All materials along with the information on diseases are listed in Table 2.

Four antibiotics were selected for the cluster analysis: ampicillin (AMP), cefotaxime (CTX), ciprofloxacin (CIP) and gentamicin (GEN). They are included in the test panels of ZoMo and GE*RM*-Vet and likewise frequently tested in the medical laboratories reporting to ARS. Other relevant antibiotics for *E. coli* such as colistin, carbapenems, co-trimoxazol, tetracycline could not be included in this study because of the limited data available in the different systems. This will be further explained in the discussion section. All isolates from ARS, which had not been tested against all of these four antibiotics, were excluded from the analysis. EUCAST clinical breakpoints for human clinical isolates (S2 Table) were used to interpret the MIC-values from animal and food isolate data.

Statistical analysis

All MIC values were coded as 0 for susceptible and 1 for resistant. Intermediate results of human AST were interpreted as susceptible. Once the coding was complete, the relative

Table 2. *Escherichia coli* data for different populations collected from *Zoonosis-Monitoring*, GE*RM*-Vet and ARS from 2014 to 2017.

Isolate type	Origin	Populations	Materials	Year			
				2014	2015	2016	2017
Non-clinical animal isolates (27 populations, incl 9 food)	Farm (F)	Broilers, F	Faeces			X	
		Broilers Conv, F	Faeces			X	
		Broilers Org, F	Faeces			X	
		Laying hens, F	Faeces	X			
		Breeder chickens, F	Faeces	X			
		Turkeys, F	Faeces	X		X	
		Growers <50 kg, F	Faeces				X
		Weaners, F	Faeces from waiting area		X		
		Sows, F	Faeces of pregnant sows		X		
		Bovine milk, Conv, F	Bulk tank milk	X			
		Bovine milk, Org, F	Bulk tank milk	X			
		Bivalves, F	Both of shells meat			X	
	Slaughter (S)	Broilers, S	Pool from ten caecals	X		X	
		Turkeys, S	Pool from ten caecals	X		X	
		Bovines <1year, S	Caecals		X		X
		Fattening pigs, S	Caecals		X		X
	Retail (R)	Venisons, R	Fresh Meat				X
		Shrimps, R	Shrimps Meat		X		
		Broiler meat, R	Fresh meat with skin	X		X	
		Table eggs, R	Pool from ten eggshells	X			
		Turkey meat, R	Fresh meat with skin	X		X	
		Bovine meat, R	Fresh meat		X		
		Pork, R	Fresh meat		X		X
		Raw sausages, R	Fresh meat				X
		Bivalves, R	Both of shells meat	X		X	
	Wild/Game	Roe deer hunted, W	Faeces				X
		Wild boar hunted, W	Faeces			X	
Clinical animal isolates (C) (11 populations)	Farm/veterinary practice	Piglets, C	Faeces / Intestines/ Swab (Enteritis)	X	X	X	X
		Growers, C	Faeces / Intestines/ Swab (Enteritis)	X	X	X	X
		Pigs, C	Faeces / Intestines/ Swab (Enteritis)	X	X	X	X
		Sows, C	Not specified[+] (Mastitis-Metritis-Agalactie—MMA)	X	X	X	X
		Broilers, C	Not specified[+] (Septicemia)	X	X	X	X
		Laying hens, C	Not specified[+] (Septicemia)	X	X	X	X
		Turkeys, C	Not specified[+] (Septicemia)	X	X	X	X
		Bovines <1year, C	Faeces / Intestines/ Swab (Enteritis)	X	X	X	X
		Cattle, C	Faeces / Intestines/ Swab (Enteritis)	X	X	X	X
		Dairy cows, C	Not specified[+] (Mastitis)	X	X	X	X
		Small animals, C	Not specified[+] (Enteritis/Urinary Tract Infection)	X	X	X	X
Clinical human isolates (3 populations)	Outpatient	Humans, A	All kind of swabs, blood, punctate, respiratory tract samples, wound samples, urine and other samples[***]	X	X	X	X
	General Ward	Humans, Gw		X	X	X	X
	Intensive care unit (ICU)	Humans, ICU		X	X	X	X

[+]Clinical specimens are not specified, only disease information was obtained.

[*]Data collected from conventional (conv) and organic (org) farms.

[**]Small animals are cats and dogs.

[***]All details of materials are listed in S3 Table.

Materials indicate where the specimen that the isolates originated from. Year indicates the different sampling year plan for the non-clinical and clinical animal isolates and the food isolates.

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frequency of all 16 possible combinations of resistance to the four antimicrobials was calculated for each population using the permutation function ($2^4 = 16$). Resistance proportions were calculated using number of tested isolates for each population as denominator. The relative frequency of the resistance combinations (in %) was determined for each population. Building on Jasper et al. [31], we modified hierarchical clustering based on relative frequencies of the resistance combinations for phenotypical AR data. We did not use the suggested principal component analysis (PCA) for choosing the resistance combinations, since we had only four antibiotics included. We tested hierarchical clustering using numerous distance measures: single (nearest neighbor), complete (furthest neighbor), and average linkage (average between nearest and furthest neighbor) and Ward's method [32]. However, average linkage with Euclidean distance was selected since it produced the most meaningful results. A dendrogram and a heatmap were used to visualize the results. In addition to cluster descriptions based on the visualization in a dendogram, we used the elbow method and silhouette plot [33] for confirming the number of clusters. All analyses were run with R 3.5.1 (Rstudio 1.1.442).

Sensitivity analysis

In an attempt to test the robustness of the result we performed sensitivity analyses. We carried out four analyses, during which one antibiotic at a time was removed from the data. Thus, the total number of antibiotics in these reduced models was three, resulting in eight different resistance combinations each. Then, we used our clustering approach to further analyze the reduced models. Results were compared to clustering using all four antibiotics (complete model).

Results

Description of included isolates

333,496 *E. coli* isolates were included from ARS, ZoMo and GE*RM*-Vet between January 2014 and December 2017. 324,304 isolates (97.2%) originated from human populations, 5,743 isolates (1.7%) from healthy food-producing animals and food and 3,449 isolates (1.0%) from diseased animals. Extraction of the data for each surveillance and monitoring system is described in Fig 1A–1C. Most human isolates (210,005 isolates (64.8%)) originated from urine samples (S3 Table). Forty-one different populations were defined including 3 human populations, 18 healthy food producing animal populations, 9 food items and 11 diseased animal populations contributing clinical *E. coli* isolates (Table 2).

Resistance to the four antimicrobials in isolates from the different populations

Table 3 demonstrates individual resistance proportions of *E. coli* from the different human and animal and food populations to each antibiotic. Overall, resistance proportions were highest to ampicillin, followed by ciprofloxacin, cefotaxime and gentamicin. They ranged from 43% to 55% in human clinical isolates, from 1% to 70% in healthy food-producing animals including wild animals (game) and food and from 16% to 64% in clinical animal isolates. Human clinical isolates from ICU, isolates from several healthy poultry populations (broilers and turkeys from farm, and slaughterhouse and their meats at retail), and clinical isolates from bovines <1 year showed the highest resistance proportions to all included antibiotics.

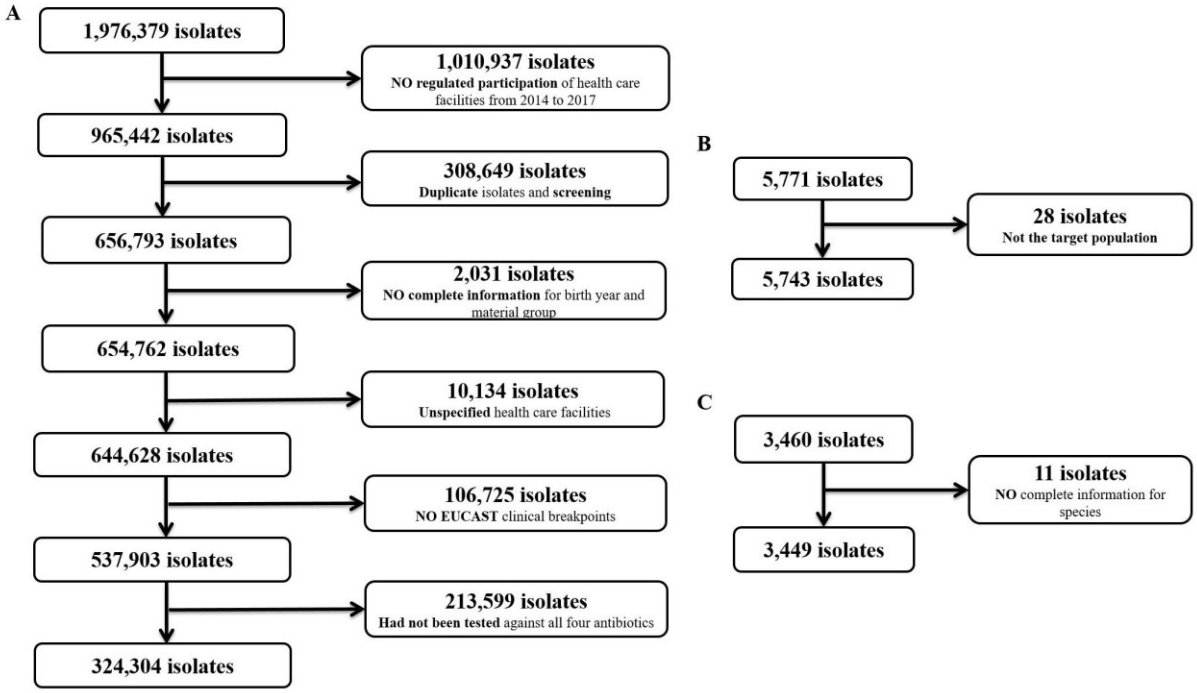

Fig 1. Data extraction from three surveillance and monitoring systems for AR. A) ARS system; B) Zoonosis-Monitoring and C) GE*RM*-Vet.

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Cluster analysis and overall relative frequencies of resistance combinations in the populations

Three clusters were detected within our dataset (Figs 2 and 3) by visualizing the dendogram and confirming with the elbow method and silhouette plot (S1 Fig). The heatmap (Fig 2) highlights 16 resistance combinations; starting from "susceptible to all" to "resistant to all" (left to the right). Each column represents the relative frequency of a resistance combination for each population. Human isolates were mostly exclusively resistant to ampicillin (26–29%), followed by resistance to ampicillin and ciprofloxacin (6–7%), and resistance to ampicillin, cefotaxime and ciprofloxacin (4–7%). Isolates from most healthy broiler and turkey populations reported higher resistance proportions to ampicillin only (46–50%) and to ampicillin and ciprofloxacin (14–19%) compared to most other populations.

Human isolates of all three populations clustered closely together in the first cluster (Figs 2 and 3). The isolates from the three human populations had similar relative frequencies of resistance combinations. The cluster also included isolates from 14 animal/food populations in two sub-clusters. Six of these were clinical isolates including subpopulations of all major food producing animal species (i.e. cattle, pigs, broilers and turkeys) and companion animals. Clinical isolates from cattle and piglets and non-clinical isolates from weaned piglets clustered closest to the human isolates. Two of the healthy poultry populations (broilers from organic farms and breeder chicken) are included in this cluster. They are separated from other healthy poultry populations in cluster three. The second cluster mainly included populations, which had low relative frequencies of resistance combinations (<25%) for all tested antibiotics and high proportions of isolates that were susceptible to all tested antimicrobials. The cluster mostly included food at retail, wild animals, laying hens, and bulk tank milk from dairy herds

Table 3. Individual resistance proportions (%) from different populations against four selected antibiotics; ampicillin (AMP), cefotaxime (CTX), ciprofloxacin (CIP) and gentamicin (GEN).

Isolate Type	Origin	Populations	Number of tested isolates [a] (N)	Resistance Proportions [95% CI] (%)			
				AMP	CTX	CIP	GEN
Non-clinical animal isolates (27 populations, incl 9 food)	Farm (F)	Weaners, F	250	50.8 [44.4; 57.2]	4.4 [2.2; 7.7]	4.0 [1.9; 7.2]	2.4 [0.9; 5.2]
		Laying hens, F	347	13.3 [9.9; 17.3]	3.2 [1.6; 5.6]	2.0 [0.8; 4.1]	0.9 [0.2; 2.5]
		Broilers, F	184	72.3 [65.2; 78.6]	2.2 [0.6; 5.5]	22.8 [17.0; 29.6]	1.1 [0.1; 3.9]
		Broilers Conv, F	299	70.2 [64.7; 75.4]	1.0 [0.2; 2.9]	18.7 [14.5; 23.6]	1.0 [0.2; 2.9]
		Broilers Org, F	31	22.6 [9.6; 41.1]	0 [0; 11.2]	9.7 [2.0; 25.8]	3.2 [0; 16.7]
		Turkeys, F	346	62.4 [57.1; 67.6]	0.6 [0.1; 2.1]	23.1 [18.8; 28.0]	8.4 [5.7; 11.8]
		Growers <50 kg, F	210	34.8 [28.3; 41.6]	1 [0.1; 3.4]	2.4 [0.8; 5.5]	1.9 [0.5; 4.8]
		Bovine milk, Conv, F	122	5.7 [2.3; 11.5]	0 [0; 3]	0.8 [0; 4.5]	0 [0; 3]
		Bovine milk, Org, F	74	1.4 [0; 7.3]	0 [0; 5.0]	0 [0; 5.0]	0 [0; 5.0]
		Breeder chickens, F	56	25.0 [14.4; 38.4]	0 [0; 6.4]	7.1 [2.0; 17.3]	5.4 [1.1; 14.9]
		Sows, F	24	26.5 [21.3; 32.1]	1.5 [0.4; 3.7]	1.8 [0.6; 4.2]	2.2 [0.8; 4.7]
		Bivalves, F	42	9.5 [2.7; 22.6]	0 [0; 8.4]	0 [0; 8.4]	2.4 [0; 12.6]
	Slaughter (S)	Broilers, S	404	57.2 [52.2; 62.1]	0.3 [0.1; 1.4]	10.6 [7.8; 14.1]	6.4 [4.3; 9.3]
		Bovines <1year, S	433	34.2 [29.7; 38.9]	1.8 [0.8; 3.6]	2.8 [1.4; 4.8]	0.9 [0.3; 2.3]
		Turkeys, S	372	63.7 [58.6; 68.6]	1.6 [0.6; 3.5]	19.6 [15.7; 24.0]	8.1 [5.5; 11.3]
		Fattening pigs, S	439	31.2 [26.9; 35.8]	2.5 [1.3; 4.4]	2.1 [0.9; 3.9]	0.5 [0; 1.6]
	Retail (R)	Venisons, R	150	2 [0.4; 5.7]	0 [0; 2.4]	0 [0; 2.4]	0 [0; 2.4]
		Shrimps, R	20	20 [5.7; 43.7]	10 [1.2; 31.7]	10 [1.2; 31.7]	5 [0; 24.9]
		Broiler meat, R	363	54.8 [49.5; 60.0]	4.4 [2.5; 7.1]	19.0 [15.1; 23.4]	3.0 [1.5; 5.4]
		Table eggs, R	90	11.1 [5.5; 19.5]	0 [0; 4]	1.1 [0; 6.0]	1.1 [0; 6.0]
		Turkey meat, R	356	67.4 [62.3; 72.3]	3.4 [1.8; 5.8]	21.3 [17.2; 26.0]	8.7 [6.0; 12.1]
		Bovine meat, R	115	11.3 [6.2; 18.6]	2.6 [0.5; 7.4]	0.9 [0; 4.8]	0 [0; 3.2]
		Pork, R	155	25.2 [18.5; 32.8]	2.6 [0.7; 6.5]	0 [0; 2.3]	0.6 [0; 3.5]
		Raw sausage, R	69	20.3 [11.6; 31.7]	1.5 [0; 7.8]	7.3 [2.4; 16.1]	1.5 [0; 7.8]
		Bivalves, R	58	8.6 [2.9; 19.0]	0 [0; 6.1]	3.5 [0.4; 11.9]	0 [0; 6.1]
	Wild/Game (W)	Roe deer hunted, W	269	1.5 [0.4; 3.8]	0.4 [0.1; 2.1]	0 [0; 1.4]	0 [0; 1.4]
		Wild boar hunted, W	217	0.5 [0; 2.5]	0 [0; 1.7]	0.9 [0.1; 3.3]	0 [0; 1.7]
Clinical animal isolates (C) (11 populations)	Farm/veterinary practice	Piglets, C	417	61.4 [56.5; 66.1]	6.5 [4.3; 9.3]	8.9 [6.3; 12.0]	7.2 [5.0; 10.1]
		Laying hens, C	557	15.6 [12.7; 18.9]	0.9 [0.3; 2.1]	2.3 [1.3; 4.0]	1.8 [0.9; 3.3]
		Bovines <1year, C	534	71.4 [67.3; 75.2]	30.5 [26.6; 34.6]	36.0 [31.9; 40.2]	29.4 [25.6; 33.5]
		Small animals, C	312	34.3 [29.0; 39.9]	10.3 [7.1; 14.2]	16.4 [12.4; 20.9]	5.8 [3.5; 9.0]
		Growers, C	129	63.6 [54.6; 71.9]	7.0 [3.2; 1.3]	2.3 [0.5; 6.6]	4.7 [1.7; 9.8]
		Broilers, C	232	35.4 [29.2; 41.9]	3.9 [1.8; 7.2]	5.2 [2.7; 8.9]	4.7 [2.4; 8.3]
		Dairy cows, C	378	18.5 [14.7; 22.8]	7.1 [4.8; 10.2]	6.1 [3.9; 9.0]	3.4 [1.8; 5.8]
		Turkeys, C	327	40.4 [35.0; 45.9]	0.3 [0; 1.7]	5.5 [3.3; 8.6]	4.3 [2.4; 7.1]
		Cattle, C	193	47.2 [39.9; 54.5]	14.5 [9.9; 20.3]	22.8 [17.1; 29.4]	15.0 [10.3; 20.9]
		Pigs, C	346	49.7 [44.3; 55.1]	5.2 [3.1; 8.1]	6.1 [3.8; 9.1]	4.6 [2.7; 7.4]
		Sows, C	24	29.2 [12.6; 51.1]	0 [0; 14.2]	4.2 [0.1; 21.1]	4.2 [0.1; 21.1]
Clinical human isolates (3 populations)	Outpatient (A)	Humans, A	96,455	42.7 [42.4; 42.9]	7.3 [7.2; 7.4]	15.2 [15.0; 15.3]	4.8 [4.7; 4.9]
	General Ward (Gw)	Humans, Gw	197,521	49.2 [49.2; 49.4]	11.5 [11.3; 11.6]	19.5 [19.4; 19.6]	5.8 [5.7; 5.9]
	Intensive Care Unit (ICU)	Humans, ICU	30,328	54.9 [54.4; 55.5]	15.8 [15.4; 16.1]	22.0 [21.5; 22.4]	6.9 [6.6; 7.2]

[a]Number of tested isolates is the sum of all sensible (0) and all resistant (1) isolates.

The denominator was number of isolates from the respective population tested against each antibiotic from 2014 to 2017.

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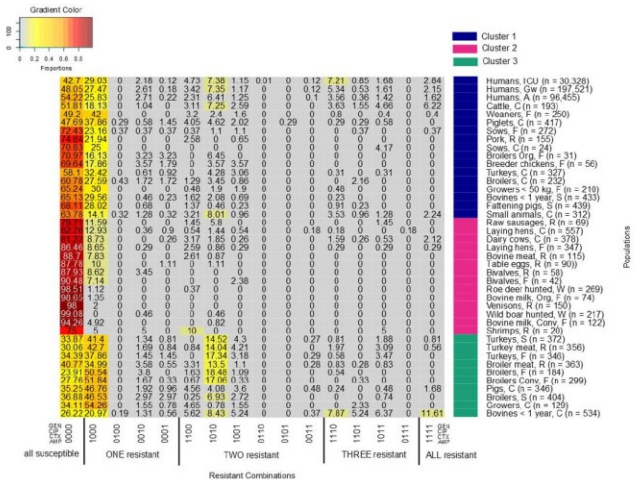

Fig 2. Heatmap describes different relative frequencies of resistance combinations for each population. From grey to red it shows the lowest to highest relative frequencies of resistance combinations. The x-axis explains the resistant combinations; from all susceptible (left) to all resistant (right). Antibiotics are described with '0' as 'susceptible' and '1' 'resistant'. The order is GEN (gentamicin), CIP (ciprofloxacin), CTX (cefotaxime) and AMP (ampicillin). The y-axis denotes each population together with their clusters.

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including from organic dairy production. The third cluster contained mostly healthy poultry (7 out of the 10 populations). Besides healthy poultry the cluster contained isolates from poultry meat, clinical isolates from pigs and, slightly separated, clinical isolates from young cattle.

