Emergence of antimicrobial resistant bacteria in and beyond companion animal medicine

Habilitation thesis

to receive the Venia Legendi for Microbiology

at Freie Universität Berlin

Department of Veterinary Medicine

submitted by

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Berlin 2021

Well we know what makes the flowers grow but we don't know why

And we all have the knowledge of DNA but we still die

White coats, New Model Army, 1987

Preliminary Note

This habilitation thesis emerged from 10 years of scientific work, covered by the 10 papers introduced here. More publications tackling the research field have been released from our working group during that time, but the papers marked in bold letters (**paper1-10)** through the text have been particularly selected to state the main results of this solely cumulative habilitation thesis. Its content crosses multiple disciplines and research areas, resulting in a cross-sectional approach illustrated in figure 1. Since detailed material and methods sections are provided in each of these papers, this thesis is confined to a brief introduction about common methods used for molecular typing and epidemiology of bacteria, with a focus on those elaborated in the papers presented.

The ten selected publications are focused on I) an overview on antimicrobial resistant (AMR) including multidrug resistant (MDR) bacteria challenging veterinary infection control in terms of hospital associated infections (HAI) in recent years, II) improving methicillin resistant staphylococci (MRS) diagnostics and genome-based insights into the genetic elements conferring methicillin resistance, III) infection epidemiology key aspects of AMR bacteria with a special focus on extended host spectrum genotype (EHSG) lineages in - and beyond - veterinary medicine culminating in a closer look at IV), bacterial niche adaptation capabilities of an example of MDR, namely methicillin resistant *Staphylococcus aureus* (MRSA).

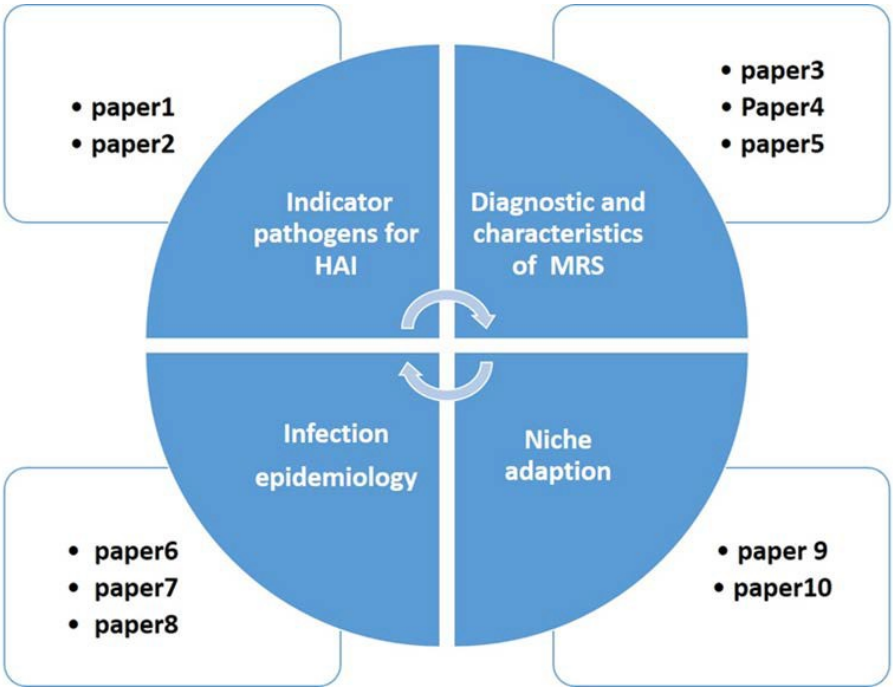

Fig. 1 Research areas addressed by this cumulative habilitation thesis

The backbone of this thesis is based on the molecular and phenotypic characteristics of methicillin resistant *Staphylococcus* spp. (MRS) and extended-spectrum beta-lactamase - producing *Escherichia coli* (ESBL-*E. coli*), which are commonly characterized by their ability to colonize and/or infect a broad range of different animal species and humans (**paper1**, **paper2**, **paper9 and paper10**). The majority of the data presented in this work refer to bacteria isolated from samples of companion animals, especially dogs, cats and horses, using culture-dependent microbiology. While the intention of figure 1 is to provide the reader with a comprehensive overview of the main topics, the 10 selected publications all clearly bridge different areas. While there is a timeline of publications for this thesis, the particular arrangement of this work does not follow a historical sketch but a rather didactical composition. A recent comprehensive review (**paper1**) was chosen to introduce the reader to my field of research, which addresses the following five main research questions:

I. Which are the important MDR indicator pathogens bacteria in companion animal medicine?

What do we know about the history of MDR bacteria in veterinary medicine and its impact on clinical settings providing health care services for companion animals? What bacterial species, also referred to as indicator pathogens, have been frequently reported as a cause of HAI in companion animals? Are there differences between bacteria causing HAI in facilities providing health-care for dogs and cats compared to those involving horses? To which extent are horses colonized or even infected with indicator pathogens when entering a horse clinic?

II. How can we improve MRS diagnostics and trace methicillin resistance in staphylococci of (animal) origin?

Is it possible to improve species identification of staphylococci frequently associated with MDR phenotypes in companion animal medicine, especially *S. pseudintermedius*, using matrix-assisted laser desorption ionization - time of flight mass spectrometry (MALDI-TOF MS) technique? Are the genes conferring methicillin resistance in Staphylococci of companion animal origin others than the originally described *mec*A gene? Do the genomic integration sites for the mobile elements harboring the *mec* genes share similarities in different Staphylococcal species? What is the putative reservoir for these novel variants mediating methicillin resistance in staphylococci?

III. Which factors influence transmission of MRSA and ESBL-*E. coli* in companion animals and beyond?

Can staphylococci cross the species barriers between dogs and humans outside of clinical settings, which are frequently associated with enhanced selective pressure? What risk factors are commonly associated with MRSA infections in companion animals? Are drug-resistant *E. coli* transferred from horse to horse in veterinary clinics through hospital-associated spread? Do MRSA and ESBL-*E. coli* isolated from horses in Germany share genetic similarity with those reported from other countries?

IV. Which factors influence niche adaptation of zoonotic MRSA of extended host spectrum genotypes (EHSG)?

Do successfully spreading MRSA and ESBL-*E. coli* belong to extended host spectrum genotype (EHSG) lineages? Do MRSA frequently associated with cases of infection in human- and companion animal medicine share common features? Which mobile genetic elements are generally capable of fostering niche- or even host adaptation in *S. aureus*, especially horses? Is there evidence for an ongoing adaptation to different hosts in MRSA belonging to the epidemic sequence type (ST) lineage 398?

The ten publications addressed above have been deposited as

Attachment

0 Abstract

0.1 Abstract (English)

This habilitation thesis summarizes the results of 10 scientific publications (references: **paper1-10**) addressing the topic "Emergence of antimicrobial resistant bacteria in and beyond companion animal medicine".

The first chapter's intention is to introduce my research area to the reader by providing basic information on the occurrence of antimicrobial resistance (AMR) in bacteria, including general resistance mechanisms, distribution and spread in different ecological environments and the commonly applied clinical classifications relevant for the veterinary medicine field. The following section characterizes the nature of health care associated infections (HAIs) in human and veterinary medicine, including baseline similarities and differences. Subsequently, bacterial pathogens exhibiting particular antibiotic resistances are introduced. Here, a strong focus is set on AMR bacteria frequently associated with HAIs in human and veterinary medicine, which are therefore also considered as "indicator pathogens". Since β-lactams such as penicillins and cephalosporins are known for their excellent pharmacokinetic properties which is commonly accompanied by a low toxicity for mammals, this group of active substances is of particular importance for antimicrobial treatment of bacterial infectious diseases in both humans and animals. Infections with indicator pathogens harboring resistance towards β-lactams such as *Staphylococcus aureus* [methicillin resistant *S. aureus*, (MRSA)] and *Escherichia coli* [Extended-Spectrum β-Lactamase (ESBL)-producing *E. coli* (ESBL-*E. coli*)] are therefore often accompanied by treatment failure. Since antibiotics belonging to this group are among the "first choices" for empirical treatment of infectious diseases, including life-threatening scenarios such as septicemia, collaborative efforts to limit spread of β-lactam resistance are currently an important goal in human- and veterinary medicine. Additional resistances towards further classes of antibiotic substances are frequently associated with MRSA and ESBL-*E. coli*, leading to a further reduction of therapeutic possibilities in case of infection.