Sensitivity analysis

Elimination of individual antibiotics from the model led to changes in the clusters (S2A–S2D Fig). In each elimination process, human inpatient isolates (ICU and general ward) always

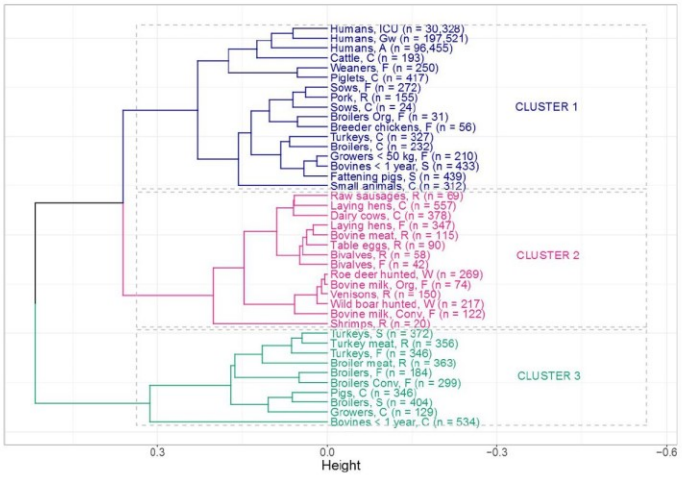

Fig 3. Cluster dendogram of different animal and human populations based on the relative frequency of resistance combinations to ampicillin, cefotaxime, ciprofloxacin and gentamicin. The x-axis describes the averaged similarities between the populations and between clusters. The y-axis shows each population and different clusters.

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clustered together. Likewise, the sub-clustering of isolates from some of poultry isolates (broilers from conventional farms and all isolates from turkeys (farm, slaughterhouse and meat)); wild animals (roe deer hunted, wild boar hunted and venisons); bovine milk from organic and conventional farms remained in same sub-cluster.

After eliminating ampicillin data, most healthy poultry isolates (except broilers from organic farms, breeder chickens and laying hens from farms and broilers from the slaughterhouse) clustered together with human isolates. Human isolates from outpatient care clustered closely with clinical isolates from small animals instead of with clinical isolates from cattle but remained the next neighbor to the inpatient isolates (S2A Fig).

After eliminating cefotaxime data, human isolates from ICU and general ward likewise clustered with most of healthy poultry populations, again with the exception of broilers from organic farms, breeder chickens and laying hens from farms. In this model, we found that human isolates from ICU and general ward cluster closely with broiler meat at retail. Without considering cefotaxime, human isolates from outpatient care clustered separately from those of inpatients indicating that resistance to cefotaxime might be important for their close association in the full model. As in the full model, they clustered with clinical isolates from cattle.

Eliminating ciprofloxacin data, human isolates from ICU and general ward clustered with isolates from weaners, clinical isolates from piglets and broiler meat at retail. Human isolates from outpatient care stayed in one cluster with human isolates from ICU and general ward but not as their closest neighbor. They clustered together with clinical isolates from turkeys. All healthy poultry populations, again except broilers from organic farms, breeder chickens and laying hens from farms, clustered separately.

By eliminating gentamicin, the model outcome did not differ substantially from the complete model.

Discussion

Cluster analysis provided information on similarities of *E. coli* isolates from humans and different animal populations based on their resistance combinations.

Human isolates from ICU and general ward always clustered together in cluster 1. Isolates from outpatient care were the next closest link in the full model and in two of the four reduced models. This finding supports the hypotheses that most ICU isolates are related to isolates from other parts of the hospital and from outpatients [34]. Studies on transmission within health-care-network and patient transfers have also supported this idea [35–37]. The slightly larger distance of the outpatient populations in comparison to the inpatient populations (general ward and ICU) might be explained by the specific situation in hospitals, with dominant hospital strains that differs from the outpatient setting [8]. Moreover, in the full model, isolates from the three human clinical populations clustered with clinical isolates from most (6/11) animal populations; i.e. cattle, piglets, sows, turkeys, broilers and small animals. The reason for these similarities between clinical isolates from human and different animal populations remains unclear as transmission of clinical isolates from animals to humans by contact or food is unlikely.

Isolates from most pig populations clustered together with the human clinical isolates (Cluster 1). This included clinical and non-clinical isolates from pigs. Prevalence of AR in pigs is associated with overall country-specific antimicrobial usage in livestock [38] Penicillins and tetracyclines are among the most frequently used antibiotics in pigs in Germany [39, 40]. This might explain the high proportions of resistance only to ampicillin in our study. Tetracyclines had to be excluded from this study as they were only tested in few medical laboratories. Their inclusion would have been associated with a substantial loss of data on the medical side as only

isolates tested against all study antimicrobials could be included. The highest proportion of ampicillin-resistance in this cluster was found in weaners (fattening piglets, up to 30kg body weight) from farms (42%) (Fig 2). Higher single ampicillin resistance in weaners in comparison to other pig populations may have been caused by a higher treatment frequency with penicillins in piglets around and weaning time to address streptococcal infections in comparison to older fattening pigs [39, 40]. Two groups of clinical isolates from pigs and growers clustered separately from the other pig populations in cluster 3. This separation was associated with higher proportions of resistance only to ampicillin in these two populations and lower proportions of susceptibility to all four studied antimicrobials than in the other pig populations.

As for healthy pigs, the transmission of bacteria from pigs to humans could be explained via food consumption. Pork is occasionally consumed raw in Germany. It is in line with our study findings, which found isolates from pork and human clinical isolates in the same cluster. However, the clinical isolates are not likely to be transmitted via food as food is harvested from healthy animals. Another possible explanation is the similar antimicrobial usage (AMU)-pattern between humans and pigs for the antimicrobials included which may create similar resistance patterns, as penicillins are also frequently used in humans. In that case, the clustering would have been caused by parallel developments rather than by transmission of isolates. This explanation could also embrace the clinical isolates.

Our study indicates separate clusters for clinical human isolates (cluster 1) and isolates from most healthy broilers (except broilers from organic farms), and turkey populations and their meat (cluster 3) and for laying hens (cluster 2). It has been reported that extended-spectrum cephalosporin-resistant *E. coli* from healthy poultry are unlikely to be the causative agents of human UTI [41]. Another study revealed low similarities of ESBL/AmpC genes between broilers and the general human population with the exception of the broiler farming communities [8] In line with that, our study indicates a lack of similarities in resistance to the four antimicrobials of *E. coli* from human and healthy broiler and turkey populations and laying hens.

In the third cluster, healthy broilers and turkeys along with their meats clustered together. AR in non-clinical *E. coli* isolates from broilers is associated with antimicrobial use in poultry production. Resistance proportions in *E. coli* to penicillins and fluoroquinolones are reported to be 40% higher in countries which have allowed the use of these two antibiotics in poultry than countries which have not [42]. In Germany, ampicillin and enrofloxacin, a fluoroquinolone with a similar chemical structure as ciprofloxacin, are authorized antibiotics for the treatment of poultry [43]. The total treatment frequencies of penicillins and fluoroquinolones in fattening turkeys and chickens are higher compared to pigs and cattle [39]. This might be the reason for higher individual resistance proportions against ampicillin and ciprofloxacin and the higher relative frequencies of the combinations of resistance to both substances compared to other populations [43].

Three non-clinical poultry populations: broilers from organic farms, laying hens and breeder chickens, and two clinical poultry populations: broilers and turkeys were not included in this third cluster (Fig 3). Broilers from organic farms, laying hens and breeder chickens have lower individual resistance proportions against the studied antimicrobials compared to the other healthy poultry populations. This is in line with earlier work on lower resistance proportions in broilers and turkeys from organic farms [44–46]. Lower antibiotic resistance rates might be caused by lower antibiotic usage in organic farming. EU legislation governing organic farming (Reg. (EC) No. 834/2007) foresees the use of antibiotics solely for diseased animals, if phytotherapeutic drugs, homeopathy and other products are not working. This includes the restriction on number of treatments and longer duration of withdrawal periods [47, 48]. This may contribute to a lower use of antibiotics in organic broiler farming compared

to conventional farming. However, valid specific use data from organic poultry farms are not available for Germany.

For breeder chickens and laying hens, low relative frequencies of resistance combinations were detected with resistance in laying hens even lower than in breeder chickens. Low single resistant proportions to the four chosen antibiotics in these two populations have been previously reported [49, 50]. Laying hens and breeder chickens received less antibiotic treatment than broilers, with the lowest antibiotic treatment in laying hens [51]. We, therefore, assume that the low relative frequencies of resistance combinations are associated with less antibiotic treatments received in laying hens and breeder chickens compared to broilers. Breeder chickens, i.e. parents and grand-parent flocks of production chicken, and laying hens live longer than broilers that only have a lifespan of approximately 4–6 weeks. It seems reasonable that the microbiome of breeder chickens and laying hens has matured [52, 53]. These microbiomes may be more competitive and resilient than those in young broilers contributing to less disease and therefore fewer treatments. Moreover, the housing conditions of breeder chickens are strictly controlled [54]. A controlled housing management might reduce the prevalence of pathogens and their transmission, which also results in fewer antibiotic treatments.

Clinical isolates from broilers and turkeys have lower resistance proportions to ampicillin compared to non-clinical isolates from broilers and turkeys (Fig 2). This applies also for the combined resistance proportions to ampicillin and ciprofloxacin. The reasons for these lower resistance rates in clinical isolates are however unclear and should be further investigated.

Isolates from wild animals, i.e. wild boars, wild roe deer and venison, clustered closely together with bulk tank milk both from conventional and organic farms. Isolates from these five populations showed the lowest individual resistance proportions and relative frequency of resistance combinations of all populations. Wild animals receive no antibiotic treatment, and therefore are not directly exposed to antimicrobials. However, wild animals were reported to carry AR commensal *E. coli* (non-clinical *E. coli* isolates) and play a role as sentinels of environmental transmission of AR [55, 56]. The presence of AR in wild animals has been associated to geographical distance to AR sources, such as wastes of antibiotic treated animals or humans [55], and also to human population density [57].

E. coli from bulk tank milk from both conventional and organic farms had low resistance rates and relative frequencies of resistance combinations. Low presence of AR in commensal *E. coli* (non-clinical *E. coli* isolates) from bulk tank milk has been previously reported [58, 59]. Low use of antibiotics in dairy cattle [51, 60] might result in low AR in the bacteria in milk. However, as *E. coli* is not part of the healthy milk microbiota and milk from *E. coli* mastitis is as a rule discarded, the most common source of *E. coli* in bulk tank milk is environmental, i.e. fecal contamination, mostly originating from the dairy herd [61] Improper milking-system hygiene also plays a role in milk contamination with coliform bacteria from the environment [62], but probably has no impact on their resistance patterns.

Clinical isolates from bovines <1 year had the highest individual proportions of AR for all four antibiotics as well as the highest relative frequency of the resistance combinations (Table 3 and Fig 2). This resulted in higher proportions of resistance combinations in comparison to other populations. Many of the isolates originated from young calves with enteritis. Use of waste milk may have contributed to the high resistance rates [63–65], given that penicillins and cephalosporins are frequently used in the treatment of mastitis of dairy cows [66, 67]. Waste milk is likely to contain residues of antimicrobials especially after intramammary treatment of dairy cows. This however cannot explain the comparatively high resistance rates to gentamicin and ciprofloxacin, as these substances are not frequently used in intramammary treatment. Further research into the dynamics of AR in calves is needed to improve the understanding of our study results.

Clinical animal isolates frequently clustered separately from their healthy animal counterparts. Our animal samples originated from two different independent datasets. There is no information whether they originated from the same farms. However, given the large number of farms and the limited number of isolates a large overlap of the source is unlikely. The separation might be caused by differences in selection pressure between the clinical and non-clinical isolates, although they originated from the same animal species and type of population. Non-clinical food-producing animal incl. food isolates were randomly sampled from each federal state in Germany. Clinical food-producing and companion animal isolates might be particular isolates from ill animals that form a specific subpopulation of *E. coli* strains. The GE*RM*-Vet study protocol states that the animals of origin should not have been treated with antibiotics within a month prior to sampling. However, it seems possible that these pathogenic isolates had prior specific antibiotic selection pressure in the animal population before the sampling time. An earlier study found the same tetracycline and aminoglycosides resistance genes in commensal (non-clinical isolates) and clinical *E. coli* [68]. Further research into the two different bacterial populations is necessary to better understand the reasons for the differences in AR.

With the sensitivity analysis we aimed to look into consistency of clusters built from the complete model (Fig 3). Some populations, i.e. human isolates from inpatient care (ICU and general ward) and isolates from wild animals and bovine milk from organic farm; remained in the same sub clusters consistently. This underlines their very close similarity with respect to resistance to the four antimicrobials and a distance to isolates from the other populations.

Removal of individual antimicrobials from the analysis also resulted in changes in cluster distributions compared to the complete model. The removal of one of the three antimicrobials —ampicillin, cefotaxime and ciprofloxacin—at a time made human clinical isolates from outpatient care change their position and nearest neighbors. This indicates a certain distance to the inpatient isolates. On the other hand, the change in the closest neighbor depending on the antimicrobial that was removed indicates that there was no clear relation to any individual other population. Removal of one antibiotic influenced the relative frequency proportions of resistance combinations. Resistance rates to ampicillin and ciprofloxacin were high in our study populations. Therefore, the removal of these two antibiotics substantially influenced the cluster order. In contrast, removal of gentamicin did not influence the clusters much. While a full analysis of these findings is outside the scope of this paper, we propose further analyses including additional antibiotics in order to understand the importance of different antibiotic usages in human and animal sectors.

There are a number of limitations to this study that must be acknowledged. Due to differences in the antimicrobials tested in the three systems, we had to choose four common antibiotics that overlapped between the three systems and for which sufficient data were available in ARS. Inclusion of further antimicrobials (e.g. tetracycline), would have reduced the number of available isolates in ARS substantially and would have excluded data from several laboratories, as those did not test *E. coli* for tetracycline resistance routinely. In ZoMo trimethoprim and sulfonamides are tested as individual substances, while in GE*RM*-Vet and human clinical isolates frequently a combination of a sulfonamide and trimethoprim is tested. Colistin and carbapenems have also not been taken into consideration. Colistin is used as a last resort antibiotic in the human sector. However, for methodological reasons phenotypical resistance data to colistin generated with automated methods are not considered reliable. Regarding carbapenems, different substances were used for animal clinical (imipenem) and non-clinical isolates (meropenem) and therefore data were not considered comparable. Moreover, resistance to carbapenem is extremely rare in animals [69] and also rare in humans in Germany [70].

We used SIR results based on clinical evaluation criteria for humans from EUCAST, as we could re-evaluate the quantitative data from the animal monitoring systems based on these

breakpoints. As for the human data, either no quantitative data were available or the tested range was so narrow that a re-evaluation according to ECOFFs was not possible.

This study highlights substantial differences between the three monitoring and surveillance systems (Table 1). Differences in data collection (surveillance versus monitoring), participation system (mandatory versus voluntarily), observed populations (humans versus different animal populations), AST (panel, methods and results) and evaluation criteria (clinical breakpoints and epidemiological cut-off values) should be carefully considered for comparative analysis. For the purpose of comparing resistance proportions, it would be desirable that the One Health community strives towards harmonized evaluation criteria for each antimicrobial in isolates from humans, food-producing animals and food. Alternatively, quantitative data, such as MIC values, need to be collected for allowing the interpretation using different standards based on any required analysis. Rational criteria should be shaped based on various purposes, such as for treatment decisions and comparative analysis of different resistant proportions across different sectors. Joint harmonized MIC value ranges for comparative analyses of human and animal data would better fit for the analysis.

Since routine standardized diagnostics differ between human and animal sectors, it needs to be investigated whether the different laboratory methods yield comparable results. Routine methods are always a compromise between scientific accuracy and economic needs. Increasing costs might discourage widespread use of costly and laborious AST methods in routine laboratories, an aspect that is less relevant in monitoring programs with limited numbers of isolates.

To the best of our knowledge, this is the first study that systematically compares the routine laboratory surveillance and monitoring systems for AR in humans with different animal populations and food of animal origin in Germany using cluster analysis. Within the limitations noted above, our results indicate that patterns of resistance combinations are able to provide insights in similarities and discrepancies between isolates from different human and animal populations. Given the current situation on surveillance and monitoring for AR in Germany, we considered it the best approach to compare the national data on AR in *E. coli* from humans, different animal populations and food based on their phenotypical resistance combinations. Regional analyses within the country and across countries might provide valuable additional insights. However, further stratification of the data would lead to very small strata for some of the populations. This would likely lead to exclusion of several populations from the analysis. In this study, we would like to avoid this type of exclusion to be able to validly compare as many populations as possible. Although phenotypic datasets are able to promote the study on resistance combinations, the findings of this study suggest a number of directions, which future studies on molecular level on AR might profitably take. Integration of whole genome sequencing (WGS) into surveillance might help further research into resistance genes similarities. Initiatives on implementation of WGS in AR monitoring system for animals have been already started [71, 72]. As genomic information provides better insights into resistance mechanisms, mobile genetic elements, chromosomal mutations and intrinsic resistance, its inclusion in the comparative analysis should be further promoted.

Conclusion

This study provides insights into possible analyses of AR phenotypical data from routine surveillance and monitoring in Germany. Despite differences in collected variables within the different surveillance and monitoring systems, cluster analysis has shown similarities and discrepancies between resistance patterns in isolates from humans and different animal populations for four frequently tested antibiotics. Using our datasets and analytical approach, we are not able to substantiate any transmission between humans, animals and foods. However, if the observed

populations clustered separately, it is unlikely that a substantial amount of transmission between the populations has occurred. Initiatives built based on these results might promote successful 'One Health' improvements across human and different animal populations in Germany.

Supporting information

S1 Table. Fixed antibiotic panel from *Zoonosis-Monitoring* for non-clinical (commensal)—and from GE*RM*-Vet for clinical (pathogen)—*E. coli*.
(DOCX)

S2 Table. EUCAST clinical breakpoints for humans (resistant).
(DOCX)

S3 Table. Details on all clinical samples from ARS systems along with absolute numbers and percentages.
(DOCX)

S4 Table. Raw data from 41 different human and animal populations from 2014 to 2017 in Germany.
(DOCX)

S1 Fig. Determination of clusters. Results of the A) elbow method and B) silhouette plot to determine and confirm the optimum number of clusters.
(DOCX)

S2 Fig. Sensitivity analysis. Results of cluster analysis considering A) only cefotaxime, ciprofloxacin and gentamicin (i.e. without ampicillin), B) only ampicillin, ciprofloxacin and gentamicin (i.e. without cefotaxime), C) only ampicillin, cefotaxime and gentamicin (i.e. without gentamicin) and D) only ampicillin, cefotaxime and ciprofloxacin (i.e. without gentamicin).
(DOCX)

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3.1. Role of the authors for the second publication

Name	Author's Role	Author's contribution
Beneditta Suwono	First author	Conceptualisation, data curation, methodology, data validation, visualization, project management, literature researches, statistical analysis, and preparation the manuscript, incl. writing, reviewing and editing the manuscript.
Tim Eckmanns	Co-author	Conceptualisation, resources for data from ARS, data validation, methodology (supervision), reviewing and editing the manuscript.
Heike Kaspar	Co-author	Resources for data from GE*RM*-Vet, data validation for GE*RM*-Vet, reviewing and editing the manuscript.
Roswitha Merle	Co-author	Statistical analysis (methodology validation and review), reviewing and editing the manuscript.
Benedikt Zacher	Co-author	Statistical analysis (supervision), reviewing and editing the manuscript.
Chris Kollas	Co-author	Data validation for Zoonosis monitoring, reviewing and editing the manuscript.
Armin A. Weiser	Co-author	Data validation for Zoonosis monitoring (mentoring), reviewing and editing the manuscript.
Ines Noll	Co-author	Data validation for ARS (mentoring), and reviewing the manuscript.
Marcel Feig	Co-author	Data validation for ARS (mentoring), and reviewing the manuscript.
Bernd-Alois Tenhagen	Corresponding author	Conceptualisation, data validation, study supervision, resources for data from Zoonosis monitoring, reviewing and editing the manuscript.

4. Third Publication

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A joint regional analysis of resistance combinations in *Escherichia coli* in humans and different food-producing animal populations in Germany between 2014-2017

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A Joint Regional Analysis of Resistance Combinations in *Escherichia coli* in Humans and Different Food-Producing Animal Populations in Germany Between 2014 and 2017

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A joint comparative regional analysis of different resistance combinations across human and veterinary medicine has not been previously conducted in Germany. This study analyses 16 resistance combinations from four antibiotics in *E. coli* from different human and food-producing animal populations in three German regions: East, North West and South West. The *E. coli* data were collected from the three national surveillance and monitoring systems for antimicrobial resistance (AMR) bacteria in humans (ARS), food-safety (Zoonosis Monitoring) and animal pathogens (GE*RM*-Vet) from January 2014 to December 2017. Analyses were performed using cluster analysis (hierarchical clustering, average linkage) in R. We included data from 537,215 *E. coli* isolates from human clinical isolates, from clinical as well as non-clinical isolates from food-producing animals and from food. The majority of the data originated from the North West region. There were two main clusters built on 54 different human and animal populations. We observed close similarities of resistance combinations in human isolates from the different regions within the same human populations from outpatient cares, general wards and ICUs. These resistance combinations clustered separately from non-clinical isolates from broilers, turkeys, cattle and pigs; except for some of clinical isolates from these populations which clustered closely to isolates from human populations. Frequently, the resistance combinations in *E. coli* isolates from farms clustered closely to the resistance combinations in isolates from slaughterhouses from broilers and turkeys over all regions. However, the resistance combinations in *E. coli* isolates from retail meat populations tended to cluster separately within their respective populations in between all regions.

Keywords: antimicrobial resistance, *Escherichia coli*, regional analyses, surveillance and monitoring systems, resistance combinations

INTRODUCTION

In Germany, regional differences in the prevalence of antimicrobial resistant (AMR) bacteria have previously been observed. In humans, the occurrences of carbapenem resistant *Klebsiella pneumoniae* and *Acinetobacter baumanii* (1, 2), vancomycin resistant *Enterococcus faecium* (3), methicillin resistant *Staphylococcus aureus* (MRSA) (4), and uropathogenic *Escherichia coli* (5) varied between German regions. Such regional differences have also been observed in e.g., occurrence of MRSA in dairy cows in Germany (6). Although regional differences in Germany were previously studied for the resistance patterns in different human and food-producing animal pathogens, a comparative regional analysis of resistance combinations between human and different food-producing animal populations has not previously been conducted. This interregional comparison analysis is important, since regional differences in resistance of bacteria from humans and different animal populations might be associated with exchange of bacteria between humans and animal populations within region (7). Therefore, the goal of this study is to compare the resistance patterns in *E. coli* isolates from humans and different food-producing animal populations considering four antibiotics—ampicillin (AMP), cefotaxime (CTX), ciprofloxacin (CIP), and gentamicin (GEN)—between three different regions of Germany: East, North West and South West. It will challenge the hypothesis that similar patterns of resistance are observed in different populations of the same region along with differences in patterns between regions in Germany.

METHODS

Data Selection

We divided Germany into three regions based on the population structure of different animal populations as previously described by Tenhagen et al. (6). The "East" region is characterized by a low number of herds with a large herd size and an overall low regional animal density. It included Berlin, Brandenburg, Mecklenburg Western Pomerania, Saxony-Anhalt, Saxony, and Thuringia. The "North West" region is characterized by a high number of animal populations with a high regional animal density and a smaller, but still rather large, herd size compared to region East. It includes Schleswig Holstein, Lower Saxony, North Rhine Westphalia, Bremen, and Hamburg. The "South West" region, which is characterized by a high number of animal populations with a high regional animal density and a comparatively small herd size, represents Bavaria, Baden-Wuerttemberg, Hesse, Rhineland Palatinate, and Saarland.

The data for this study were collected between January 2014 and December 2017. For the same study period, we previously studied similarities in resistance patterns of *E. coli* isolates from different human and animal populations for the whole of Germany (8). Data on human isolates originated from the *Antibiotika Resistenz Surveillance* (ARS) system (9). All data on non-clinical *E. coli* isolates from food-producing animals and food came from the Zoonosis Monitoring that were collected in Germany (10). There were no non-clinical isolates collected from cattle from farms during this study period. Data on clinical isolates from animals were taken from GE*RM*-Vet (11), the system for the monitoring of resistance in animal pathogens in Germany. Detailed information on these systems was summarized in a previous study (8).

Four frequently tested antibiotics in ARS, Zoonosis Monitoring and GE*RM*-Vet - ampicillin, cefotaxime, ciprofloxacin, and gentamicin—were included. The sixteen resistance combinations to these four antibiotics—were calculated using the permutation function. Detailed information on the inclusion criteria has previously been described (8). This study included *E. coli* isolates from humans, broilers, turkeys, pigs, and cattle populations stratified by their origins: for human populations outpatient care (A), intensive care unit (ICU), general ward (GW); for non-clinical animal populations, farm (F), slaughterhouse (S) and retail (R) and for clinical animal populations (C) (8) (**Supplementary Table 1**).

For the purpose of cluster analysis, each population was split into three different regional sub-populations: East, North West and South West. In total, there were 54 regionally stratified populations derived from the in total 18 populations for human and different animal populations in each region. All 54 regional populations were included in one model. For the analysis several pig populations (growers, sows, fattening pigs, piglets and weaners) and cattle populations (bovines <1 year and dairy cows) had to be grouped into "pigs" and "cattle" respectively to account for small sample sizes in the sub-populations. Eleven non-clinical animal populations and two clinical animal populations from the national model (9) were excluded from this study on account of too few isolates in the regions (**Supplementary Table 2**).

Statistical Analysis

This study used cluster analysis to analyze similarities of sixteen resistance combinations between different human and animal populations in three German regions. Cluster analysis on resistance combinations was performed with the hierarchical clustering using Euclidian distance and the average linkage. This method was adapted from the previous study on statistical methodology for analysis of multi-drug resistant bacteria by Jasper et al. (12). For the purpose of our study, the step "multiple correspondence analysis" to reduce number of resistance combinations was excluded. This was not necessary for our datasets since there were only 16 resistance combinations built from four antibiotics. Similar to the previous study, average linkage was chosen because of inclusion of all study populations. The number of clusters was determined visually by the silhouette plot (8) and elbow method. In this study, we defined main clusters (Cluster) and sub-clusters (SC) to support the readers for differentiating the populations in the cluster visualization. All analyses were conducted with R 3.5.1 (Rstudio 1.1.442). The same R-packages as previously described were used (8).

RESULTS

Descriptive Analysis

Data were collected from 537,215 *E. coli* isolates from ARS, Zoonosis Monitoring, and GE*RM*-Vet. The data extraction from each system has been previously described (8). The number

of farms, animals, animals per farms, human populations, participating hospitals, and general practices in ARS systems, and numbers of *E. coli* isolates from different systems for different populations in each study region are summarized in **Supplementary Table 3**. After the exclusion of non-target populations for this study (**Supplementary Table 1**), 327,416 isolates were included in this study. Out of these isolates, 320,555 isolates (98%) originated from human populations: 30,328 isolates from ICU (9.3%), 197,521 isolates from general ward (60.3%) and 92,706 isolates (28.3%) from outpatient care; 4,298 isolates were non-clinical isolates (1%) from food-producing animals and food, and 2,563 isolates (1%) were clinical isolates from food-producing animals (**Supplementary Table 3**).

Cluster Analysis

The elbow and silhouette methods suggested two main clusters (**Supplementary Figure 1**). Cluster 1 includes the majority of all populations (33 populations, 61%) including all nine populations of clinical isolates from humans, 17 populations of non-clinical animal isolates from food-producing animals and foods, and seven populations of clinical isolates from food-producing animals. The second cluster contains 21 different food-producing animal populations (39%) with 16 populations of non-clinical animal isolates from food producing animals and foods and five populations of clinical isolates from food-producing animals (**Figure 1**).

All isolates from the different **human populations** from all three different regions clustered next to each other in one sub-cluster (**Figure 1**, Cluster 1, SC 1.1.2). Within SC 1.1.2 there were two slightly separated groups. One of those contained all human isolates, while the other contained mainly poultry isolates. Isolates from humans in general wards from the three regions clustered closely together (Humans_Gw_South_West, Humans_Gw_North_West, and Humans_Gw_East). Isolates from ICUs were their closest neighbor. The isolates from humans in outpatient care facilities from the North West (Humans_A_North_West) and the East (Humans_A_East) clustered closely together separated only slightly from the other human isolates. Clinical isolates from two food-producing animal populations clustered in the same part of the sub-cluster with the isolates from humans: clinical isolates from broilers in the North West (Broilers_C_North_West) and clinical isolates from pigs from the South West (Pigs_C_South_West). At a slightly larger distance, this part of the sub-cluster contains also isolates from broilers and turkeys on farms in the South West (Broilers_F_South_West and Turkeys_F_South_West).

The isolates from broilers on farms in the North West (Broilers_F_North_West) and the East (Broilers_F_East) clustered together in one sub-cluster that predominantly contained poultry isolates (Cluster 1, SC 1.1.1). It also included the respective regional isolates collected at slaughter. The isolates from broilers on farms in the South West (Broilers_F_South_West) clustered (Cluster 1, SC 1.1.2) separately from broilers from the same region at the slaughterhouse (Cluster 2, SC 2.1.1). Isolates from broiler meat at retail from the three different regions all clustered together (Cluster 1, SC 1.1.2). In contrast, clinical isolates from broilers from the three regions clustered separately from each other in different main clusters (SC 1.1.2, 2.1.1 and 2.2 respectively).

Most isolates from **turkeys** clustered in two different sub-clusters in cluster 1. All non-clinical isolates from turkeys in the North West on farms, in slaughterhouses and at retail clustered together in SC 1.1.1 (Turkeys_F_North_West, Turkeys_S_North_West, and Turkeys_R_North_West). The SC 1.1.2 contains all non-clinical isolates from turkeys in the South West (i.e., farm, slaughterhouse, retail). It also contained isolates from farms and from the slaughterhouses from the East (Turkeys_F_East and Turkeys_S_East). Clinical isolates from turkeys from the three regions all appeared in different subclusters (Turkeys_C_North_West in SC 1.1.2, Turkeys_C_South_West in SC 1.1.1 Turkeys_C_East in SC 2.1.1).

Nearly **all non-clinical isolates from pigs** clustered together in Cluster 2, SC 2.1.1. Only isolates from pork at retail in East (Pigs_R_East) clustered separately (SC 2.2). They all clustered separately from the isolates from the "human cluster" (SC 1.1.2). In SC 2.1.1, the non-clinical isolates from pigs clustered together with clinical isolates from broilers in the South West (Broilers_C_South_West) and turkeys in the East (Turkeys_C_East). It clustered also together with isolates from two cattle populations (Cattle_R_South_West and Cattle_S_North_West). The clinical isolates from pigs clustered separately from the non-clinical isolates from pigs. Clinical isolates from pigs from two different regions (Pigs_C_South_West and Pigs_C_East) clustered in the same sub-cluster with the isolates from humans (SC 1.1.2). The clinical isolates from pigs from the North West (Pigs_C_North_West) clustered in SC 1.1.1 and were the only non-poultry isolates in that cluster.

The isolates from cattle clustered in both clusters, one population in Cluster 1 and eight populations in Cluster 2. Interestingly, all clinical isolates from cattle clustered separately from the isolates from other food-producing animal populations. The clinical isolates from cattle from the North West (Cattle_C_North_West) clustered alone in one sub cluster (SC 1.2), those of the other regions were alone in SC 2.1.2. The isolates from cattle in slaughterhouses in the South West and the East, (Cattle_S_South_West and Cattle_S_East) clustered together in the same sub-cluster (Cluster 2.2) that also included the isolates of bovine meat from the East. The isolates from these two regions clustered separately from the isolates from the slaughterhouse and from meat at retail in the North West (Cluster 2, SC 2.1.1). The isolates from bovine meat at retail were separated according to region: Cattle_R_South_West in SC 2.1.1, Cattle_R_North_West and Cattle_R_East in SC 2.2.

DISCUSSION

This study compared resistance combinations in *E. coli* from different populations in three German regions. It built upon a previous study (8) to investigate potential regional associations of AMR bacteria between isolates from different human and food-producing animal populations. As observed in the earlier

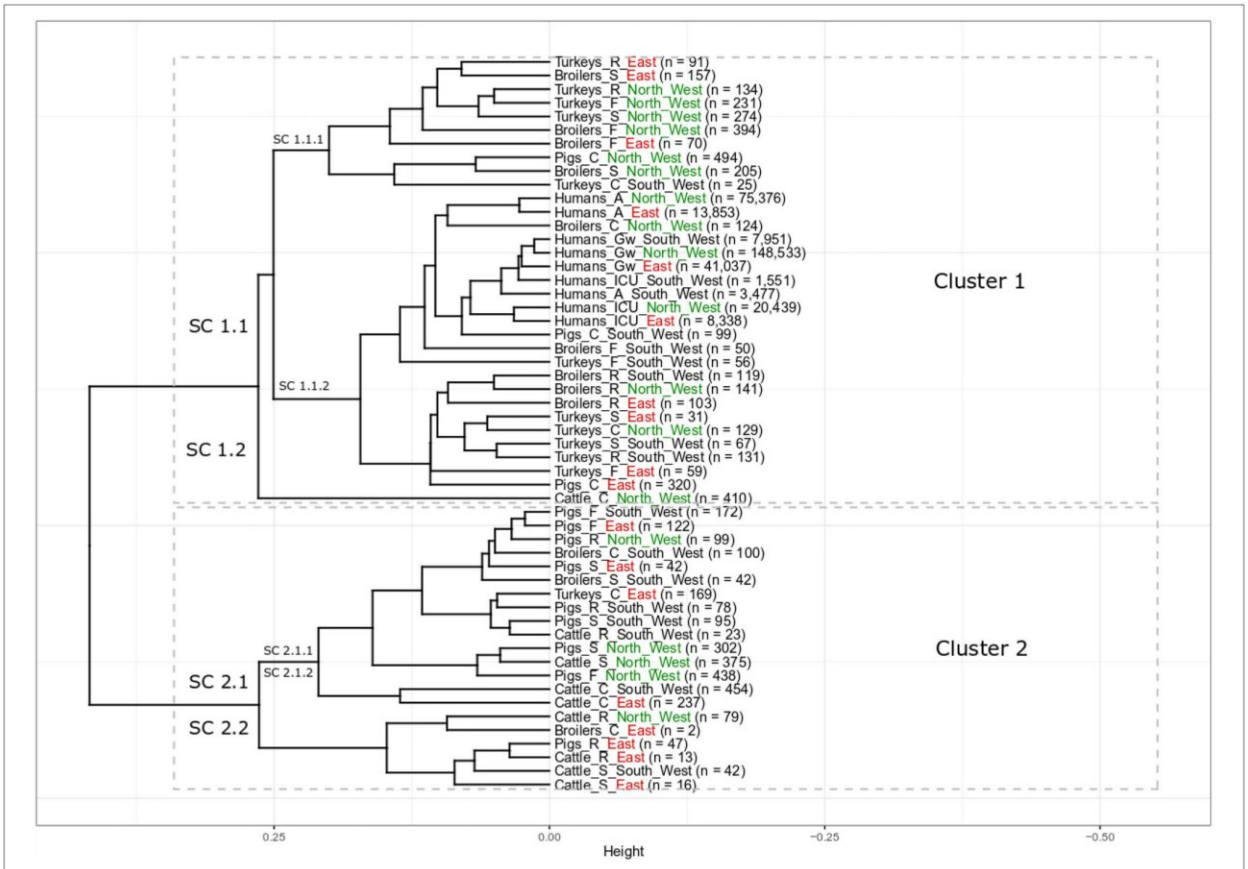

FIGURE 1 | Results of cluster analysis of the resistance combinations in *E. coli* isolates from 18 grouped populations from different human and food-producing animal populations between 2014 and 2017. There are two main clusters with eight sub-clusters (SC). Human population: A: isolates from outpatient care, Gw: isolates from general ward and ICU: isolates from intensive care unit. Animal population: F: isolates from farms, S: isolates from slaughterhouse and R: isolates from retail, C: clinical isolates from animals. n is the total number of included isolates. For better regional separation, the regions are additionally color coded with North West in green, East in red and South West in black.

study, all human isolates from different health care facilities clustered together. This was confirmed regardless of the different regions of origin. However, the different levels of the health care facilities (outpatient, general ward and intensive care) tended to cluster together across regions indicating a stronger association of the level of health care as compared to the regional stratification. This effect was less pronounced with the isolates from outpatient care where isolates from the North West and the East were slightly separated from those from the South West. This separation remains however unclear and should be considered in the further comparative analyses between German regions. As the level of antimicrobial use tends to differ between the different levels of health care facilities, more differences between samples from these subpopulations might have been expected. However, detailed data on antimicrobial use in these populations in Germany are not available and therefore cannot be used in the analysis. This should be addressed in future studies. The close similarities of human isolates reported from this study confirmed previous study that was carried out addressing ESBL/AmpC genes specifically (13). In addition to that, a population-based study in Netherland reported that most of ESBL producing bacteria in the general population of the Netherlands was probably originated from other human populations (7).

Closer regional associations were seen for some of the food-producing animal populations. Most poultry populations were in cluster 1 and often isolates from farms clustered in the same sub-cluster with isolates from the slaughterhouses from the same region. These animals will frequently be slaughtered in the same region that they are raised in to avoid long transport. An exception was observed for the South West, where broilers at farm and at slaughter were in two different main clusters (1 and 2, respectively). All isolates from pigs at farm and pigs at slaughter from the same region were observed in the same sub-cluster (2.1). For cattle, this association could not be studied as no non-clinical isolates had been collected at the farm level in the period. The non-clinical food-producing animal isolates coming from farms

and slaughterhouses were collected in the framework of food-safety monitoring in Germany. These isolates are mandatorily collected from the German domestic primary productions, i.e., excluding slaughter batches from neighboring countries that may have different levels of antimicrobial resistance (14, 15).

In contrast, all samples from broiler meat at retail from all regions were in the same cluster as closest neighbors indicating that isolates from broiler meat sold in different parts of the country share similar AMR patterns. This was not observed for turkey meat (two populations in SC 1.1.1, one in 1.1.2), pigs (two in SC 2.1.1, one in 2.2) or cattle (one in SC 2.1.1 and two in 2.2). Retail samples were not restricted to domestic production and therefore may include products originating for other EU-Member states or even third countries. Moreover, some isolates on meat may originate from contamination at slaughter or during further processing. This might explain some differences between the slaughterhouse and the retail level. Proximity of broiler meat isolates from different regions might indicate trade of broiler meat across the country, irrespective of region. This indicated a more regional trade of turkey, pig and bovine meat (15). In line with that, in two regions turkey meat clustered closely with turkeys at slaughter. However, trade data to confirm this are not available.

For the regional model, we gathered the isolates from different cattle and pig populations to the species level, to account for the small sample sizes in the three regions (**Supplementary Table 2**). The monitoring programs in the food chain are not designed for regional stratification but for national estimates. Therefore, samples are assigned to regions proportionate to the size of the respective population in the region to better reflect the national population. In consequence, sample sizes may be small in some regions, if most of the food-producing animals are housed in other regions.

In this study, the clinical isolates from cattle (Cattle_C) predominantly contain the clinical isolates from bovines <1 year. In the previous national model, the clinical isolates from bovine <1 year clustered separately in one main cluster due to their higher relative frequencies of all resistance combinations than other isolates from cattle populations (8). In this study clinical isolates from cattle in the North West, also formed a cluster of their own and those from the East and the South West formed a separate sub-cluster, indicating that resistance patterns in clinical isolates from cattle differ substantially from the other bacterial populations and between the North West where most of the veal calves are raised and the South West and East.

This study addresses the similarity of resistance combinations of AMR bacteria between human and different animal populations. These data were obtained in three different systems and it could be speculated that differences in the resistance combinations reflect differences in the systems. However, we recently retested isolates from medical laboratories using broth microdilution as used in the animal and food isolates. We found a good agreement of the results (16). As previously described in the national model (8), the close similarities of resistance combinations between clinical isolates from different animal populations and clinical isolates from human in different levels of health care facilities were also observed in this study. Additionally, we observed close similarities of resistance combinations between pigs- and poultry populations in different German regions. These similarities were also reported in the national model and earlier study that reported high ampicillin resistance in pigs and poultry populations (8, 17). However, the transmission of AMR bacteria between humans and different animal populations in different German regions remains complex and cannot be unraveled with our datasets. The data was mostly collected in the North West, both for the different human populations and different food-producing animal populations. This is in line with the high density of livestock production in the North West (18) and high number of participating health care facilities in the human surveillance system (1–3) (**Supplementary Table 3**). We studied only resistance to four antibiotics that were routinely included in all three monitoring and surveillance systems in one country. The situation and the clustering of isolates from the different populations may differ substantially in other countries with different treatment patterns and level of antimicrobial use as indicated by data on antimicrobial consumption in food-producing animals and humans provided by the European institutions (19, 20). A number of antimicrobials had to be excluded as they were only tested in one or two of the studied systems. This calls for a better harmonization of resistance testing in the one health context. Due to the structure of surveillance and monitoring datasets the regional analyses are limited. Additional indicators such as trade (21), and animal movement (22) should be considered in further studies. Further investigations on the food-chain network between the European countries will support as well further explanations on the variation of resistance combinations between the countries, as these countries have different regulations on monitoring systems (23).

To the best of our knowledge, this study is the first study on regional analyses of resistance combinations in different human and food-producing animal populations in Germany. Regional cluster analysis with the routine phenotypical AMR data underlines the complexity of the relationship between AMR in human and different animal populations. It also underlines that the human isolates tend to cluster together and separate from most of the healthy food-producing animal isolates. Further regional analyses should consider additional information such as structures of counties, e.g. rural and urban, other relevant antibiotics, and information on trade and animal movement in the country.

DATA AVAILABILITY STATEMENT

The aggregated data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

BS and B-AT conceptualized the study. B-AT, TE, and HK provided the datasets. BS analyzed the datasets and wrote the manuscript. B-AT, TE, and HK read and reviewed the manuscript. All authors have read and agreed on submission of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2022.823613/full#supplementary-material

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4.1. Role of the authors for the third publication

Name	Author's Role	Author's contribution
Beneditta Suwono	First author	Conceptualisation, data curation, methodology, data validation, visualization, project management, literature researches, statistical analysis, and preparation the manuscript, incl. writing, reviewing and editing the manuscript.
Tim Eckmanns	Co-author	Conceptualisation, resources for data from ARS, data validation, methodology (supervision), reviewing and editing the manuscript.
Heike Kaspar	Co-author	Resources for data from GE*RM*-Vet, data validation for GE*RM*-Vet, reviewing and editing the manuscript.
Bernd-Alois Tenhagen	Corresponding author	Conceptualisation, data validation, study supervision, resources for data from Zoonosis monitoring, reviewing and editing the manuscript.