In the second chapter, the publications' results are summarized into four distinct sections. First, the importance of HAI in small animal and equine medicine is illustrated by a literature review considering outbreak events reported so far.

A brief historical sketch reveals that problems due to HAI in animal clinics, including cases of zoonotic transmission, have been known since at least 1961. While there have been numerous reports in the past highlighting the technical and scientific developments during the

last decades in companion-animal medicine, other important fields such as hygiene management, infection control and occupational safety of employees still lack comparable achievements.

The current challenges faced by companion animal clinics are described by studies evaluating the introduction rates for multi drug resistant (MDR) indicator pathogens via colonized horses in a large German university clinic. Overall, n = 341 horses representing distinct medical indications (i.e. 233 horses with "colic symptoms" and 108 with "open wounds") were sampled immediately upon hospital admission. The overall screening results showed that MRSA was detected in 3.5% of the nasal swabs and ESBL-Enterobacteriaceae in 10.3% (mainly ESBL-*E. coli*) of the fecal samples. Subsequent molecular typing of the isolates showed that all MRSA belonged to sequence type (ST) 398, the currently predominating MRSA lineage identified in clinical samples of horse origin in Europe. In contrast, ESBL-*E. coli* showed a broad heterogeneity in genomic backgrounds associated with the β-lactam resistance. However, isolates belonging to ST complexes (STC) 1250 (31.7%) and STC10 (19.5%) clearly dominated the collection. Notably, all MRSA and ESBL- *E. coli* isolates isolated in these studies showed additional antimicrobial resistances to a minimum of three further classes of antibiotics.

For assessment of a particular AMR situation in certain bacterial pathogens and/or environments, e.g. if surveillance of AMR in a pathogen causing HAI is needed, an unambiguous species identification is mandatory. Species identification is essential for sound interpretation of antimicrobial sensitivity testing (AST) results and therefore a prerequisite to classify the resistance phenotype of the pathogen correctly. Moreover, species identification is also needed for precise interpretation of additional results gained by other molecular biological methods. A combination of bacterial genome analysis with matrix-assisted laser desorption ionization - time of flight mass spectrometry (MALDI-TOF MS) allowed us to differentiate between the species *Staphylococcus intermedius*, *S. delphini* and *S. pseudintermedius*, which was previously a time-consuming and uncertain approach. Since methicillin resistant *S. pseudintermedius* (MRSP) are often associated with resistances towards several other antimicrobials, a rapid and reliable species identification using MALDI-TOF MS promotes early and targeted antimicrobial therapy in cases of infection and, if necessary, the initiation of additional hygiene measures to prevent its spread. The following results demonstrate the overwhelming importance of constant adjustments of microbiological diagnostic screening methods for identification of MDR: In 2011, a novel variant of the methicillin resistance gene (later: *mec*C) was described for MRSA of cattle and human origin. A study we immediately initiated identified *mec*C-MRSA in clinical samples from horses, dogs, cats, wild animals and rodents. Here, the “weak” expression of the resistance phenotype, now known to be a

common feature of *mec*C-MRSA, was a challenge to classify an isolate as MRS, which was achieved after diagnostic adjustments. As described above, methicillin resistance is often associated with other resistances, e.g. towards tetracyclines, zinc and arsenic compounds. Our detailed analysis of the integration site for the methicillin resistance-mediating mobile genetic element in staphylococci was identified as a "hot spot" for recombination events: We discovered a mosaic-like genomic structure which is prone to integration of mobile resistance-mediating elements of different types.

The combination of up-to-date microbiological diagnostics with classical principles of infection epidemiology allowed us to answer important questions concerning possible transmission scenarios for MDR pathogens in companion animal clinics and identify risk factors for MRSA infections in these animals. In 2012, the ability of MRSA to cause HAI in companion animals and its importance as an indicator pathogen for veterinary medicine was already known. At that time however, similar information was not available for ESBL- *E. coli*. To address this deficit, we retrospectively investigated several cases of serious infections caused by ESBL-*E. coli* in hospitalized horses in a veterinary clinic. Comparative analysis of molecular typing results obtained for 13 ESBL-*E. coli* together with the horses' clinical data revealed a putative spatio-temporal relationship of the isolates, which probably reflected several transmission events between these patients.

In another case-control study, we have examined the possible risk factors for wound infections caused by MRSA in horses, dogs and cats. Multivariable logistic regression identified the following variables as risk factors for MRSA infection compared to methicillin susceptible infection: (i) the number of employees working at the veterinary setting ($n>10$; $p<0.001$), (ii) antibiotic treatment prior to sampling (systemic: $p=0.002$; local: $p=0.049$, both: $p=0.011$) and (iii), surgical site infection ($p<0.001$). *Spa* typing revealed clonal complexes (CC) previously known for hospital-associated lineages spreading in human health-care settings in Germany (i.e. CC5 and CC22) as the dominating lineages among isolates of dog and cat origin. Equine MRSA belonged nearly exclusively to CC398, a CC previously described as a nosocomial pathogen in equine clinical settings.

While performing the studies mentioned above, we considered whether transmission of relevant AMR bacteria between pets and their owners (e.g. MRS) occur outside the typical hospital environment, and further, which circumstances and human behavioral patterns might influence such events. Therefore, we investigated different aspects of the human-to-dog relationship together with MRS carriage at a dog show in 2009. We introduced a questionnaire accompanied by a screening program of the nasal swabs of dog owners (108) and their dogs (108) with respect to staphylococci. *S. aureus* was identified in swabs obtained from 20

(18.5%) humans and two dogs (1.8%), respectively. 15 dogs (13.9%) and six owners (5.6%) harbored *S. pseudintermedius*, including one MRSP. Interestingly, 68.5% of the dog owners allowed their dog(s) to rest on the sofa, 39.8% allowed their dog(s) to lay on their bed, 93.5% let them lick their hands, and 52.8% let them lick their face. A bivariate analysis of putative risk factors showed that dog owners who kept more than two dogs were significantly more likely to become colonized with *S. pseudintermedius* than those who kept only one or two dogs ($p<0.05$).

The results section closes with the most recent results, which demonstrated the continuing adaptation of MDR bacteria to the companion animal hospital environment. We showed that equine MRSA-ST398 have acquired different mobile genetic elements (i.e. pathogenicity islands, phages) harboring immune-modulating factors which impede the activation of the complement system, which is among the most important defense measures of the innate immune system towards invasive *S. aureus* infections. The equine MRSA harbored genes encoding variants of the staphylococcal complement inhibitor protein (SKIN), known for its function in subverting the complement system in plasma of humans, cattle, pigs and horses. These results clearly demonstrated the existence of extended host (EHSG) lineages and their characteristic flexibility with respect to niche- and host-adaptation.

Chapter 3's discussion section presents the general concepts of surveillance of antibiotic consumption, HAI and antibiotic resistance in human medicine, and expands the context with respect to the results of this habilitation thesis. The importance of HAI and MDR pathogens in companion animals for human health is pointed out and current developments in this area are discussed. With regard to integrated surveillance for antibiotic resistance, antibiotic consumption and HAI, a "best practice" model featuring the One Health concept is outlined. This is followed by a discussion of prospects for small animal and horse medicine, taking into account realistic human and financial resources to establish fundamental surveillance structures.