5. General Discussion

5.1. Key findings

This thesis has conducted the first joint comparative analysis of phenotypical AMR data from the selected German surveillance and monitoring systems in human and veterinary medicine and in the food chain. *E. coli* was used as a model organism due to its importance in human and animal populations as well as the availability of relevant data over time. The five research questions were analysed in three publications (Suwono et al. 2022, Suwono et al. 2021a, Suwono et al. 2021b) and the answers to these questions are summarised below.

In the first publication, we found that the MICs of *E. coli* isolates from different methods used in the medical laboratories and in the National Reference Laboratory for AMR (NRL-AR) at BfR for food safety monitoring were comparable **(research question number 1)**. This was highlighted by the strong agreement between MICs resulting from automated AST and broth microdilution for 11 overlapping antibiotics. The comparability of the MICs allowed us to further analyse the integrated phenotypical *E. coli* datasets from the selected surveillance and monitoring systems to address the second to fifth research question.

In the second publication, different variables that are routinely collected by surveillance and monitoring systems for bacterial AMR in human (ARS) and veterinary medicine (Zoonosis Monitoring and GE*RM*-Vet) were first identified and compared **(research question number 2)**. This analysis aimed to examine overlapping variables that should be considered or adjusted when studying the possible transmission of *E. coli* isolates between human and different animal populations. It revealed the comparability of origin of *E. coli* isolates (humans: clinical isolates from outpatient care, general ward and intensive care unit (ICU); animals: non-clinical isolates from farm, slaughterhouse, foods in retail outlets and clinical isolates from farms or veterinary practices), AST panels (harmonised vs. not harmonised panels), AST methods (automated AST with kinetic growth curves vs. broth-microdilution), AST results (SIR or MICs), evaluation criteria (EUCAST vs CLSI) and federal states (*Bundesländer*). Using these comparable variables, this thesis has focused on resistance combinations against four antibiotics that were frequently tested in all the selected surveillance and monitoring systems. The resistance combinations of *E. coli* isolates were then investigated in the selected human and different animal populations and studied with cluster analysis **(research question number 3).** Detailed information regarding the methodological approach is explained in the next chapter (Chapter 5.2.). The cluster analysis highlighted the similarities in the resistance combinations in the human and different animal populations **(research question number 4)**. The similarities

in the resistance combinations demonstrated the possible transmission of *E. coli* isolates within and between human and different animal populations. However, this should be cautiously interpreted since the direction of transmission, such as animals to humans, humans to animals, animals to animals or humans to humans, could not be identified. By applying the same statistical methods as in the second publication, the third publication assessed different resistance combinations in *E. coli* isolates in relation to three German regions: East, North West and South West **(research question number 5)**. These findings highlighted the potential inter- and intraregional transmission of *E. coli* between the human and different animal populations.

By using *E. coli* as a model organism this thesis was able to highlight comparable phenotypical AMR datasets from different surveillance and monitoring systems in Germany. It allowed detailed analyses regarding the possible transmission of *E. coli* isolates within and between human and different animal populations based on the similarities in the resistance combinations. Although some challenges of comparison analyses were described in the three publications, these thesis findings will enable a better understanding of intersectoral transmission pathways and integration of bacterial AMR data from surveillance and the monitoring systems in Germany within the One Health framework in Germany.

5.2. Methodological Approach

AMR data from surveillance and monitoring systems for human and different animal populations have been compared in a range of cases, especially within European countries (ECDC et al. 2021). Identifying the standardised methods for comparing these data is crucial in light of the varying AST methods for routine diagnostics within human and veterinary medicine. In medical laboratories, AST is often conducted with automated methods based on broth microdilution with kinetic growth curves, while semi-automated broth microdilution is used for food safety monitoring in veterinary laboratories. This difference could result in minor discrepancies in AST results (MICs) for different antibiotics.

For the purpose of the first publication (Suwono et al. 2021b), an analysis was conducted with laboratories that routinely submit MICs determined by automated AST systems to the ARS system. The study's protocol and report are attached in Supplementary File 9.1. In August 2018, 40 laboratories out of 66 (61%) sent MICs from between 2014 and 2017 to the ARS system. The six laboratories that tested tetracycline before the study period (2019) were selected for the comparison study of MICs. One additional laboratory was included due to the large number of isolates that originated from this laboratory, although this laboratory did not conduct AST for tetracycline. In total, seven laboratories were invited to the study, and six out

of seven were willing to participate (Figure 3). The study of MICs demonstrated the comparability of *E. coli* resistance data from the human surveillance and monitoring systems for different animal populations, including food. This was a validation of the approach for further comparative analyses of the phenotypical data regarding *E. coli* isolates.

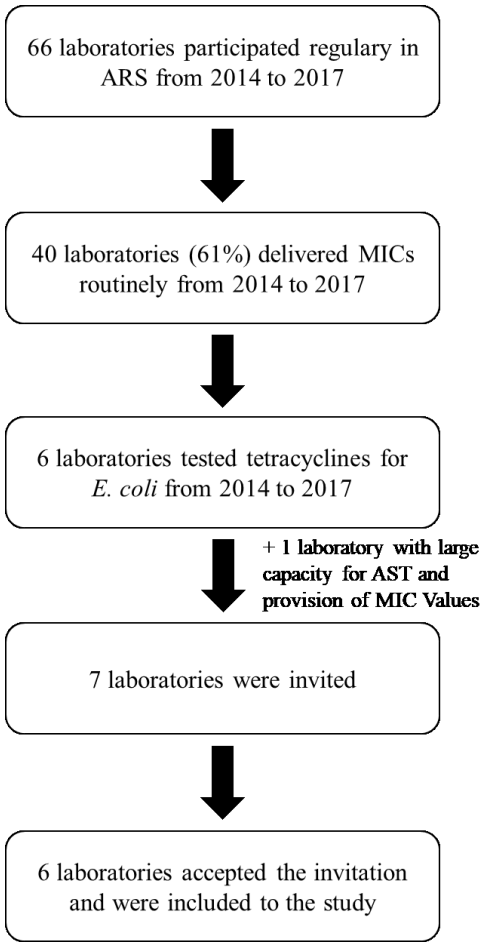

Figure 2. Selection of laboratories included for the first publication of the comparison of MIC values.

In the second publication, the second, third and fourth research question (Chapter 1.5.2.) were examined. The second thesis objective aimed to further investigate the available and overlapping variables in ARS, Zoonosis Monitoring and GE*RM*-Vet. After a thorough assessment of these three systems, a subset of variables was selected, including the origin of the *E. coli* isolates (i.e. farms, slaughterhouses, retail outlets, veterinary clinics, outpatient care facilities, general medical wards and ICUs), results of resistance testing of four antibiotics (ampicillin, cefotaxime, ciprofloxacin and gentamicin) and information regarding the federal states where the *E. coli* isolates were collected.

To compare the *E. coli* data in these different human and animal populations, an analysis of resistance combinations was conducted based on the four antibiotics. This approach was

selected because bacteria are often resistant to more than one antibiotic (Magiorakos et al. 2012). Sixteen resistance combinations were created from the four antibiotics that were frequently tested for *E. coli* in ARS, Zoonosis Monitoring and GE*RM*-Vet. In order to analyse the similarities of sixteen resistance combinations in humans and different animal populations, we tested different methodological approaches introduced by CenStat et al. that aimed to define multidrug-resistant bacteria by using varied approaches to cluster different resistance patterns (CenStat et al. 2016). The AMR datasets used by CenStat et al. (CenStat et al. 2016) were similar to the datasets used for the second publication, which originated from national surveillance and monitoring systems. Thus, these approaches might also suitable for our datasets.

CenStat et al. suggested principal component analysis (PCA) to determine resistance patterns based on single resistance proportions. PCA aims to reduce the number of resistance combinations built from numerous antibiotics. This method involves analysing the distances between the respective resistance combinations and excludes the irrelevant resistance combinations to create the clustering model. However, for the purpose of the second publication in this thesis, PCA was not conducted due to the small number of resistance combinations between the four antibiotics selected (2^4 = 16). Rather, all 16 resistance combinations were included for the second publication.

K-means clustering and latent class analysis methods were also explored for the second publication's analysis in this thesis. For the k-means clustering, the analysis results were not reliable due to the large variance of resistance combinations in *E. coli* isolates between human and different animal populations. On the other hand, latent class analysis aims to build a model depicting the effect of covariates, such as time to study the probability of resistance combinations for each of the observed study populations (CenStat et al. 2016). This method also did not align with the goal of the second publication, as it did not allow for the observation of changes in resistance combinations over a period of time.

Cluster analysis was selected to further examine the similarities between the resistance combinations in *E. coli* isolates amongst the different human and animal populations. The cluster analysis aimed to classify different objects based on their similarities and distinguish them if they were not similar. The distances between the objects were measured, for example, with Euclidean metrics, to determine the objects for each group (Murtagh and Contreras 2012). This approach has been used in numerous studies to analyse the similarities between objects, including for clustering the resistance patterns of bacterial AMR (CenStat et al. 2016). These authors used hierarchical clustering with Ward's distance method to describe the similarities in the resistance patterns of bacterial AMR in different European countries. Varied hierarchical clustering approaches were introduced for different categories of AST results: one using the

SIR (susceptible, intermediary, resistance) interpretation and another using the observed MICs. In hierarchical clustering, the distances between two clusters are measured using varied approaches such as the linkages method, namely single (minimum distance or nearest neighbour), complete (farthest neighbour), and average (average distances), or Ward's method (minimum variance method) (Murtagh and Contreras 2012). The hierarchical clustering method suited the goal of the second publication and was therefore chosen for the cluster analysis. For the second publication, different clustering distances were also tested for single, complete, average and Ward's method to determine suitable distances for the datasets. After comparing the dendrograms produced from these methods, the average linkage was selected because it presented superior content interpretation regarding differences in the clusters' objects compared with the other three methods. Similar statistical methods were then applied to the third publication. The study protocol is documented in the Supplementary File 9.2.

5.3. Limitations

5.3.1. Different antibiotics tested in the three surveillance and monitoring systems: Limited comparability between data sources

The limited availability of overlapping antibiotics in the studied testing panels of human and veterinary medicine, including in food safety was observed in all three publications. In human and veterinary medicine including in food safety, different antibiotic panels have been used for different purposes: 1) routine diagnostics and 2) epidemiological studies. This led to different availability of the data of antibiotic resistance and will hamper the comparative analyses of these data. As an example, two important antibiotics in human and veterinary medicine, such as colistin and tetracycline, could not be further studied in this thesis due to this matter. However, these two antibiotics remain important within these two sectors because of the following reasons.

Colistin is one of the oldest antibiotics, and its use is restricted in human medicine due to its toxicity (Ling et al. 2020), while in veterinary medicine it is yet frequently used for different purposes (Binsker et al. 2022). Despite its restriction in human medicine it remains as an therapeutic option due to limited treatment options for MDR organisms (Baron et al. 2016, El-Sayed Ahmed et al. 2020), especially for the treatment of carbapenem-resistant Enterobacteriaceae (Binsker et al. 2022). However, the use of colistin in both human and veterinary medicine has been increasing globally (Binsker et al. 2022, Janssen and van Schaik

2021). Resistance to colistin is marked by the mobile colistin resistance (*mcr*) gene, which varies from *mcr*-1 to *mcr*-10 (Ling et al. 2020, Wang et al. 2020a, Xu et al. 2022). These genes have already been found in different animal (Timmermans et al. 2021) and human populations (Köck et al. 2021, Neumann et al. 2020). Moreover, the bacteria that carried colistin genes have been also found in processed waters and wastewaters from poultry and pig slaughterhouses (Savin et al. 2020). Thus, it is important to continuously monitori the development of colistin-resistant bacteria in human and different animal populations within the One Health approach, since colistin resistance genes could be transmitted between these populations. In food safety and veterinary medicine, colistin resistance is tested and monitored routinely due to possible transmission of colistin resistance genes through food consumption and its frequent use in veterinary medicine, whereas in human medicine, it is less frequently tested in routine medical laboratories (Chapter 1.4.1, Figure 1). The limited testing in routine medical laboratories was caused by the challenging AST of colistin (Matuschek et al. 2018, Pfennigwerth et al. 2019) that necessitate lyophilised broth microdilution, which is more frequently conducted at the national level for routine food safety monitoring in NRL-AR, monitoring for diseased animals in BVL (GERM-Vet) and in the National Reference Centers (NRCs) for human medicine (Beermann et al. 2015, Robert Koch-Institut 2021b). Therefore, the results of AST for colistin from routine medical laboratories could not be further used for comparative analyses in the second and third publications. However, the comparative analyses of colistin-resistant bacteria in humans and different animal populations are yet important. For this purpose, it is necessary to consider another source of data that resulted from AST that used lypophilised broth microdilution, such as from the NRCs for human medicine.

Tetracycline was initially used and, in some countries, is still frequently used in animal husbandry as a growth promoter (OIE 2021). However, the use of tetracyclines as a growth promoter has been banned in the European Union since 2006 (European Commission 2005). In Germany, the use of tetracyclines in animal husbandry has been annually monitored. Over the past years the use of tetracyclines decreased drastically between 2011 and 2017, especially between 2014 and 2017 (BMEL 2019), and further declined until 2020 (Gefeller et al. 2021). Similar to routine monitoring of the use of tetracyclines in animal husbandry, resistance to tetracycline in bacteria from different food-producing animals in European countries has been also annually monitored and reported (ECDC et al. 2021). In these routine monitoring activities the association between the consumption of tetracycline and tetracycline-resistant bacteria in food-producing animals, such as in commensal *E. coli*, was already observed (ECDC et al. 2021). In human medicine nowadays, tetracycline is less commonly used than in animal husbandry (ECDC et al. 2021). Particularly within European countries, it

is not recommended for treating human *E. coli* infections, such as UTIs (ECDC et al. 2021, Ny et al. 2019). Tetracycline is also not a component of the routine surveillance of *E. coli* infections in human medicine, which resulted to limited data on resistance to tetracycline in *E. coli*. However, the association between the consumption of tetracycline and tetracycline resistance was observed in *Salmonella* Enteriditis in 2017 within European countries (ECDC et al. 2021). Moreover, in low- and middle-income countries (LMIC), high tetracycline resistance in *E. coli* from UTIs (Bunduki et al. 2021) and commensal *E. coli* in healthy humans in community settings (Nji et al. 2021) have also been previously reported. This highlights the importance to continuously monitor tetracycline resistance in human medicine and further analyse the association between the use of tetracycline in food-producing animals and tetracycline-resistant bacteria in humans and food-producing animals.

To overcome the limited availability of overlapping antibiotics in the studied testing panels mentioned above, it is necessary to consider a harmonised antibiotic panel for human and veterinary medicine that incorporates relevant antibiotics from both areas, such as colistin and tetracycline. Moreover, harmonised evaluation criteria for both sectors might also support comparable interpretations of AST results. These actions will support further joint analyses of bacterial AMR and the integrated surveillance of bacterial AMR in the context of One Health.

5.3.2. Model organisms

This thesis presents comparative analyses of AMR data using *E. coli* isolates as model organisms. Thus, the results of the comparative analyses of the AST results and the similarities in the resistance combinations in *E. coli* isolates between human and different animal populations are not representative of other AMR profiles in other bacteria.

E. coli was selected as a model organism because of the frequently collected number of isolates across the ARS, Zoonosis Monitoring and GE*RM*-Vet systems. For other important bacteria in human and food safety monitoring, such as *Campylobacter* spp., *Enterococcus* spp. (*E. faecalis* and *E. faecium*) and *Salmonella* spp., the comparison of AMR data collected within the three systems is more limited. A comparison of AMR data of *Salmonella* spp., *Campylobacter* spp. and *Enterococcus spp*. could only be conducted within two systems (ARS and Zoonosis Monitoring), as these pathogens are not part of the routine monitoring of GE*RM*-Vet (BVL 2018). Nonetheless, efforts to harmonise AMR monitoring for *Campylobacter* spp. and *Salmonella* spp. from clinical human isolates on the European level (EFSA et al. 2019) were initiated in 2019 to support the integrated analysis of AMR data from *Salmonella* spp. and *Campylobacter* spp. isolates from humans and animals (EFSA and ECDC 2020).

If further joint comparative analyses of other zoonotic bacterial AMR data from ARS, Zoonosis Monitoring and GE*RM*-Vet should be conducted, these analyses should focus on *S. aureus*, as explained in the next chapter (Chapter 5.4.1.)

5.4. Outlook

5.4.1. Another pathogen for comparative analysis: *S. aureus*

S. aureus colonizes the skin and mucus of humans and animals. It can also cause a multitude of infections, which are frequently caused by methicillin-resistant *S. aureus* (MRSA) (Cuny et al. 2013, Spoor et al. 2013). The occurrence of MRSA in humans, animals and the environment has indicated the possible transmission of *S. aureus* isolates between these populations, highlighting the importance of comparing the AMR data for *S. aureus* from ARS, Zoonosis Monitoring and GE*RM*-Vet. Similar to *E. coli* isolates, the resistance profiles of *S. aureus* have also been monitored over time within ARS, Zoonosis Monitoring (MRSA) and GE*RM*-Vet. A similar approach relative to the comparative analyses of the *E. coli* could be used for *S. aureus*: 1) assessment of the comparability of AST results using automated AST and broth microdilution and 2) the cluster analysis of resistance combinations using other relevant antibiotics for *S. aureus* that overlap within the three systems. The statistical methods should, however, be adapted based on the number of overlapping antibiotics and the variance of relative frequencies across resistance combinations. If necessary, additional methods for reducing the number of resistance combinations should be conducted, as previously discussed. For cluster analyses, it is important to test different distances, namely single, complete, average and Ward's, for the available datasets. Moreover, other methods of cluster analysis, such as k-means clustering, should be tested, as different datasets might result in different variances of relative frequencies in resistance combinations. Further additional analyses using elbow and silhouette methods might support the determination of the number of clusters.

These investigations would not only further contribute to research on bacterial AMR in a One Health context; they would also contribute important findings to the results of the comparative analyses of bacterial AMR presented in this study.

5.4.2. A web-based platform for bacterial AMR from ARS, Zoonosis Monitoring and GE*RM*-Vet

An open-access and interactive web-based platform with standardised variables across the three systems, namely ARS, Zoonosis Monitoring and GE*RM*-Vet, would better support future integrated epidemiological analyses. This platform should aim to map the resistance patterns of all relevant pathogen bacteria, starting with *E. coli* and *S. aureus*, for different human and animal populations. This should be an interface extension between ARS, Zoonosis Monitoring and GE*RM*-Vet, requiring no additional data collection from either sector. For human populations, the information should include the health care settings (outpatient care, general wards or ICUs), antibiogram for each patient, clinical specimens, gender, age, county and federal state. This information should be described over years. As for different animal populations, the information regarding species, origins (farms, slaughterhouses, retail outlets or veterinary clinics), and federal states with information on urban and rural area should be included. Additionally, the period of time (in years) should be included. Until joint clinical breakpoints are available, the AST results from Zoonosis Monitoring and GE*RM*-Vet should be interpreted using EUCAST clinical breakpoints for human isolates since this guideline is used in almost all routine medical laboratories that participated in the ARS.

However, there are some barriers in relation to establishing this platform. It will require additional data extraction for ARS, which could necessitate further resources such as time and personnel. Moreover, the different annual sampling plan of food-producing animals in Zoonosis Monitoring and the animals' pathogens (GE*RM*-Vet), which are taken for different species each year, can only be included as two-year periods of resistance patterns for each species.

On the other hand, this platform could also offer advantages: 1) continuous monitoring of trends of bacterial AMR for overlapping antibiotics in human (yearly) and different animal populations (two-year trends for non-clinical animal isolates), 2) continuous monitoring of trends in resistance combinations in bacterial AMR to specific antibiotics in human and different animal populations, 3) early detection of novel resistance combinations in bacterial AMR in human and different animal populations, 4) support of research questions within the scope of One Health AMR in Germany, and 5) support of the knowledge exchange on AMR data nationally and internationally. This platform will support the German strategies for One Health AMR (BMG et al. 2015).

Additional sub-analyses of *Campylobacter* spp. and *Enterococcus* spp., drawn from exclusively ARS and Zoonosis Monitoring, might facilitate an improved overview of other important zoonotic agents in Germany. If such a joint platform can offer a broader scope than the three systems used for this thesis, additional data from the NRC for colistin-resistant

Enterobacteriaceae and the molecular AMR data, such as information regarding the ESBL/AmpC gene for Enterobacteriaceae, could also be included.

5.4.3. Comparative analyses of AMR data using the stratification of human age groups

In the second publication, different animal population stratifications were considered based on animal categories (species, age or different types of food-producing animals) and their origins (farms, slaughterhouses, retail outlets and clinics), whereas in human populations, stratification based on healthcare settings (general ward, ICUs and outpatient care) was considered. Bacterial AMR was previously observed within different age groups of humans (Robey et al. 2017) and animals (Gaire et al. 2020). In children, the high resistance of *E. coli* isolates was previously observed along with the frequent use of commonly prescribed antibiotics, such as ampicillin (Bryce et al. 2016, Vazouras et al. 2020). High ciprofloxacin resistance in community-acquired UTI *E. coli* isolates amongst elderly groups has also been reported, with a connection to age, frequent use of ciprofloxacin and consumption of pork and chicken (Mulder et al. 2016). Further comparative analyses should include the examination of the different human age groups to identify the appropriate age stratification for each pathogen and group of antibiotics. The selection of different clinical specimens, e.g. only blood (invasive isolates) or other clinical specimens, should be also considered. Such stratified cluster analyses of resistance combinations provide more detailed information on the similarities between the human age groups and different animal populations.