The following section summarizes the key factors for HAI prevention in veterinary clinics. In particular, technical progress (i.e. genomics/bioinformatics) in the field of molecular epidemiology has contributed to these findings, a development which is reflected in the publications presented.

Finally, possible reasons for the occurrence of MDR pathogens in animal clinics are given, using horse clinics as an example. The occurrence of ESBL-*E. coli* and MRSA belonging to EHSG-lineages explain why further research on these MDR pathogens is necessary, especially with respect to further host- and niche-adaptation.

In summary, the publications presented make it clear that research on EHSG pathogens and their importance has only just begun. New methods of molecular epidemiology currently (2020) offer completely new opportunities to study adaptive changes in bacterial genomes in large, representative studies. However, care is needed with respect to establishment of a suitable, representative isolate collection, which is of crucial importance for the validity of the studies. Integrated One Health surveillance, as outlined in this document, could reflect the current importance of MDR EHSG pathogens for human and veterinary medicine. An integrated approach would lead to a better understanding of the bacterial adaptation processes to ecological niches as a whole and reveal new, epidemiologically significant developments in a timely manner, whereas currently we can often only trace these types of developments in retrospect.

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0.2 Abstract (Deutsch)

Diese Habilitationsschrift ist eine Zusammenfassung der Ergebnisse aus insgesamt 10 Publikationen mit dem Titel „Auftreten von Antibiotika-resistenten Bakterienspezies in der Kleintier- und Pferdemedizin und darüber hinaus“, welche aufgrund der jeweiligen inhaltlichen Schwerpunkte in vier Themenkomplexe untergliedert sind. Die den Ergebnissen vorangestellte Einleitung stellt das Forschungsgebiet insgesamt vor und bietet grundlegende Informationen zum Vorkommen von Antibiotikaresistenzen in Bakterien, deren Verbreitungswege sowie die für die Veterinärmedizin klinisch relevanten Klassifikationen. Es folgt eine kurze Einführung in das Thema „Health care“ assoziierte Infektionen (HAI) in der Human- und Veterinärmedizin, einschließlich einer Gegenüberstellung von grundsätzlichen Gemeinsamkeiten und Unterschieden. Im Anschluss werden die bakteriellen Pathogene mit Antibiotikaresistenzen vorgestellt, die von besonderer Bedeutung für HAIs in der Human- wie auch in der Veterinärmedizin sind und daher auch als „Indikatorpathogene“ gelten. Da β-Lactame wie Penicilline oder Cephalosporine im Allgemeinen hervorragende pharmakokinetische Eigenschaften besitzen bei gleichzeitig guter Verträglichkeit und geringer Toxizität, ist diese Wirkstoffgruppe besonders wichtig für die empirische Therapie von bakteriellen Infektionskrankheiten bei Menschen und Tieren. Resistenzen gegen β-Lactame in Indikatorpathogenen wie *Staphylococcus aureus* [Methicillin-resistente *S. aureus*, (kurz MRSA)] und *Escherichia coli* [(engl.) „Extended-Spectrum β-Lactamase“ (ESBL)- bildende *E. coli* (ESBL-*E. coli*)] wirken sich oftmals nachteilig auf den Behandlungserfolg aus, zumal Antibiotika dieser Gruppe für viele medizinische Indikationen Mittel der ersten Wahl darstellen. Zusätzliche Resistenzen gegenüber weiteren Wirkstoffklassen sind häufig mit diesen MRSA und ESBL-*E. coli* assoziiert, so dass sich die therapeutischen Möglichkeiten im Falle einer Erkrankung weiter verringern.