This would contribute to a further understanding of the characterisation of resistance proportions across different human groups and of the extent of similarities between different animal populations.

6. Zusammenfassung

Antibiotikaresistenzen (eng. Antimicrobial resistance, AMR) bei Bakterien stellen eine globale Gefahr für die Gesundheit von Menschen und Tieren dar. Durch diese Bakterien verursachte Infektionen können zu schweren Erkrankungen und Todesfällen führen und sind zudem mit hohen Behandlungskosten assoziiert. Da antibiotikaresistente Bakterien in Menschen, verschiedenen Tierpopulationen und in der Umwelt vorkommen können, ist es wichtig diese mit einem sektorübergreifenden Ansatz, wie dem „One Health-Ansatz“, zu untersuchen.

Das Ziel dieser Dissertation war der Vergleich von Antibiotikaresistenzdaten aus unterschiedlichen Surveillance- und Monitoring Systemen für Menschen und verschiedene Tierpopulationen in Deutschland. Mit *Escherichia coli* (*E. coli*) als Beispielerreger wurden fünf Fragestellungen in dieser Dissertation adressiert:

1) Wie vergleichbar sind die routinemäßig erhobenen bakteriellen Antibiotikaresistenzdaten aus humanen und tierärztlichen Surveillance- und Monitoring-Systemen in Deutschland?
2) Was charakterisiert die Monitoring- und Surveillance-Systeme für bakteriellen Antibiotikaresistenzen bei Menschen, Tieren und Lebensmitteln in Deutschland? Welche Arten von Daten werden in der Antibiotika-Resistenz-Surveillance (ARS), dem Zoonosen-Monitoring und in dem Nationalen Resistenzmonitoring tierpathogener Bakterien (GE*RM*-Vet) erhoben? Sind die erhobenen Daten zwischen diesen drei Systemen ähnlich?
3) Welche Art von Analyse könnte, basierend auf den unterschiedlichen Variablen der verfügbaren Surveillance- und Monitoring-Systeme, zum Vergleich der Daten aus dem Human- und Tierbereich genutzt werden?
4) Was können die Ergebnisse der Analyse von Frage 3 zum Verständnis der Antibiotikaresistenzdaten im Human- und Tierbereich beitragen? Können Ähnlichkeiten von Resistenzmustern der Isolate von Menschen und Tieren analysiert und darüber hinaus Erkenntnisse zu Erregerübertragungen zwischen Menschen und Tieren generiert werden?
5) Gibt es eine weitere demografische Stratifizierung, wie z. B. die Region basierte Stratifizierung, welche die Vergleichbarkeit der Resistenzdaten beeinflusst?

Zur Beantwortung der ersten Fragestellung zur Vergleichbarkeit der bakteriellen Antibiotikaresistenzdaten aus humanen und tierärztlichen Surveillance- und Monitoring-Systemen in Deutschland wurde eine Vergleichsanalyse der minimalen Hemmkonzentrationen (MHK) von *E. coli*-Isolaten als Ergebnis verschiedener Methoden der antimikrobiellen Empfindlichkeitsprüfung (AST) (automatisierte AST und Bouillon-

Mikrodilution) erstellt und in der ersten Publikation veröffentlicht. Diese Studie wurde mit Unterstützung von sechs humanmedizinischen Laboren, die routinemäßig automatisierte AST verwenden, durchgeführt. Fünf der Labore nutzen das VITEK®2 und ein Labor den MicroScan. Die Labore mit dem VITEK®2 stellten insgesamt 106 *E. coli* Isolate mit deren jeweiligen MHKs zur Verfügung. Die MHKs dieser Isolate wurden mittels Bouillon-Mikrodilution, der Methode welche im Rahmen des deutschen Monitorings für Lebensmittelsicherheit im Nationalen Referenzlabor für Antibiotikaresistenz (NRL-AR) eingesetzt wird, erneut getestet. Im Anschluss wurden die erhaltenen MHK-Werte aus dem VITEK®2 und der Bouillon-Mikrodilution miteinander verglichen. Die Analyse ergab für elf Antibiotika hohe Übereinstimmungen (> 90%) mit einer niedrigem Fehlerquote (< 20%). Diese Studie zeigte somit, dass für *E. coli*-Isolate die ermittelten MHK-Werte der in humanmedizinischen Laboren angewendeten automatisierten Empfindlichkeitsprüfung mit denen der Bouillon-Mikrodilution des Monitorings für Lebensmittelsicherheit vergleichbar sind. Diese Vergleichbarkeit der MHK-Werte erlaubte weitere Analysen der integrierten Datensätze aus der Human- und Veterinärmedizin inklusive dem Bereich Lebensmittelsicherheit in Deutschland.

Die Analysen zu den Fragestellungen 2 bis 4 dieser Dissertation wurden in einer zweiten Publikation veröffentlicht. Diese widmete sich dem Vergleich von Resistenzkombinationen bei *E. coli*-Isolaten aus verschiedenen Human- und Tierpopulationen gegenüber vier häufig getesteten Antibiotika. Zur Beantwortung der zweiten Fragestellung wurde die verschiedenen Datensätzen aus ARS, dem Zoonosen-Monitoring und GE*RM*-Vet zunächst verglichen. Diese Analyse ergab, dass folgende Datensätze der verschiedenen Surveillance- und Monitoring Systeme für einen Vergleich geeignet sind: 1) Herkunft der Isolate (klinische Humanisolate aus ambulanten-, Normal- und Intensivstationen; nicht-klinische Tierisolate aus Erzeugerbetrieben und Schlachthöfen; Lebensmittelisolate aus dem Einzelhandel, und klinische Tierisolate aus Erzeugerbetrieben oder Tierkliniken), 2) Beurteilung der Ergebnisse der Empfindlichkeitsprüfung auf Grundlage der EUCAST-Kategorien „sensibel“, „intermediär“ und „resistent“ (SIR), 3) die vier am häufigsten getesteten Antibiotika: Ampicillin (AMP), Cefotaxim (CTX), Ciprofloxacin (CIP) und Gentamicin (GEN) und 4) Bundesländer. Diese Datensätze ermöglichten weitere Vergleichsanalysen, welche im Rahmen der dritten und vierten Fragenstellung dieser Dissertation adressiert wurden. Für diese Untersuchungen wurden *E. coli*-Datensätze aus ARS, dem Zoonosen-Monitoring und GE*RM*-Vet aus den Jahren 2014 bis 2017 in 41 verschiedenen Populationen analysiert. Es handelte sich um Isolate aus drei Humanpopulationen, 27 nicht-klinischen Tierpopulationen, inklusive Lebensmittel und 11 klinischen Tierpopulationen. Die Resistenzmuster der *E. coli*-Isolate wurden für die oben genannten Antibiotika erstellt. Die aus diesen vier Antibiotika resultierenden sechzehn Resistenzkombinationen wurden mithilfe einer Clusteranalyse und

basierend auf einem hierarchischen Clustermodell für die 41 Populationen analysiert. Die Anzahl der Cluster wurde anschließend mit der Ellbogen- und der Silhouettenmethode bestimmt und die Haupt- und Subcluster zur Interpretation der Ergebnisse visuell definiert. Die darauffolgenden zusätzlichen Sensitivitätsanalysen dienten der Testung der Robustheit des Modells. Dafür wurde nach und nach ein Antibiotikum aus der jeweiligen Analyse entfernt. Durch die Clusteranalysen konnten, basierend auf den Resistenzmustern von *E. coli*-Isolaten unterschiedlicher Human- und Tierpopulationen, folgende Ähnlichkeiten festgestellt werden: 1) Clusterung verschiedener Humanpopulationen aus ambulanten Stationen, Normalstationen und Intensivstationen (ITS), 2) Clusterung klinischer Isolate von Rindern, Schweinen, Masthähnchen und Puten mit klinischen Isolaten vom Menschen, 3) Clusterung nicht-klinischer Isolate von Masthähnchen und Puten, allerdings getrennt von den verschiedenen Humanpopulationen und 4) Clusterung nicht-klinischer Isolate von verschiedenen Tierpopulationen, einschließlich Lebensmitteln mit niedrigen relativen Häufigkeiten von Resistenzkombinationen. Die Ergebnisse dieser Studie zeigten somit mögliche Übertragungen von *E. coli*-Isolaten zwischen Menschen und Tieren im Kontext von One Health. Es muss jedoch betrachtet werden, dass hierbei keine Rückschlüsse über die Richtung der Übertragung gezogen werden konnten. Dies sollte Gegenstand zukünftiger Forschung zu diesem Thema sein.

Die Vergleichsanalyse im Rahmen der dritten Publikation diente der Beantwortung der fünften Fragestellung dieser Dissertation. Im Rahmen dieser Analyse wurden, aufbauend auf der vorangegangenen Studie und unter Verwendung derselben Populationen, der vier oben genannten Antibiotika und den Methoden der zuvor erwähnten Clusteranalyse, Resistenzkombinationen bei *E. coli*-Isolaten in verschiedenen deutschen Regionen (Ost, Südwest und Nordwest) betrachtet. Erneut wurde zur Bestimmung der Anzahl der Cluster die Ellbogen- und Silhouettenmethode verwendet. Für diese Analyse standen insgesamt 51 unterschiedliche Populationen aus verschiedenen Human- und Tierpopulationen der drei Regionen zur Verfügung, bei denen *E. coli*-Isolate untersucht wurden. Dabei wurden ähnliche Resistenzkombinationen bei *E. coli*-Isolaten der unterschiedlichen Humanpopulationen der drei Regionen festgestellt. Auch clusterten die Resistenzkombinationen der klinischen *E. coli*-Isolate aus unterschiedlichen Humanpopulationen der verschiedenen Regionen mit klinischen *E. coli*-Isolaten von Schweinen der Region Südwest und Masthühnern der Region Nordwest. Weiterhin clusterten die Resistenzkombinationen der nicht-klinischen *E. coli*-Isolate von Masthühnern aus Erzeugerbetrieben und Schlachthöfen derselben Regionen. Keine Clusterungen wurden dagegen festgestellt bei Resistenzkombinationen nicht-klinischer *E. coli*-Isolate von Fleisch aus dem Einzelhandel und den jeweiligen lebensmittelproduzierenden

Tieren derselben Regionen. Diese Studie offenbarte somit mögliche Übertragungswege von *E. coli*-Isolaten innerhalb und außerhalb der Regionen.

Folgende Limitationen der vorliegenden Dissertation sollten beachtet werden. Die Vergleichsanalysen basieren lediglich auf *E. coli*-Isolaten und sind daher nicht repräsentativ für die nationale Situation von Antibiotikaresistenzdaten verschiedener Surveillance- und Monitoring-Systeme in Deutschland. Darüber hinaus war es nicht möglich, andere wichtige Antibiotika der Human- und Veterinärmedizin, wie z. B. Colistin, und weitere bedeutende Zoonoseerreger, wie z. B. *Salmonella* spp., *Campylobacte*r spp. und *Enterococcus* spp. auf Grundlage zur Verfügung stehenden Datensätze zu untersuchen. So wären für die Betrachtung phänotypischer Resistenzdaten zu Colistin zusätzliche Datenquellen erforderlich. Würde man Antibiotikaresistenzdaten von *Salmonella* spp., *Campylobacter* spp. und *Enterococcus* spp. vergleichen wollen, so wäre das nur für Daten aus ARS und dem Zoonosen-Monitoring möglich.

Die vorliegende Dissertation konnte anhand der aktuellen und verfügbaren Antibiotikaresistenzdaten die Vergleichbarkeit der getesteten Antibiotika, Routinelabormethoden und erhobenen demografischen Variablen für *E. coli*-Isolate in nationalen Surveillance- und Monitoring-Systemen der Human- und Veterinärmedizin aufzeigen. Mittels Clusteranalyse konnte in dieser Arbeit erstmals eine Methode zur Untersuchung der möglichen Übertragung von Antibiotikaresistenzen in *E. coli* aus unterschiedlichen Human- und Tierpopulationen entwickelt werden. Sie zeigte somit, dass differenzierte und integrierte Analysen aus etablierten Surveillance- und Monitoringsystemen im Rahmen des One-Health-Ansatzes trotz gewisser Limitationen möglich sind. Weitere Untersuchungen basierend auf phänotypischen bakteriellen Antibiotikaresistenzdaten sollten andere Bakterien wie *S. aureus* und Reserveantibiotika wie Colistin berücksichtigen. Darüber hinaus sollte der Aufbau einer gemeinsamen webbasierten Plattform mit phänotypischen Resistenzdaten aus ARS, dem Zoonosen-Monitoring und GE*RM*-Vet und genotypischen Resistenzdaten aus anderen Datensätzen sowie weitere Vergleiche von Humanpopulationen verschiedener Altersgruppen angestrebt werden.

7. Summary

Bacterial antimicrobial resistance (AMR) in both human and animal health is associated with a substantial global threat to public health. Infections caused by these bacteria can lead to severe illnesses, costly treatments and even death. As bacterial AMR can be found in humans, different animal populations and the environment, it is important to study this area using a multidisciplinary One Health approach.

This thesis has presented comparative analyses of phenotypical AMR data from surveillance and monitoring systems for human and different animal populations in Germany. Using *E. coli* as a model organism, the following five research questions have been addressed:

1. How comparable are the bacterial AMR data from surveillance systems for humans and monitoring systems for animals and food safety in Germany?
2. What are the characteristics of the surveillance and monitoring systems for bacterial AMR in humans, animals and food safety in Germany? What kind of variables are collected in *Antibiotika-Resistenz*-Surveillance (ARS), Zoonosis Monitoring and German Resistance Monitoring for Veterinary Medicine (GE*RM*-Vet)? Are the collected variables similar across these three systems?
3. Based on the existing variables related to the available surveillance and monitoring systems, what kind of analyses could be used to compare data between the human and animal sectors?
4. How will the analyses outlined in question three contribute to understanding bacterial AMR situations in the human and animal sectors? Will these analyses be capable of identifying the similarities in resistance patterns between humans and different animal populations, thereby improving the understanding of transmission between populations?
5. Is there any further demographical stratification, such as region-based stratification, that affects the comparative analyses?

The first research question warranted a comparative analysis of bacterial AMR data from surveillance systems for human and monitoring systems for animals and food safety in Germany. This was studied in the first publication concerning the comparison of minimum inhibitory concentrations (MICs) yielded by different methods of antimicrobial susceptibility testing (AST) across systems (automated AST and broth microdilution). In this study, six human medical laboratories that routinely used automated AST were included. Five laboratories used VITEK®2, and one laboratory used MicroScan. One hundred and six *E. coli* isolates, with their MICs from five human medical laboratories with VITEK®2, were collected. These isolates were retested with the broth microdilution method used by the National

Reference Laboratory for AMR (NRL-AR) for German food safety monitoring. The resulting MICs from automated AST and broth microdilution were analysed to test their agreement. The findings indicated high agreement (> 90%) with a low rate of errors (< 20%) for 11 antibiotics that were tested in five human medical laboratories and NRL-AR. This study highlighted the comparability of MICs between automated AST from medical laboratories and broth microdilution from food safety monitoring for *E. coli* isolates. These comparable MICs allowed further integrated analyses using AMR datasets from German surveillance and monitoring systems.

The second through fourth research questions were analysed in the second publication. This publication performed a comparative analysis of resistance combinations in different human and animal populations from three surveillance and monitoring systems in relation to four frequently tested antibiotics. Firstly, the variables in the ARS, Zoonosis Monitoring and GE*RM*-Vet systems were thoroughly compared to assess potential overlap. This analysis yielded the following overlapping variables: origin (humans: clinical isolates from outpatient care, general ward and intensive care unit (ICU); animal: non-clinical isolates from animals from farm, slaughterhouse, and foods from retail outlets and clinical isolates from farms or veterinary clinics), AST results with sensible, intermediate, and resistance (SIR) interpretation with EUCAST guideline for human medicine, four frequently tested antibiotics (ampicillin (AMP), cefotaxime (CTX), ciprofloxacin (CIP) and gentamicin (GEN)), and federal states. These results presented the comparable variables and were used to inform the second comparative analysis that addressed the third and fourth research question. To answer these two research questions, data from *E. coli* isolates between 2014 and 2017 were analysed. During these years, there were 41 populations of humans (three populations) and different animal populations (27 populations of non-clinical isolates, including food, and 11 populations of clinical isolates) of *E. coli* isolates in ARS, Zoonosis Monitoring and GE*RM*-Vet between 2014 and 2017. The 16 resistance combinations were built from four antibiotics (AMP, CTX, CIP and GEN) and clustered using the hierarchical clustering model with average linkages. The number of clusters was determined via the elbow- and silhouette methods. The main and sub-clusters were also analysed graphically. Additionally, sensitivity analyses were performed to test the robustness of the complete model. These sensitivity analyses were conducted by eliminating one antibiotic at a time. Through cluster analysis, the similarities in the resistance combinations in *E. coli* isolates from different human and animal populations were observed as follows: 1) close similarities between different human populations from outpatient care, general wards and ICUs; 2) close similarities between clinical isolates from cattle, pigs, broilers and turkeys that were present in one cluster with different human populations; 3) close similarities between non-clinical isolates from poultry (broilers and turkeys) that were clustered separately from different

human populations; and 4) close similarities between non-clinical isolates from different animal populations, including foods that exhibited low relative frequencies in resistance combinations. This investigation was the first joint comparative analysis of bacterial AMR data from human and veterinary medicine. The similarities in the resistance combinations highlighted the possible transmission of *E. coli* isolates within and between human and different animal populations within a One Health context. However, the direction of transmission between these populations could not be studied within these datasets. Further investigations are therefore necessary.

The third comparative analysis was an in-depth sub-analysis following the second comparative analysis with the same resistance combinations. This sub-analysis aimed to answer the fifth research question. For this analysis, the human and different animal populations were stratified into three different German regions based on the structure of their animal populations: East, South West and North West. The same methods of hierarchical clustering (average linkage) were performed. Elbow- and silhouette methods were again used to determine the number of clusters. In total, 51 populations in three German regions from human and different animal populations were clustered. The close similarities between different human populations in the three different regions were observed, as were the close similarities between clinical isolates from pigs in the South West and broilers in North West and human populations in different regions. Furthermore, the close similarities in resistance combinations in *E. coli* isolates from non-clinical isolates in poultry from farms and slaughterhouses within the same regions were observed. However, the resistance combinations in *E. coli* isolates from meats in retail outlets clustered separately with their respective food-producing animals from all three regions. The regional stratification based on the structure of animal populations revealed similarities in resistance combinations within the same populations and regions, except for the majority of non-clinical animal isolates from foods from retail outlets. These results demonstrated the possibility of the transmission of *E. coli* isolates within and between the regions.

There are some limitations to be acknowledged for this thesis. Although this investigation presents the results of the first joint comparative analyses of AMR data between human and different animal populations from three selected surveillance and monitoring systems in Germany, it is not fully representative of the current national situation regarding AMR data originating from different surveillance and monitoring systems in Germany. The comparative analyses presented in this thesis were limited to a model organism using *E. coli* isolates. Moreover, it was not possible to assess other important antibiotics in both human and veterinary medicine, such as colistin and other zoonotic pathogens, such as *Salmonella* spp., *Campylobacter* spp. and *Enterococcus* spp., due to the limited data availability across the three surveillance and monitoring systems. Further sources of data are necessary to examine the

phenotypical AMR data for colistin. For *Salmonella* spp., *Campylobacter* spp. and *Enterococcus* spp., the comparative analysis could only be conducted for AMR data from ARS and Zoonosis Monitoring.

In conclusion, by using the current and available AMR data, this thesis was able to highlight the comparability of tested antibiotics, routine laboratory methods and collected demographical variables for *E. coli* isolates in German surveillance and monitoring systems in human and veterinary medicine including food safety. By using cluster analysis this thesis was able to present first feasible method for investigating the possible transmission of AMR in *E. coli* isolates from human and different animal populations. This demonstrated that the integrated analyses within the One Health context were, in spite of some challenges, possible. Further investigations with phenotypical bacterial AMR data should consider other bacteria, such as *S. aureus, Salmonella* spp. and *Campylobacter* spp., other important antibiotics, such as colistin, and additional stratifications, such as human age group. Future initiatives could aim to establish a joint web-based platform for phenotypical AMR data from ARS, Zoonosis Monitoring and GE*RM*-Vet and genotypical AMR data from other surveillance systems as well to analyse the different age groups in different human populations to better support integrated comparative analyses.

8. References for Introduction and Discussion

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9. Supplementary Files

9.1. MIC study: The study proposal and an example of laboratory report (in German)

Projektantrag

Untersuchung humaner Isolate aus der Routine-Diagnostik mit einem standardisierten Panel von Antibiotika im Rahmen einer gemeinsamen Doktorarbeit am Bundesinstitut für Risikobewertung (BfR) und Robert Koch-Institut (RKI)

Hintergrund

Im Rahmen von nationalen und internationalen interdisziplinären Forschungsprojekten wird intensiv an „One Health“ Fragestellungen gearbeitet. Schwerpunkt ist hier stets die Abschätzung des Beitrags von resistenten Erregern aus der landwirtschaftlichen Tierhaltung zur Resistenzproblematik in der Humanmedizin. Die „German One Health Initiative“ (GOHI) ist eine Initiative des Bundesinstituts für Risikobewertung (BfR), des Robert Koch-Instituts (RKI), des Friedrich Loeffler-Instituts (FLI) und des Paul Ehrlich Instituts (PEI) zur Entwicklung einer abgestimmten Strategie im Bereich One Health und Zoonosen.

Im aktuellen Projekt führen wir eine Analyse der bestehenden Systeme zur Resistenzsurveillance aus der Human- und Veterinärmedizin durch. Dies erfolgt unter der Betreuung der Fachgruppe 43 des BfR (Fachgruppe für Epidemiologie, Zoonosen und Antibiotikaresistenz) und des Fachgebiets 37 des RKI (Nosokomiale Infektionen, Surveillance von Antibiotikaresistenz und –verbrauch). Das Ziel des Projektes umfasst die Vergleichsanalyse von phänotypischen Resistenzdaten von Bakterien von Menschen und Tieren aus nationalen Surveillance- und Monitoringprogrammen.

Ziel der Untersuchung

Im Rahmen des Projekts vergleichen wir die an das ARS-System gelieferten Resistenzdaten mit Daten aus Monitoringprogrammen in der Veterinärmedizin. Hierfür werden Isolate aus der humanmedizinischen Routine-Diagnostik mit den Testmethoden weiter untersucht, die im Rahmen des Monitorings im Veterinärbereich verwendet werden und auf dem Durchführungsbeschluss der EU-Kommission 2013/652/EU basieren. Dazu werden die Isolate mit Hilfe der Bouillon-Mikrodilution auf Resistenz gegen 14 Referenzantibiotika untersucht.

Untersuchungsumfang und Logistik

Die Untersuchung wird im Nationalen Referenzlabor für Antibiotikaresistenz am BfR erfolgen. Insgesamt sollen pro Labor zwanzig humane *E. coli*-Isolate untersucht werden. Diese sollten ausgewählt werden aus Isolaten, die ab Januar 2019 aus Urinproben isoliert werden. Es soll maximal ein Isolat aus einer Urinprobe pro Patient untersucht werden. Soziodemographische Daten benötigt das BfR nicht, lediglich eine eindeutige Identifikation des Isolates, des Labors und die Antibiogrammergebnisse des Labors (MHK Werte) zu diesem Isolat. Die Bakterienisolate werden dann im BfR gemäß Beschluss 2013/652/EU getestet. Zunächst werden die Bakterienspezies durch MALDI-ToF bestätigt. Die Untersuchungsergebnisse werden mit den Routineergebnissen qualitativ anhand der definierten cut offs/breakpoints, sowie quantitativ anhand der MHK Werte verglichen. Gegebenenfalls werden weitere molekular-biologische Charakterisierungen bei bestimmten Isolaten durchgeführt insbesondere im Fall unterschiedlicher Untersuchungsergebnisse mit den verschiedenen Methoden. Die Ergebnisse der Untersuchungen am BfR werden an die Einsender-Labore zurück übermittelt. Die Versandkosten und die Kosten der Vergleichsuntersuchung werden vom BfR getragen.

LABORBERICHT

***E. coli* Isolate aus den humanen Urinproben von den privaten Laboren aus dem ARS System**

German One Health Initiative (GOHI) Projekt von 2017-2020

Promotionsarbeit

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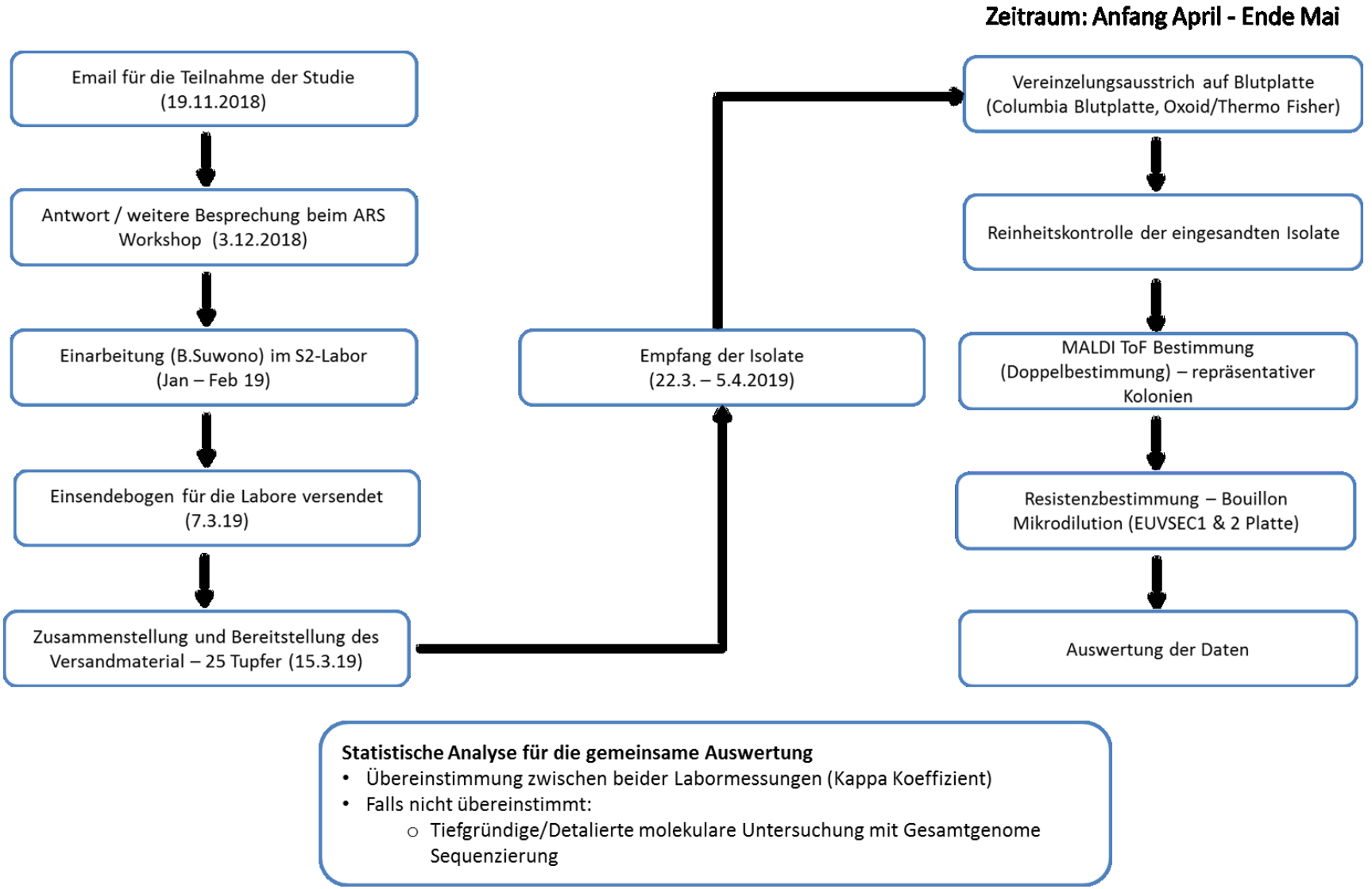
Arbeitsschema



Methode

Vereinzelungsausstrich auf Blutplatte

Die gesammelten Isolate (eingesandten Transporttupfer) wurden auf Blutplatten (Columbia Blutplatte, Oxoid/Thermo Fisher) ausgestrichen und über Nacht bei 37°C bebrütet. Alle Isolate, die unterschiedliche Koloniemorphotypen zeigten, wurden weiter charakterisiert.

MALDI-ToF Bestimmung

Zum Zweck der Spezies-Bestätigung der eingesandten Isolaten, haben wir zusätzlich eine Identifizierung von Mikroorganismen mittels MALDI-ToF Massenspektrometrie (MALDI Biotyper Systems, Microflex™ LT MALDI-TOF System mit Software-Paket MALDI Biotyper und Datenbanken ReferenceLibrary und SecurityLibrary, Bruker Daltonik GmBH) vorgenommen.

Resistenzbestimmung

Die Resistenzbestimmung wurde mittels Bouillon Mikrodilution (ISO 20776-1:2006 bzw. CLSI M31-A3) durchgeführt. Dieses Verfahren ist durch den Beschluss der EU-Kommission zur Überwachung und Meldung von Antibiotikaresistenzen bei zoonotischen und kommensalen Bakterien (2013/652/EU) aus der Lebensmittelkette festgelegt. Darunter sind 14 Antibiotika von den kommerziellen Platten des Formates EUVSEC (Tab 1) und zehn Antibiotika von der EUVSEC2 Platte (Tab. 2), die für *E. coli* untersucht werden. Diese Platten stammen von der Firma TREK Diagnostic Systems (Magellan Biosciences, Inc). Für unsere Routinediagnostik wird die EUVSEC2 Platte für eine weitere Testung Cefotaxim-resistenter Isolate verwendet. In dieser Studie wurden die Isolate einheitlich mit beiden Plattenformaten getestet. Für Antibiotika die auf beiden Platten vorhanden sind wird grundsätzlich die zweite Platte für die Interpretation herangezogen.

Vergleichsanalyse

Der direkte Vergleich der ermittelten Konzentrationsbereiche wurde analysiert. Dafür wurden die MHK Ergebnisse der einzelnen Untersuchungslabore mit den MHK Ergebnissen aus der Testung des NRL-Antibiotikaresistenz am BfR verglichen. Trotz der unterschiedlichen Konzentrationsbereiche die in den verschiedenen Testungen abgefragt werden, lassen sich die ermittelten Daten aus den Untersuchungslaboren anhand der BfR Ergebnisse bestätigen. Ein direkter Vergleich der Ergebnisse außerhalb der verwendeten Konzentrationsbereiche der antimikrobiellen Substanzen ist nicht möglich, da hierfür keine experimentellen Daten generiert werden. Der Vergleich der MHK Daten innerhalb des getesteten Spektrums kann geringfügig, z.B. im Rahmen einer MHK Stufe, schwanken. Diese Schwankung kann die methodische

Ungenauigkeit der Testung bzw. den Einfluss der ausübenden Person betreffen. Isolate die im Vergleich zur ursprünglichen Testung mehr als eine MHK-Stufe Unterschied aufwiesen sind in den nachfolgenden Tabellen markiert.

Tabelle 1. Erstes Panel zur Indikator kommensalen *E. coli*

Antibimikrobielles Mittel	Konzentrationsbereiche (mg/l)
Ampicillin	1-64
Cefotaxim	0,25-4
Ceftazidim	0,5-8
Meropenem	0,03-16
Nalidixinsäure	4-128
Ciprofloxacin	0,015-8
Tetracyclin	2-64
Colistin	1-16
Gentamicin	0,5-32
Trimetophrim	0,25-32
Sulfamethoxazol	8-1024
Chloramphenicol	8-128
Azithromycin	2-64
Tigecyclin	0,25-8

Tabelle 2. Zweites Panel zur Indikatorkommensalen *E. coli*

Antibimikrobielles Mittel	Konzentrationsbereiche (mg/l)
Cefoxitin	0,5-64
Cefepim	0,06-32
Cefotaxim + Clavulansäure	0,06-64
Ceftazidim + Clavulansäure	0,125-128
Meropenem	0,03-16
Temocillin	0,5-64

Imipenem	0,12-16
Ertapenem	0,015-2
Cefotaxim	0,25-64
Ceftazidim	0,25-128

Ergebnisse

Speziesbestätigung ihrer eingesandten Isolate durch MALDI-ToF

Insgesamt haben wir mittels MALDI-ToF 19 *E. coli* und zwei *Shigella dysenteriae* nachgewiesen.Ein Isolat (Nr. 913B-1033) wurde doppelt getestet, da bei dem Vereinzelungsausstrich zwei unterschiedliche Kolonien identifiziert wurden.

MHK Werte Verteilung von den unterschiedlichen Antibiotika

Für einen Großteil der bewerteten Antibiotika konnte kein signifikanter Unterschied festgestellt werden. Für Ciprofloxacin wurden bei zwei Isolaten Diskrepanzen über eine MHK Stufe festgestellt. Während das Isolat 913B-1153 innerhalb des getesteten Spektrums in der ursprünglichen Untersuchung kein Wachstum zeigte (MHK <=0.25), wurde im NRL-AR eine geringe Toleranz (MHK 0,5) nachgewiesen. Im Gegensatz dazu zeigte das Isolat 913B-1620 ursprünglich einen höheren MHK Wert (MHK 1) als der im NRL-AR festgestellt wurde (MHK 0.25). Trotz der geringen Unterschiede führen die ermittelten Diskrepanzen nicht zu einer abweichenden Bewertung nach EUCAST (> 0,5 µg/mL), CLSI (≥ 1 µg/mL) und ECOFF (≤ 0,06 µg/mL).

ORI: originale Laborwerte
BfR: BfR Laborwerte
BfR_1: Erstes Antibiotikapanel
BfR_2: Zweites Antibiotikapanel

Ampicillin

Originalnummer	AMP_ORI	AMP_BfR	Diskrepanz
913B-403	>=32	>64	nein
913B-515	>=32	>64	nein
913B-536	>=32	>64	nein
913B-544	>=32	>64	nein
913B-965	>=32	>64	nein
913B-986	>=32	>64	nein

913B-1153	>=32	>64	nein
913B-1353	>=32	>64	nein
913B-8052	>=32	>64	nein
913B-884	>=32	>64	nein
913B-886	>=32	>64	nein
913B-1033A	>=32	>64	nein
913B-1033B	>=32	>64	nein
913B-7805	>=32	>64	nein
913B-7955	>=32	>64	nein
913B-1609	>=32	>64	nein
913B-1614	>=32	>64	nein
913B-1620	>=32	>64	nein
913B-1658	>=32	>64	nein
914B-96	>=32	>64	nein
914B-113	>=32	>64	nein

Cefotaxim

Originalnummer	FOT_ORI	FOT_BfR_1	FOT_BfR_2	Diskrepanz
913B-403	>=64	>4	>64	nein
913B-515	>=64	>4	>64	nein
913B-536	<=1	<=0.25	<=0.25	Nein
913B-544	2	4	4	Nein
913B-965	>=64	>4	>64	Nein
913B-986	>=64	>4	>64	Nein
913B-1153	<=1	<=0.25	<=0.25	Nein
913B-1353	<=1	<=0.25	<=0.25	Nein
913B-8052	<=1	<=0.25	<=0.25	Nein
913B-884	<=1	<=0.25	<=0.25	Nein
913B-886	>=64	>4	>64	Nein
913B-1033A	<=1	<=0.25	<=0.25	Nein
913B-1033B	<=1	<=0.25	<=0.25	Nein
913B-7805	<=1	<=0.25	<=0.25	Nein
913B-7955	<=1	<=0.25	<=0.25	Nein
913B-1609	<=1	<=0.25	<=0.25	Nein
913B-1614	<=1	<=0.25	<=0.25	Nein
913B-1620	<=1	<=0.25	<=0.25	Nein
913B-1658	<=1	<=0.25	<=0.25	Nein

914B-96	<=1	<=0.25	<=0.25	Nein
914B-113	<=1	<=0.25	<=0.25	Nein

Ciprofloxacin

Originalnummer	CIP_ORI	CIP_BfR	Diskrepanz
913B-403	>=4	>8	Nein
913B-515	<=0.25	0.25	Nein
913B-536	<=0.25	<=0.015	Nein
913B-544	1	0.5	Nein
913B-965	>=4	>8	Nein
913B-986	0.5	0.25	Nein
913B-1153	<=0.25	0.5	Ja
913B-1353	<=0.25	<=0.015	Nein
913B-8052	<=0.25	0.03	Nein
913B-884	<=0.25	<=0.015	Nein
913B-886	>=4	>8	Nein
913B-1033A	>=4	8	Nein
913B-1033B	>=4	8	Nein
913B-7805	<=0.25	<=0.015	Nein
913B-7955	<=0.25	0.06	Nein
913B-1609	<=0.25	<=0.015	Nein
913B-1614	<=0.25	<=0.015	Nein
913B-1620	1	0.25	Ja
913B-1658	<=0.25	<=0.015	Nein
914B-96	<=0.25	<=0.015	Nein
914B-113	1	0.5	Nein

Meropenem

Originalnummer	MERO_ORI	MERO_BfR_1	MERO_BfR_2	Diskrepanz
913B-403	<=0.25	<=0.03	<=0.03	Nein
913B-515	<=0.25	<=0.03	<=0.03	Nein
913B-536	<=0.25	<=0.03	<=0.03	Nein
913B-544	<=0.25	<=0.03	<=0.03	Nein
913B-965	<=0.25	<=0.03	<=0.03	Nein
913B-986	<=0.25	<=0.03	<=0.03	Nein
913B-1153	<=0.25	<=0.03	<=0.03	Nein
913B-1353	<=0.25	<=0.03	<=0.03	Nein

913B-8052	<=0.25	<=0.03	<=0.03	Nein
913B-884	<=0.25	<=0.03	<=0.03	Nein
913B-886	<=0.25	<=0.03	<=0.03	Nein
913B-1033A	<=0.25	<=0.03	<=0.03	Nein
913B-1033B	<=0.25	<=0.03	<=0.03	Nein
913B-7805	<=0.25	<=0.03	<=0.03	Nein
913B-7955	<=0.25	<=0.03	<=0.03	Nein
913B-1609	<=0.25	<=0.03	<=0.03	Nein
913B-1614	<=0.25	<=0.03	<=0.03	Nein
913B-1620	<=0.25	<=0.03	<=0.03	Nein
913B-1658	<=0.25	<=0.03	<=0.03	Nein
914B-96	<=0.25	<=0.03	<=0.03	Nein
914B-113	<=0.25	<=0.03	<=0.03	Nein

Ertapenem

Originalnummer	ERTAPE_ORI	ERTAPE_BfR	Diskrepanz
913B-403	<=0.5	0.06	Nein
913B-515	<=0.5	<=0.015	Nein
913B-536	<=0.5	<=0.015	Nein
913B-544	<=0.5	<=0.015	Nein
913B-965	<=0.5	0.03	Nein
913B-986	<=0.5	0.06	Nein
913B-1153	<=0.5	<=0.015	Nein
913B-1353	<=0.5	<=0.015	Nein
913B-8052	<=0.5	<=0.015	Nein
913B-884	<=0.5	<=0.015	Nein
913B-886	<=0.5	0.06	Nein
913B-1033A	<=0.5	<=0.015	Nein
913B-1033B	<=0.5	<=0.015	Nein
913B-7805	<=0.5	<=0.015	Nein
913B-7955	<=0.5	<=0.015	Nein
913B-1609	<=0.5	<=0.015	Nein
913B-1614	<=0.5	<=0.015	Nein
913B-1620	<=0.5	<=0.015	Nein
913B-1658	<=0.5	<=0.015	Nein
914B-96	<=0.5	<=0.015	Nein
914B-113	<=0.5	<=0.015	Nein

Weitere Anmerkung

Es gibt keine weiteren Anmerkungen

Zusammenfassung

Die MHK-Werte zeigen größtenteils zwischen der Routine Labordiagnostik im Humanbereich und dem Lebensmittelsicherheitsbereich. Alle eingesandten MHK-Werte stimmen mit den von uns mittels Bouillon Mikrodilutionen gewonnenen MHK-Werten überein. Alle MHK-Werte, die sich nur um eine MHK-Stufe unterscheiden, wurden als übereinstimmend angesehen, da eine solche Schwankung zur tolerierten Streuung der Methode gehört.

9.2. Cluster analysis protocol in R

Resistance Pattern Analysis - Cluster Protocol

Beneditta Suwono

17 September 2020

Escherichia coli data from 2014 - 2017 in Germany

This protocol aims to report every steps on the cluster analysis for the GOHI AMR Project 2017-2020. Data was collected from German national surveillance and monitoring system for AMR. The data were originated from ***Antibiotika-Resistenz-Surveillance* (ARS)** system for human clinical isolates, ***Zoonosis Monitoring* (ZoMo)** system for zoonoses and commensal bacteria in animal and food, and ***GERM-Vet* study** for the animal pathogen.

1 Libraries

```
library(plyr)
library(reshape2)
library(gtools)
library(gplots)
library(factoextra)
library(NbClust)
library(readxl)
```

2 Data Cleaning

The data stored within the three systems have different infrastructures. Thus, prior data cleaning for each dataset is necessary.