Im 2. Kapitel werden die Ergebnisse der hier vorgestellten Publikationen in thematisch gegliederten Abschnitten zusammengefasst und eingeordnet. Zunächst wird die Bedeutung von HAI in der Kleintier- und Pferdemedizin durch Literatur zu Ausbruchsgeschehen mit den jeweils verantwortlichen Infektionserregern verdeutlicht. Diese Analyse zeigte, dass seit 1961 die Problematiken durch HAI in Tierkliniken sowie der Aspekt der möglichen wechselseitigen Übertragung von HAI-assoziierten Infektionserregern zwischen Mensch und Tier bekannt sind. Vielfach wurde in der Vergangenheit geschildert, dass im Hinblick auf Hygienemanagement, Infektionskontrolle sowie Arbeitsschutz der beschäftigten Mitarbeiter in der Veterinärmedizin ein vergleichsweise zu geringer Fortschritt zu verzeichnen ist.

Mit welcher tatsächlichen Erregerlast Tierkliniken konfrontiert sein können, zeigen Ergebnisse zum Eintrag von multi-resistenten Infektionserregern (MRE) in eine große Deutsche Universitätspferdeklinik durch kolonisierte Pferde. Insgesamt wurden n= 341 Pferde, davon

233 mit dem Vorbericht „Kolik-Symptomatik“ und 108 mit dem Vorbericht „offene Wunde“ unmittelbar bei Klinikaufnahme beprobt. Die Screening-Ergebnisse insgesamt zeigten 3,5% MRSA-positive Nüsternabstriche sowie ESBL-Enterobacteriaceae in 10,3% (überwiegend ESBL-*E. coli*) der Kotproben. Die nachfolgende molekulare Typisierung der Isolate ergab, dass alle MRSA dem Sequenztyp (ST)398 zuzurechnen waren, der derzeit prädominanten Linie in Europa in Verbindung mit klinischen MRSA-Nachweisen beim Pferd. Bei den ESBL-*E. coli* zeigte die phylogenetische Analyse eine Vielzahl von unterschiedlichen Genotypen, es dominierten jedoch Isolate der ST Komplexe (STC)1250 (31,7%) und STC10 (19,5%). Alle MRSA und ESBL-*E. coli* Isolate zeigten zudem Resistenzen gegenüber mindestens drei weiteren Antibiotika-Wirkstoffklassen.

Für die Bewertung der Resistenzsituation von Bakterien in beispielsweise einem bestimmten Umfeld, im Zusammenhang mit HAIs, oder für deren systematische Erfassung im Rahmen einer Surveillance, ist zunächst eine eindeutigen Speziesidentifizierung unerlässlich. Erst danach können die Ergebnisse der Empfindlichkeitstestung gegen Antibiotika und ggf. molekularbiologischen Ergebnisse sachgerecht für ein bestimmtes Isolat interpretiert werden. Durch Kombination von Genomanalyse mit (engl.) matrix-assisted laser desorption ionization - time of flight mass spectrometry (MALDI-TOF MS) ist es gelungen, die für die veterinärmedizinische Diagnostik wichtige, eindeutige und schnelle Differenzierung zwischen den Spezies *S. intermedius*, *S. delphini* und *S. pseudintermedius* per MALDI-TOF MS vorzunehmen, die zuvor sehr zeitaufwendig und unsicher war. Da Methicillin-resistente *S. pseudintermedius*-Isolate (MRSP) häufig mit zahlreichen weiteren Resistenzen assoziiert sind und es Berichte über HAI durch MRSP in Kleintierkliniken gibt, ermöglicht nun die schnelle Diagnostik dieser Spezies per MALDI-TOF MS u.a. eine rasche und gezielte Therapie sowie ggf. die Einleitung von zusätzlichen Hygienemaßnahmen. Wie wichtig auch die ständige Anpassung der diagnostischen Screening-Methoden für MRE ist, zeigen die folgenden Ergebnisse: 2011 wurde erstmals eine neue Variante des Methicillin-Resistenzgens (später: *mec*C) bei MRSA von Rindern und Menschen beschrieben. Eine von uns umgehend eingeleitete Studie wies *mec*C-MRSA auch in klinischen Proben von Pferden, Hunden, Katzen, Wildtieren und Nagetieren nach, wobei die schwache Expression des Resistenzphänotyps bei *mec*C-MRSA eine Herausforderung für die Diagnostik insgesamt darstellt und Anpassungen erforderte. Methicilin-Resistenz ist häufig mit anderen Resistenzen, z.B. gegen Tetrazykline, Zink und Arsen, assoziiert. Unsere detaillierte Analyse der Integrationsstelle für das Methicillinresistenz-vermittelnde mobile genetische Element im Genom von Staphylokokken identifizierte einen „Hot-spot“ für Rekombinationsereignisse, wodurch diese Region häufig eine mosaikartige genomische Struktur aufweist, in die sich mobile, resistenzvermittelnde Elemente, insgesamt leicht integrieren können.