2.1. *Antibiotika-Resistenz-Surveillance* (ARS)

The ARS dataset was extracted from the cube for ARS provided by FG 37. Since ARS system collected all the clinical data that are daily sent to the system, there are several important measures that needed to be conducted for ARS dataset. These steps are explained as described in the first paper Fig. 1

1. Sorting the species

```
Ecoli14_17 = read.csv("P:/GOHI/DATEN/2018-11-29-ESCCOL_2014To2017.csv",
                      sep = ";",row.names = NULL, header = T, as.is = T)
## this dataset was given from M.Feig (RKI) in November 2018

# subsetting the data based on the pathogen name #
Ecoli14_17 = Ecoli14_17[Ecoli14_17$Pathogen == "Escherichia coli",]
```

Total isolates at this stage: **1,976,379 isolates** (all *E. coli* isolates collected from 2014 to 2017)

2. routine participation from health care facilities from 2014-2017

```
# subsetting the data based on the participation years #
Ecoli14_17 = Ecoli14_17[Ecoli14_17$Teilnahme2014_17 == "ja",]
```

Total isolates at this stage: **965,442 isolates** (1,010,937 isolates were eliminated because not regulated participation)

3. de-duplication rules (copy strains). In this study, only first isolates per patinet per year per material group were chosen.

```
# subsetting the data based on the copy strain #
Ecoli14_17 = Ecoli14_17[Ecoli14_17$CSYMG == 1,]
```

Total isolates at this stage: **656,793 isolates** (308, 649 isolates were excluded because it were duplicate isolates and screening isolates)

4. Exclusion for birth year and material group

```
#exclusion of the people who born in 1900, 1902, 1903 #
Ecoli14_17 <- Ecoli14_17[which(Ecoli14_17$BirthYear != "1900"),]
Ecoli14_17 <- Ecoli14_17[which(Ecoli14_17$BirthYear != "1902"),]
Ecoli14_17 <- Ecoli14_17[which(Ecoli14_17$BirthYear != "1903"),]
Ecoli14_17 <- Ecoli14_17[which(Ecoli14_17$BirthYear != "-1"),]

##Exclusion von MaterialgroupRKILO
Ecoli14_17 = Ecoli14_17[which(Ecoli14_17$MaterialgroupRkiL0 != "Screening"),]
Ecoli14_17 = Ecoli14_17[which(Ecoli14_17$MaterialgroupRkiL0 != "unbekannt"),]
```

Total isolates at this stage: **654,762 isolates** (10,134 isolates were excluded because of non-complete information for birth year and material group).

5. Exclusion for unspecified health care facilities

```
#exclusion of the irrelevant information in material group
Ecoli14_17 = Ecoli14_17[which(Ecoli14_17$Stationstyp != "sonstige Behandlungsart"),]
```

Total isolates at this stage: **644,628 isolates** (10,134 isolates were excluded because of unspecified health care facilities)

6. Norm selection

```
# Auswahl EUCAST norm #
Ecoli14_17 = Ecoli14_17[Ecoli14_17$Norm == "EUCAST",]
```

Total isolates at this stage: **537,903 isolates** (106,725 isolates were excluded because of no use of EUCAST clinical breakpoints (EUCAST Version 2018))

7. Antibiotics selection and antibiotics coding In ARS system, the antibiotics were coding as:
   a. 0 = not tested
   b. 3 = resistant isolates
   c. 4 = intermediate isolate
   d. 5 = susceptible isolates

This paper interpret the "intermediate" AST result as susceptible isolates.

```
#Selected antibiotic test based on the gemeinsame getesten Antibiotikas #
mdr_categories_coli =list(Carbapeneme=c("IMP","MER", "ERT"),
                          Penicilline=c("AMP"),
                          Cephalosphorine=c("CTX", "CEP"),
                          Flurochinolone=c("CIP"),
                          Aminoglycoside=c("GEN"),
                          Tetrazykline=c("TET"),
                          CoTrimo = c("SXT"),
                          Polymxyin = c("COL"))
```

```
category = c(NA, 1, 0, 0)
names(category) = c(0, 3, 4, 5)

for(n in unlist(mdr_categories_coli)) {
  Ecoli14_17[,n] = as.vector(category[as.character(Ecoli14_17[,n])])
}
```

For the purpose of this paper, we chose **only** isolates that were tested against **all four chosen antibiotics (AMP, CTX, CIP, GEN)**.

```
Ecoli14_17 <- Ecoli14_17[complete.cases(Ecoli14_17$AMP),]
Ecoli14_17 <- Ecoli14_17[complete.cases(Ecoli14_17$CTX),]
Ecoli14_17 <- Ecoli14_17[complete.cases(Ecoli14_17$CIP),]
Ecoli14_17 <- Ecoli14_17[complete.cases(Ecoli14_17$GEN),]
```

Total isolates at this stage: **324,304 isolates** (213,599 isolates were excluded because they had not been tested against all four antibiotics)

8. Manipulation of the data: Age Group and Date origin Ages were grouped based on the ECDC age grouped

```
#Altersgruppe#
#Muss noch mal gecheckt werden#
Ecoli14_17$Age_cut <- cut(
  Ecoli14_17$ReceiptAge,
  breaks = c(1,6,20, 30, 40, 50, 60, 70, 80, 90, Inf),
  labels = c("0-5","6-19","20-29", "30-39", "40-49", "50-59",
             "60-69","70-79","80-89",">=90"),
  right = FALSE
  )

# separate the dates #
Ecoli14_17$DateExplant = as.Date(Ecoli14_17$DateExplant, "%Y-%m-%d")

# separate the years and months #
Ecoli14_17$year = as.numeric(format(Ecoli14_17$DateExplant, "%Y"))
Ecoli14_17$month = as.numeric(format(Ecoli14_17$DateExplant, "%m"))
```

Total isolates at this stage: **324,304 isolates** (no elimination)

9.Variables selection for joint analyses

```
# choosing the variables #
Ecoli14_17_selected <- Ecoli14_17[,c(
  "Pathogen", "LaborCode", "Stationstyp", "Age_cut","Sex","year","month",
  "Bundesland","MaterialgroupRkiL0",unlist(mdr_categories_coli))]

## CSV written data
write.csv(
  Ecoli14_17_selected, file = "Ecoli14_17_Menschen_new_selected.xls", fileEncoding = "UTF-8"
  )
```

Total isolates at this stage: **324,304 isolates** (no elimination)

2.2. Zoonosis Monitoring and GERM-Vet study

Cleaning of the datasets from these two studies were necessary. Protocol of the data cleaning was attached elsewhere (Supplementary).

2.3 Joining the datasets for GOHI project

Three datasets (ARS,Zoonosis Monitoring and GERM-Vet) are integrated together.

```
## ARS ##
ARS_gohi <-
  read.csv("I:/Surveillance_Monitoring/Daten/RKI/Ecoli14_17_Menschen_new_selected.xls",
                      header = TRUE, sep=",", fileEncoding = "UTF-8")

ARS_gohi <- ARS_gohi %>%
  select("Stationstyp","AMP", "GEN", "CTX", "CIP")  %>%
  mutate(Spezies = "Menschen", Program = "ARS") %>%
  rename(Ort="Stationstyp")

## Zoonosis monitoring ##
zomo_gohi <-
  read.csv("I:/Surveillance_Monitoring/Daten/BfR/zomo_gohi.csv",
           sep = ",", header = TRUE, fileEncoding = "UTF-8")

## Germ-Vet
GERMVet_gohi <-
  read.csv("I:/Surveillance_Monitoring/Daten/BVL/GERMVet_gohi.csv",
                         sep = ",", header = TRUE, fileEncoding = "UTF-8")

zomo_gohi <- zomo_gohi %>% rename(Ort = "Herkunft")
zomo_gohi <- zomo_gohi[,-1]
GERMVet_gohi <- GERMVet_gohi[,-1]

## Datasets for further analyses
GOHI1417_20 <- rbind(zomo_gohi, GERMVet_gohi, ARS_gohi20)

## Data Manipulation
GOHI1417$Spezies = factor(paste(
  GOHI1417$Spezies,
  ifelse(GOHI1417$Ort == "tierklinik", "_K",
         ifelse(GOHI1417$Ort == "Erzeugerbetrieb","_EB",
                ifelse(GOHI1417$Ort == "Schlachthof", "_SH",
                       ifelse(GOHI1417$Ort == "Einzelhandel", "_EH",
                              ifelse(GOHI1417$Ort == "ambulant", "_M_A",
                                     ifelse(GOHI1417$Ort == "Normalstation", "_M_N", "_M_I"
                                            ))))))))

getwd()
setwd("I:/Surveillance_Monitoring/GOHI")
GOHI1417_dat <- write.csv(GOHI1417, file = "GOHI1417_dat.csv", fileEncoding = "UTF-8")
```

2.4 Additional Data Cleaning

```
#read the data#
tab = read.table("I:/Surveillance_Monitoring/GOHI/GOHI1417_dat.csv",
                 header=T,
                 as.is=T,
                 sep=",",
                 fileEncoding = "UTF-8")
```

Exclusion of non-target populations in Zoonosis Monitoring There were **5,771 isolates** collected within Zoonosis Monitoring from 2014 to 2017.

```
#exclusion for irrelevant populations in Zomo
tab <- tab[tab$Spezies != "Pflanzliche Lebensmittel _EH",] #Isolates from plants
tab <- tab[tab$Spezies != "Sprossen _EH",] #Isolates from beans sprout
```

After the exclusion of non-target populations (28 isolates), there were **5,743 isolates** that were included for the analyses.

Exclusion of non-target populations in GERM-Vet study There were **3,460 isolates** collected within GERM-Vet Study from 2014 to 2017.

```
#exclusion for irrelevant populations in GERM-Vet
tab <- tab[tab$Spezies != "NA _K",] #unspecified animal species
tab <- tab[tab$Spezies != "Gans und Ente _K",] #isolates from ducks
```

After the exclusion of non-target populations and incomplete information (11 isolates), there were **3,449 isolates** that were included for the analyses.

Renaming some target populations For the purpose of this study, some of populations were grouped and renamed.

```
#renaming the column names
tab$Spezies <- revalue(tab$Spezies, c("Wildschwein _M_I" = "Wildschwein _W"))
tab$Spezies <- revalue(tab$Spezies, c("Wildreh _M_I" = "Wildreh _W"))
tab$Spezies <- revalue(tab$Spezies, c("Menschen _M_I" = "Menschen _I"))
tab$Spezies <- revalue(tab$Spezies, c("Menschen _M_A" = "Menschen _A"))
tab$Spezies <- revalue(tab$Spezies, c("Menschen _M_N" = "Menschen _N"))
tab$Spezies <- revalue(tab$Spezies, c("Mastkalb/Jungrind _SH" = "Mastkalb _SH"))
tab$Spezies <- revalue(tab$Spezies, c("Rindfleisch (kühl) _EH" = "Rindfleisch _EH"))
tab$Spezies <- revalue(tab$Spezies, c("Rindfleisch (Tatar/Schabefleisch) _EH" = "Rindfleisch _EH"))
tab$Spezies <- revalue(tab$Spezies, c("Schweinefleisch (hackfleisch) _EH" = "Schweinefleisch _EH"))
tab$Spezies <- revalue(tab$Spezies, c("Schweinefleisch (kühl) _EH" = "Schweinefleisch _EH"))
tab <- tab[,-1]
```

3 Species Translation to English

Since our data stored in German language, there is necessary to translate the data to english

```
tab$Spezies <- revalue(tab$Spezies, c("Ferkel _K" = "Piglets, C"))
tab$Spezies <- revalue(tab$Spezies, c("Fleisch Wildwiederkäuern _EH" = "Venisons, R"))
tab$Spezies <- revalue(tab$Spezies, c("Garnelen _EH" = "Shrimps, R"))
tab$Spezies <- revalue(tab$Spezies, c("Hähnchenfleisch _EH" = "Broiler Meat, R"))
tab$Spezies <- revalue(tab$Spezies, c("Jung- und Legehenne _K" = "Laying Hens, C"))
tab$Spezies <- revalue(tab$Spezies, c("Kalb und Jungrind _K" = "Bovines <1 year, C"))
tab$Spezies <- revalue(tab$Spezies, c("Kleintier _K" = "Small Animals, C"))
tab$Spezies <- revalue(tab$Spezies, c("Konsumeier _EH" = "Table Eggs, R"))
tab$Spezies <- revalue(tab$Spezies, c("Läufer _K" = "Growers, C"))
tab$Spezies <- revalue(tab$Spezies, c("Läufer bis 30 kg _EB" = "Weaners, F"))
tab$Spezies <- revalue(tab$Spezies, c("Legehennen _EB" = "Laying Hens, F"))
tab$Spezies <- revalue(tab$Spezies, c("Masthahn/Masthahnküken _K" = "Broilers, C"))
tab$Spezies <- revalue(tab$Spezies, c("Masthähnchen _EB" = "Broilers, F"))
tab$Spezies <- revalue(tab$Spezies, c("Masthähnchen _SH" = "Broilers, S"))
tab$Spezies <- revalue(tab$Spezies, c("Masthähnchen konventionell _EB" = "Broilers Conv, F"))
tab$Spezies <- revalue(tab$Spezies, c("Masthähnchen ökologisch _EB" = "Broilers Org, F"))
```

```
tab$Spezies <- revalue(tab$Spezies, c("Mastkalb _SH" = "Bovines < 1 year, S"))
tab$Spezies <- revalue(tab$Spezies, c("Mastputen _EB" = "Turkeys, F"))
tab$Spezies <- revalue(tab$Spezies, c("Mastputen _SH" = "Turkeys, S"))
tab$Spezies <- revalue(tab$Spezies, c("Mastschwein (<50kg) _EB" = "Growers <50 kg, F"))
tab$Spezies <- revalue(tab$Spezies, c("Mastschwein _SH" = "Fattening Pigs, S"))
tab$Spezies <- revalue(tab$Spezies, c("Menschen _A" = "Humans, A"))
tab$Spezies <- revalue(tab$Spezies, c("Menschen _I" = "Humans, ICU"))
tab$Spezies <- revalue(tab$Spezies, c("Menschen _N" = "Humans, Gw"))
tab$Spezies <- revalue(tab$Spezies, c("Milchrind _K" = "Bovine Mastitis, C"))
tab$Spezies <- revalue(tab$Spezies, c("Milchrind konventionell _EB" = "Bovine Milk Conv, F"))
tab$Spezies <- revalue(tab$Spezies, c("Milchrind ökologisch _EB" = "Bovine Milk Org, F"))
tab$Spezies <- revalue(tab$Spezies, c("Pute _K" = "Turkeys, C"))
tab$Spezies <- revalue(tab$Spezies, c("Putenfleisch _EH" = "Turkey Meat, R"))
tab$Spezies <- revalue(tab$Spezies, c("Rind _K" = "Cattles, C"))
tab$Spezies <- revalue(tab$Spezies, c("Rindfleisch _EH" = "Bovine Meat, R"))
tab$Spezies <- revalue(tab$Spezies, c("Schwein _K" = "Pigs, C"))
tab$Spezies <- revalue(tab$Spezies, c("Schweinefleisch _EH" = "Pork, R"))
tab$Spezies <- revalue(tab$Spezies, c("Streifähige Rohwürste _EH" = "Raw Sausages, R"))
tab$Spezies <- revalue(tab$Spezies, c("Wildreh _W" = "Roe Deer Hunted, W"))
tab$Spezies <- revalue(tab$Spezies, c("Wildschwein _W" = "Wild Boar Hunted, W"))
tab$Spezies <- revalue(tab$Spezies, c("Zuchthühner - Legerichtung _EB" = "Breeder Chickens, F"))
tab$Spezies <- revalue(tab$Spezies, c("Zuchtsau _K" = "Sows, C"))
tab$Spezies <- revalue(tab$Spezies, c("Zuchtsauen _EB" = "Sows, F"))
tab$Spezies <- revalue(tab$Spezies, c("Zweischalige Weichtiere _EB" = "Bivalves, F"))
tab$Spezies <- revalue(tab$Spezies, c("Zweischalige Weichtiere _EH" = "Bivalves, R"))
```

4 Determination of Resistance Patterns

There are 4 antibiotics that we chose to be clustered. These antibiotics are *ampicillin, cefotaxime, ciprofloxacin and gentamicin.* From those four we have **16 different resistance combination patterns** to be determined. Here are the steps:

```
tab_spez = split(tab, tab$Spezies)

## Probability - Permutations ##
combns_spez = permutations(n=2,r=4,v=0:1,repeats.allowed=T)
combns_spez = apply(combns_spez, 1, paste, collapse="")
result_spez = list()
for(n in names(tab_spez)) {
  result_spez[[n]] = table(factor(apply(tab_spez[[n]][,c("AMP", "CTX", "CIP", "GEN")],
                                  1, paste, collapse=""), levels=combns_spez))
}
result_spez = do.call("rbind", result_spez)
result_percent_spez = t(apply(result_spez, 1, function(x) x/sum(x)))

Cluster_dat <- as.data.frame(result_percent_spez)
```

5 Row and column names

We added number of tested isolates in our rownames for 41 populations. Then, sorted the column names (resistance combinations) from all susceptible to all resistant (left to right)

```
result_percent_spez1 = result_percent_spez[,c("0000", "1000", "0100", "0010",
                                              "0001", "1100","1010", "1001",
```

```
                                          "0110","0101", "0011",  "1110",
                                          "1101","1011", "0111","1111")]

rownames(result_percent_spez1) <- c(
  "Bivalves, F (n = 42)", "Bivalves, R (n = 58)", "Bovine Mastitis, C (n = 378)",
  "Bovine Meat, R (n = 115)", "Bovine Milk Conv, F (n = 122)",
  "Bovine Milk Org, F (n = 74)","Bovines < 1 year, S (n = 433)",
  "Bovines < 1 year, C (n = 534)", "Breeder Chickens, F (n = 56)",
  "Broiler Meat, R (n = 363)", "Broilers Conv, F (n = 299)",
  "Broilers Org, F (n = 31)", "Broilers, C (n = 232)", "Broilers, F (n = 184)",
  "Broilers, S (n = 404)", "Cattles, C (n = 193)","Fattening Pigs, S (n = 439)",
  "Growers < 50 kg, F (n = 210)", "Growers, C (n = 129)", "Humans, A (n = 96,455)",
  "Humans, Gw (n = 197,521)", "Humans, ICU (n = 30,328)", "Laying Hens, C (n = 557)",
  "Laying Hens, F (n = 347)","Pigs, C (n = 346)", "Piglets, C (n = 417)",
  "Pork, R (n = 155)", "Raw Sausages, R (n = 69)","Roe Deer Hunted, W (n =  269)",
  "Shrimps, R (n = 20)", "Small Animals, C (n = 312)", "Sows, C (n = 24)",
  "Sows, F (n = 272)", "Table Eggs, R (n = 90)", "Turkey Meat, R (n = 356)",
  "Turkeys, C (n = 327)", "Turkeys, F (n = 346)","Turkeys, S (n = 372)",
  "Venisons, R (n = 150)", "Weaners, F (n = 250)", "Wild Boar Hunted, W (n = 217)"
)
```

6 Cluster Analysis

Different methods for the clustering have been previously tested for single, complete average etc (Supplementary Protocol). The chosen method is described as follows. All results were showed in the first manuscript.