Die Verbindung einer „up-to-date“ mikrobiologischen Diagnostik mit Methoden der klinischen Infektionsepidemiologie ermöglichte darüber hinaus die Beantwortung wichtiger Fragestellungen zu möglichen Transmissionsszenarien von MRE in Tierkliniken und die Identifikation von Risikofaktoren für MRE-Infektionen in der Kleintier- und Pferdemedizin – und- darüber hinaus. An den folgenden Beispielen soll dieser Zusammenhang beispielhaft erläutert werden. Während für MRSA 2012 bereits bekannt war, dass diese HAI Indikatorpathogene mit Ausbruchsgeschehen in Tierkliniken in Verbindung stehen können, war dies für ESBL-*E. coli* damals noch unklar. Daher haben wir mehrere Fälle von z.T. schweren Infektionen durch ESBL-*E. coli* bei hospitalisierten Pferden in einer Tierklinik retrospektiv untersucht. Durch die Analyse von molekularen Typisierungsergebnissen von 13 ESBL-*E. coli* mit den klinischen Daten der erkrankten Pferde konnte der wahrscheinliche spatio-temporale Zusammenhang der Isolate rekonstruiert werden, so dass es wahrscheinlich mehrere Transmissionsereignisse gab, die zu den Fällen führten.

In einer weiteren Studie wurden die möglichen Risikofaktoren für eine Wundinfektion durch MRSA bei Pferden, Hunden und Katzen in einer Fall-Kontroll-Studie untersucht. Eine starke Korrelation für MRSA (n= 106) im Vergleich zu Methicillin-sensiblen *S. aureus* (n= 102) zeigte sich, wenn i) die Anzahl Beschäftigten in einer Praxis oder Klinik mindestens 10 war ($p<0.001$), ii) zuvor eine systemische Antibiose bei den Tieren erfolgte ($p=0.002$), oder iii) der Vorbericht „postoperative Wundinfektion“ lautete ($p<0.001$). Eine weitere Erkenntnis dieser Studie war, dass MRSA-ST398 in ganz Deutschland bei Pferden die am häufigsten nachgewiesene Genotyplinie ist. Aus diesen Ergebnissen wird deutlich, dass MRE in Zusammenhang mit HAI in Tierkliniken/-Praxen auftreten, und dass mindestens einige Risikofaktoren für Tiere, eine HAI durch MRE zu entwickeln, aus der Humanmedizin seit langem bekannt sind.

Immer wieder kam während der oben genannten Untersuchungen die Frage auf, ob Bakterien mit relevanten Resistenzen, z.B. MRS, auch außerhalb des Hospitalmilieus zwischen Haustieren und ihren Besitzern übertragen werden können, sowie, welche Umstände und Verhaltensweisen dies möglicherweise begünstigen. Wir haben daher 108 Hundebesitzer nach ihren Gewohnheiten im Umgang mit Ihren Hunden gefragt und gleichzeitig Nasentupfer von allen befragten Menschen und deren Hunden auf MRSA und MRSP untersucht. *S. aureus* wurde in 18,5% (Mensch) und 1,8% (Hund) der Abstriche nachgewiesen, während *S. pseudintermedius* häufiger beim Hund (13,9%) als bei den Besitzern (5,6%) nachweisbar war. Ein MRSP wurde bei einem Hundebesitzer nachgewiesen. Viele Besitzer gaben an, sich im Gesicht ablecken zu lassen (52,8%), den Hund auf dem Sofa (68,5%) oder gar im Bett (39,8%) zu dulden. Eine bivariate Analyse möglicher Risikofaktoren ergab, dass Hundebesitzer, die mehr als zwei Hunde halten, eine signifikant höhere Wahrscheinlichkeit für