6.1. Determination of the cluster

To determine the number of clusters, elbow and silhouette methods were used (Supplementary Files S4 Figure)

```
fviz_nbclust(Cluster_dat[,1:16], kmeans, method = "wss", k.max = 10) +
  theme_minimal() +
  labs(subtitle = "Elbow Method")

fviz_nbclust(Cluster_dat[,1:16], kmeans, method = "silhouette", k.max = 10) +
  theme_minimal() +
  labs(subtitle = "Silhouette Method")
```

6.2 Hierarchical Clustering

Three clusters were determined based on the dataset. We use hclust with euclidian and average method for our resistance combination.

```
cluster_avg <- hcut(result_percent_spez1,
                    k = 3,
                    hc_func = "hclust",
                    hc_metric = "euclidian",
                    hc_method = "average")

fviz_dend(cluster_avg,
          cex = 1.2,
          rect = T,
          horiz = TRUE,
          ggtheme = theme_bw(),
```

```
          k_colors = c("#1B9E77", "#E7298A", "darkblue"),
          main ="") +
  theme(axis.title.x = element_text(size = 20),
        axis.text.x = element_text(size = 16))
```

6.3 Heatmap with Clustering

We use to the heatmap with cluster to visualize the data. In order to do better visualization, InkScape program was used to remove colour of cluster beside the population's names.

```
heatmap.2(
  as.matrix(result_percent_spez1)[cluster_avg$order,],
  Rowv=F,
  dendrogram = "none",
  trace="none",
  RowSideColors=(c("#E7298A","darkblue","#1B9E77")[cluster_avg$cluster[cluster_avg$order]]),
  col=colorRampPalette(c(
    "grey", "dark blue", "dark red", "red", "orange", "gold", "yellow")),
  cellnote=round(as.matrix(result_percent_spez1*100)[cluster_avg$order,],2),
  margins=c(10,25),
  reorderfun = function(d, w) reorder(d, w),
  key = TRUE,
  keysize = 0.8,
  key.title = "Gradient Color",
  key.xlab = "Proportions",
  key.ylab = "",
  density.info = "density",
  xlab = "Resistant Combinations",
  ylab = "Populations",
  notecol="black",
  notecex= 1.6,
  cexRow = 1.6,
  cexCol = 1.6,
  Colv = FALSE
  )
```

7 Sensitivity Analysis

For the purpose of this study, we conducted the sensitivity analyses by eliminating one antibiotic at the time. This aimed to test the robustness for built model (complete model). All the coding for eliminations were coded as described below. All results are presented in supplementary sections of the first paper (Supplemetary Files S5 Figures)

7.1 Ampicilin

```
# Resistance combination #
tab_spez_AMP = split(tab, tab$Spezies)

## Probability - Permutations ##
combns_spez_AMP = permutations(n=2,r=3,v=0:1,repeats.allowed=T)
combns_spez_AMP = apply(combns_spez_AMP, 1, paste, collapse="")
result_spez_AMP = list()
for(n in names(tab_spez_AMP)) {
  result_spez_AMP[[n]] = table(factor(apply(tab_spez_AMP[[n]][,c("CTX", "CIP", "GEN")],
```

```
                                        1, paste, collapse=""), levels=combns_spez_AMP))
}
result_spez_AMP = do.call("rbind", result_spez_AMP)
result_percent_spez_AMP = t(apply(result_spez_AMP, 1, function(x) x/sum(x)))

rownames(result_percent_spez_AMP) <- c(
  "Bivalves, F (n = 42)", "Bivalves, R (n = 58)", "Bovine Mastitis, C (n = 378)",
  "Bovine Meat, R (n = 115)", "Bovine Milk Conv, F (n = 122)",
  "Bovine Milk Org, F (n = 74)","Bovines < 1 year, S (n = 433)",
  "Bovines < 1 year, C (n = 534)", "Breeder Chickens, F (n = 56)",
  "Broiler Meat, R (n = 363)", "Broilers Conv, F (n = 299)",
  "Broilers Org, F (n = 31)","Broilers, C (n = 232)", "Broilers, F (n = 184)",
  "Broilers, S (n = 404)", "Cattles, C (n = 193)","Fattening Pigs, S (n = 439)",
  "Growers < 50 kg, F (n = 210)", "Growers, C (n = 129)", "Humans, A (n = 96,455)",
  "Humans, Gw (n = 197,521)", "Humans, ICU (n = 30,328)", "Laying Hens, C (n = 557)",
  "Laying Hens, F (n = 347)","Pigs, C (n = 346)", "Piglets, C (n = 417)",
  "Pork, R (n = 155)", "Raw Sausages, R (n = 69)","Roe Deer Hunted, W (n =  269)",
  "Shrimps, R (n = 20)", "Small Animals, C (n = 312)", "Sows, C (n = 24)",
  "Sows, F (n = 272)", "Table Eggs, R (n = 90)", "Turkey Meat, R (n = 356)",
  "Turkeys, C (n = 327)", "Turkeys, F (n = 346)","Turkeys, S (n = 372)",
  "Venisons, R (n = 150)", "Weaners, F (n = 250)", "Wild Boar Hunted, W (n = 217)"
  )

cluster_avg_AMP <- hcut(result_percent_spez_AMP,
                    k = 3,
                    hc_func = "hclust",
                    hc_metric = "euclidian",
                    hc_method = "average")

fviz_dend(cluster_avg_AMP,
          cex = 1.2,
          rect = T,
          horiz = TRUE,
          ggtheme = theme_bw(),
          k_colors = c("#1B9E77", "#E7298A", "darkblue"),
          main = "Without Ampicillin") +
  theme(title = element_text(size = 24),
        axis.title.x = element_text(size = 20),
        axis.text.x = element_text(size = 16))
```

7.2 Cefotaxime

```
# Resistance combination #
tab_spez_CTX = split(tab, tab$Spezies)

## Probability - Permutations ##
combns_spez_CTX = permutations(n=2,r=3,v=0:1,repeats.allowed=T)
combns_spez_CTX = apply(combns_spez_CTX, 1, paste, collapse="")
result_spez_CTX = list()
for(n in names(tab_spez_CTX)) {
  result_spez_CTX[[n]] = table(factor(apply(tab_spez_CTX[[n]][,c("AMP", "CIP", "GEN")],
                                        1, paste, collapse=""), levels=combns_spez_CTX))
}
```

```
result_spez_CTX = do.call("rbind", result_spez_CTX)
result_percent_spez_CTX = t(apply(result_spez_CTX, 1, function(x) x/sum(x)))

rownames(result_percent_spez_CTX) <- c(
  "Bivalves, F (n = 42)", "Bivalves, R (n = 58)", "Bovine Mastitis, C (n = 378)",
  "Bovine Meat, R (n = 115)", "Bovine Milk Conv, F (n = 122)",
  "Bovine Milk Org, F (n = 74)","Bovines < 1 year, S (n = 433)",
  "Bovines < 1 year, C (n = 534)", "Breeder Chickens, F (n = 56)",
  "Broiler Meat, R (n = 363)", "Broilers Conv, F (n = 299)",
  "Broilers Org, F (n = 31)","Broilers, C (n = 232)", "Broilers, F (n = 184)",
  "Broilers, S (n = 404)", "Cattles, C (n = 193)","Fattening Pigs, S (n = 439)",
  "Growers < 50 kg, F (n = 210)", "Growers, C (n = 129)", "Humans, A (n = 96,455)",
  "Humans, Gw (n = 197,521)", "Humans, ICU (n = 30,328)", "Laying Hens, C (n = 557)",
  "Laying Hens, F (n = 347)","Pigs, C (n = 346)", "Piglets, C (n = 417)",
  "Pork, R (n = 155)", "Raw Sausages, R (n = 69)","Roe Deer Hunted, W (n =  269)",
  "Shrimps, R (n = 20)", "Small Animals, C (n = 312)", "Sows, C (n = 24)",
  "Sows, F (n = 272)", "Table Eggs, R (n = 90)", "Turkey Meat, R (n = 356)",
  "Turkeys, C (n = 327)", "Turkeys, F (n = 346)","Turkeys, S (n = 372)",
  "Venisons, R (n = 150)", "Weaners, F (n = 250)", "Wild Boar Hunted, W (n = 217)"
  )

cluster_avg_CTX <- hcut(result_percent_spez_CTX,
                        k = 3,
                        hc_func = "hclust",
                        hc_metric = "euclidian",
                        hc_method = "average")

fviz_dend(cluster_avg_CTX,
          cex = 1.2,
          rect = T,
          horiz = TRUE,
          ggtheme = theme_bw(),
          k_colors = c("#1B9E77", "#E7298A", "darkblue"),
          main = "Without Cefotaxime") +
  theme(title = element_text(size = 24),
        axis.title.x = element_text(size = 20),
        axis.text.x = element_text(size = 16))
```

7.3 Ciprofloxacin

```
# Resistance combination #
tab_spez_CIP = split(tab, tab$Spezies)

## Probability - Permutations ##
combns_spez_CIP = permutations(n=2,r=3,v=0:1,repeats.allowed=T)
combns_spez_CIP = apply(combns_spez_CIP, 1, paste, collapse="")
result_spez_CIP = list()
for(n in names(tab_spez_CIP)) {
  result_spez_CIP[[n]] = table(factor(apply(tab_spez_CIP[[n]][,c("AMP", "CTX", "GEN")],
                                            1, paste, collapse=""), levels=combns_spez_CIP))
}
result_spez_CIP = do.call("rbind", result_spez_CIP)
result_percent_spez_CIP = t(apply(result_spez_CIP, 1, function(x) x/sum(x)))
```

```
rownames(result_percent_spez_CIP) <- c(
  "Bivalves, F (n = 42)", "Bivalves, R (n = 58)", "Bovine Mastitis, C (n = 378)",
  "Bovine Meat, R (n = 115)", "Bovine Milk Conv, F (n = 122)",
  "Bovine Milk Org, F (n = 74)","Bovines < 1 year, S (n = 433)",
  "Bovines < 1 year, C (n = 534)", "Breeder Chickens, F (n = 56)",
  "Broiler Meat, R (n = 363)", "Broilers Conv, F (n = 299)",
  "Broilers Org, F (n = 31)","Broilers, C (n = 232)", "Broilers, F (n = 184)",
  "Broilers, S (n = 404)", "Cattles, C (n = 193)","Fattening Pigs, S (n = 439)",
  "Growers < 50 kg, F (n = 210)", "Growers, C (n = 129)", "Humans, A (n = 96,455)",
  "Humans, Gw (n = 197,521)", "Humans, ICU (n = 30,328)", "Laying Hens, C (n = 557)",
  "Laying Hens, F (n = 347)","Pigs, C (n = 346)", "Piglets, C (n = 417)",
  "Pork, R (n = 155)", "Raw Sausages, R (n = 69)",
  "Roe Deer Hunted, W (n =  269)","Shrimps, R (n = 20)", "Small Animals, C (n = 312)",
  "Sows, C (n = 24)","Sows, F (n = 272)", "Table Eggs, R (n = 90)",
  "Turkey Meat, R (n = 356)", "Turkeys, C (n = 327)",
  "Turkeys, F (n = 346)","Turkeys, S (n = 372)", "Venisons, R (n = 150)",
  "Weaners, F (n = 250)", "Wild Boar Hunted, W (n = 217)"
  )

cluster_avg_CIP <- hcut(result_percent_spez_CIP,
                        k = 3,
                        hc_func = "hclust",
                        hc_metric = "euclidian",
                        hc_method = "average")

fviz_dend(cluster_avg_CIP,
          cex = 1.2,
          rect = T,
          horiz = TRUE,
          ggtheme = theme_bw(),
          k_colors = c("#1B9E77", "#E7298A", "darkblue"),
          main = "Without Ciprofloxacin") +
  theme(title = element_text(size = 24),
        axis.title.x = element_text(size = 20),
        axis.text.x = element_text(size = 16))
```

7.4 Gentamicin

```
# Resistance combination #
tab_spez_GEN = split(tab, tab$Spezies)

## Probability - Permutations ##
combns_spez_GEN = permutations(n=2,r=3,v=0:1,repeats.allowed=T)
combns_spez_GEN = apply(combns_spez_GEN, 1, paste, collapse="")
result_spez_GEN = list()
for(n in names(tab_spez_GEN)) {
  result_spez_GEN[[n]] = table(factor(apply(tab_spez_GEN[[n]][,c("AMP", "CTX", "CIP")],
                                            1, paste, collapse=""), levels=combns_spez_GEN))
}
result_spez_GEN = do.call("rbind", result_spez_GEN)
result_percent_spez_GEN = t(apply(result_spez_GEN, 1, function(x) x/sum(x)))

rownames(result_percent_spez_GEN) <- c(
```

```
  "Bivalves, F (n = 42)", "Bivalves, R (n = 58)", "Bovine Mastitis, C (n = 378)",
  "Bovine Meat, R (n = 115)", "Bovine Milk Conv, F (n = 122)",
  "Bovine Milk Org, F (n = 74)","Bovines < 1 year, S (n = 433)",
  "Bovines < 1 year, C (n = 534)", "Breeder Chickens, F (n = 56)",
  "Broiler Meat, R (n = 363)", "Broilers Conv, F (n = 299)",
  "Broilers Org, F (n = 31)","Broilers, C (n = 232)", "Broilers, F (n = 184)",
  "Broilers, S (n = 404)", "Cattles, C (n = 193)","Fattening Pigs, S (n = 439)",
  "Growers < 50 kg, F (n = 210)", "Growers, C (n = 129)", "Humans, A (n = 96,455)",
  "Humans, Gw (n = 197,521)", "Humans, ICU (n = 30,328)", "Laying Hens, C (n = 557)",
  "Laying Hens, F (n = 347)","Pigs, C (n = 346)", "Piglets, C (n = 417)",
  "Pork, R (n = 155)", "Raw Sausages, R (n = 69)","Roe Deer Hunted, W (n =  269)",
  "Shrimps, R (n = 20)", "Small Animals, C (n = 312)", "Sows, C (n = 24)",
  "Sows, F (n = 272)", "Table Eggs, R (n = 90)", "Turkey Meat, R (n = 356)",
  "Turkeys, C (n = 327)","Turkeys, F (n = 346)","Turkeys, S (n = 372)",
  "Venisons, R (n = 150)", "Weaners, F (n = 250)", "Wild Boar Hunted, W (n = 217)"
  )

cluster_avg_GEN <- hcut(result_percent_spez_GEN,
                        k = 3,
                        hc_func = "hclust",
                        hc_metric = "euclidian",
                        hc_method = "average")

fviz_dend(cluster_avg_GEN,
          cex = 1.2,
          rect = T,
          horiz = TRUE,
          ggtheme = theme_bw(),
          k_colors = c("#1B9E77", "#E7298A", "darkblue"),
          main = "Without Gentamicin") +
  theme(title = element_text(size = 24),
        axis.title.x = element_text(size = 20),
        axis.text.x = element_text(size = 16))
```

10. List of Publications, Talks and Posters

Publications for the thesis

Suwono B, Eckmanns T, Kaspar H and Tenhagen B-A (2022) A Joint Regional Analysis of Resistance Combinations in *Escherichia coli* in Humans and Different Food-Producing Animal Populations in Germany Between 2014 and 2017. Front. Public Health 10:823613. doi: 10.3389/fpubh.2022.823613

Suwono B, Hammerl JA, Eckmanns T, Merle R, Eigner U, Lümen M, Lauter S, Stock R, Fenner I, Boemke E, Tenhagen BA. Comparison of MICs in *Escherichia coli* isolates from human health surveillance with MICs obtained for the same isolates by broth microdilution. JAC Antimicrob Resist. 2021 Sep 21;3(3):dlab145. doi: 10.1093/jacamr/dlab145. PMID: 34676365; PMCID: PMC8524623.

Suwono B, Eckmanns T, Kaspar H, Merle R, Zacher B, Kollas C, Weiser AA, Noll I, Feig M, Tenhagen BA. Cluster analysis of resistance combinations in *Escherichia coli* from different human and animal populations in Germany 2014-2017. PLoS One. 2021 Jan 20;16(1):e0244413. doi: 10.1371/journal.pone.0244413. PMID: 33471826; PMCID: PMC7817003.

Talks

World One Health Congress 2020 | Virtual Edition | 31.10.2020

Regional distribution of resistance combinations in *Escherichia coli* from different human and animal populations: Data from German national surveillance and monitoring for AMR between 2014 and 2017

International Symposium for Zoonoses 2019 | Berlin | 17.10.2019

Cluster Analysis with German Antimicrobial Resistance (AMR) Data for Humans and Food-producing Animals: *Escherichia coli* data from human and different animal populations, 2014-2017

5th International Conference on Prevention and Infection Control (ICPIC) | Genf, Schweiz | 12.09.2019

Abstract is published in ARIC Journal Supplementary 2019

Cluster Analysis with Antimicrobial Resistance (AMR) data: Data from Surveillance and Monitoring Systems in Germany

German Society of Hygiene and Microbiology Annual Meeting (DGHM) | Göttingen | 26.02.2019

How should we compare the Antimicrobial Resistance (AMR) data between different populations in Germany? *Escherichia coli* data from humans and different animals

Poster

8th Symposium on Antimicrobial Resistance in Animals and the Environment (ARAE) | Tours, Frankreich | 01.-03.07.2019

B. Suwono, T. Eckmanns, H. Kaspar, B.-A. Tenhagen

Cluster Analysis with Phenotypical Antimicrobial Resistance Data (AMR): Data from German national surveillance and monitoring

National Symposium on Zoonoses Research | Berlin | 17.-19.10.2018

B. Suwono, C. Kollas, A.A. Weiser, B. Zacher, M. Feig, I. Noll, M. Abu Sin, T. Eckmanns, H. Kaspar, B.-A. Tenhagen

Antimicrobial Resistance in *Escherichia coli* isolates from humans and different animals against four common antibiotics: Data from national surveillance and monitoring – German One Health Initiative (GOHI)

Junior Scientist Meeting 2018 | Hamburg | 07.-09.06.2018

B. Suwono, C. Kollas, A.A. Weiser, B. Zacher, M. Feig, I. Noll, M. Abu Sin, T. Eckmanns, H. Kaspar, B.-A. Tenhagen

Comparison of resistant proportions in *Escherichia coli* isolates from humans and animals: Data from German national surveillance and monitoring from 2014-2016, German One Health Initiative (GOHI)

4th International Conference on Prevention and Infection Control (ICPIC) – ARIC Journal Supplementary 2017 | June 20th to 23rd 2017

B. Suwono, M. Abu Sin, B. Zacher, M. Feig, I. Noll, T. Eckmanns

Analysis of susceptibility in *Pseudomonas aeruginosa* isolates based on Antibiotic Resistance Surveillance System – data in Germany from 2010-2015 within in- and outpatient care.

Other publications

Wilking H, Beermann S, Boone I, Dreesman J, Fingerle V, Gethmann J, Lachmann R, Lamparter M, Mayer-Scholl A, Meinen A, Schöl M, **Suwono B**. Bakterielle Zoonosen mit Bedeutung für den öffentlichen Gesundheitsschutz in Deutschland – Vorkommen, Verbreitung

und Übertragungswege [Bacterial zoonoses of public health importance in Germany-incidence, distribution, and modes of transmission]. Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz. 2023 Jun;66(6):617-627. German. doi: 10.1007/s00103-023-03703-6. Epub 2023 May 23. PMID: 37221263; PMCID: PMC10204660.

Suwono B, Brandl M, Hecht J, Eckmanns T, Haller S: Epidemiology of healthcare-associated SARS-CoV-2 outbreaks in Germany between March 2020 and May 2022. J Hosp Infect 2023; 134:108-120. doi: 10.1016/j.jhin.2023.01.011. Epub ahead of print. PMID: 36738991; PMCID: PMC9894679.

Said D, **Suwono B**, Schweickert B, Schönfeld V, Eckmanns T, Haller S: SARS-CoV-2 outbreaks in care homes for the elderly and disabled in Germany—a comparative epidemiological analysis of the periods before and after the beginning of the vaccination campaign. Dtsch Arztebl Int 2022; 119. DOI: 10.3238/arztebl.m2022.017

Suwono B, Steffen A, Schweickert B, Schönfeld V, Brandl M, Sandfort M, Willrich N, Eckmanns T, Haller S. SARS-CoV-2 outbreaks in hospitals and long-term care facilities in Germany: a national observational study. Lancet Reg Health Eur. 2022 Mar;14:100303. doi: 10.1016/j.lanepe.2021.100303. Epub 2022 Jan 14. PMID: 35043103; PMCID: PMC8759004.

Hecht J, Reichert F, **Suwono B**, Reuß A, Behnke M, Gropmann A, Eckmanns T, Abu Sin M: COSIK – COVID-19-Surveillance in Krankenhäusern. Epid Bull 2022;2:19-28 | DOI 10.25646/9513

Ayobami O, Willrich N, **Suwono B**, Eckmanns T, Markwart R. The epidemiology of carbapenem-non-susceptible Acinetobacter species in Europe: analysis of EARS-Net data from 2013 to 2017. Antimicrob Resist Infect Control. 2020 Jun 19;9(1):89. doi: 10.1186/s13756-020-00750-5. PMID: 32560670; PMCID: PMC7304165.

Blaschke U, **Suwono B**, Zafari S, Ebersberger I, Skiebe E, Jeffries CM, Svergun DI, Wilharm G. Recombinant production of A1S_0222 from Acinetobacter baumannii ATCC 17978 and confirmation of its DNA-(adenine N6)-methyltransferase activity. Protein Expr Purif. 2018 Nov;151:78-85. doi: 10.1016/j.pep.2018.06.009. Epub 2018 Jun 22. PMID: 29908915.

Publications as supplementary materials

Suwono B., Eckmanns T., Kaspar H., Tenhagen B.-A. Cluster Analysis with Antimicrobial Resistance (AMR) Data: Data from Surveillance and Monitoring Systems in Germany. Abstracts from the 5th International Conference on Prevention & Infection Control (ICPIC 2019). *Antimicrob Resist Infect Control* **8,** 148 (2019). https://doi.org/10.1186/s13756-019-0567-6. O17

Suwono B., Eckmanns T., Abu Sin M., Noll I., Feig M. Analysis of susceptibility of *Pseudomonas aeruginosa* based on ARS data within in- and outpatient care in Germany from year 2010 to 2015. Meeting abstracts from International Conference on Prevention & Infection Control (ICPIC 2017). *Antimicrob Resist Infect Control* **6,** 52 (2017). https://doi.org/10.1186/s13756-017-0201-4. P280

Lectures

Guest Lecture in HAW Summer school 2022 (online) | 05.07.2022
Antimicrobial Resistance and One Health

Guest Lecture in Berlin School of Public Health | 15.06.2022
Infectious Disease Epidemiology - Antimicrobial Resistance and One Health

Guest Lecture in Berlin School of Public Health (online) | 18.11.2021
Infectious Disease Epidemiology - Antimicrobial Resistance and One Health

Guest Lecture in HAW Winter School 2021 (online) | 13.11.2021
Antimicrobial Resistance

Guest Lecture in HAW DAAD Earth School 2020 (online) | Nov 2020
Antimicrobial Resistance

Guest Lecture in LMU Munich at MSc Epidemiology | München | 13.02.2019
One Health and Antimicrobial Resistance

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13. Conflict of Interest

Im Rahmen dieser Arbeit bestehen keine Interessenskonflikte durch Zuwendungen Dritter.

14. Declaration of Independence

Hiermit bestätige ich, dass ich die vorliegende Arbeit selbstständig angefertigt habe. Ich versichere, dass ich ausschließlich die angegebenen Quellen und Hilfen in Anspruch genommen habe.

Berlin, 13.10.2023 Beneditta Suwono