einen *S. pseudintermedius*-Nachweis haben, als jene, die nur 1-2 Hunde halten ($p < 0,05$). Wir kamen zu dem Schluss, dass die Übertragung von *Staphylococcus* spp. zwischen Hundebesitzern und ihren Hunden ungeachtet der möglichen Resistenzen generell möglich ist, eine Beobachtung welche seitdem vielfach bestätigt wurde.

Der Ergebnisteil meiner Arbeit schließt mit den aktuellsten Ergebnissen, die die kontinuierliche Anpassung von MRE an das „Klinikmileu“ am Beispiel equiner MRSA-ST398 demonstrieren: Durch die Aufnahme unterschiedlicher mobiler Elemente mit immunmodulierende Virulenzfaktoren haben MRSA-ST398 von Pferden u.a. die Fähigkeit erlangt, die Aktivierung der Komplementkaskade zur Abwehr der eindringenden Bakterien, welche ein wichtiger Teil der angeborenen Immunantwort ist, zu verhindern. Die Überwindung des Komplementsystems durch *S. aureus* gilt als der entscheidende Schritt zu Beginn einer invasiven Infektion. Die von uns typisierten Isolate zeigten dabei drei vollkommen unterschiedliche Varianten des Komplement-inhibierenden (SKIN) Proteins, welche zuvor unterschiedlich starke Komplement-Inhibierungs-Aktivitäten im Plasma von Menschen, Rindern, Schweinen und Pferden gezeigt hatten. Eine Kombination dieser Faktoren in equinen Isolaten führte also unmittelbar zu verbesserten Überlebenschancen von MRSA-ST398 bei verschieden Tierarten und dem Menschen, ein klarer Beweis für die Existenz von EHSG (engl. Extended-Host Spectrum Genotype)-Linien, die sich durch ihre besonders flexible Adaptationsfähigkeit auszeichnen.

Der Diskussionssteil der Arbeit in Kapitel 3 ordnet die Bedeutung der vorgestellten Ergebnisse in einen größeren Kontext ein und stellt dabei die generellen Konzepte der Surveillance von Antibiotikaverbrauch, HAI und Antibiotikaresistenzen in der Humanmedizin vor. Es wird die Bedeutung des Auftretens von HAI und MDR Pathogenen bei „companion animals“ für die Gesundheit von Menschen diskutiert sowie aktuelle Entwicklungen auf diesem Gebiet erörtert. Ein „best practice“ One Health Model-Konzept im Sinne einer integrierten Surveillance für Antibiotikaresistenz, Antibiotikaverbrauch und HAI wird skizziert, um fundamentale Surveillancestrukturen zu etablieren, gefolgt von Strategien für die Kleintier- und Pferdemedizin, unter Berücksichtigung realistischer personeller und finanzieller Ressourcen.

Die Schlüsselfaktoren für die Prävention von HAI in Tierkliniken fasst der nachfolgende Abschnitt zusammen. Zu diesen Erkenntnissen hat vor allem der technische Fortschritt im Bereich molekulare Epidemiologie beigetragen, eine Entwicklung, die sich anhand der vorgestellten Publikationen nachzeichnen lässt.

Es werden mögliche Gründe für das Auftreten von MDR Pathogenen in Tierkliniken, am Beispiel Pferdekliniken dargelegt. Abschließend wird an den für die Arbeit gewählten Beispielen ESBL-*E. coli* und MRSA erläutert, warum weitere Forschungen zu MDR Pathogenen und Ihrer Bedeutung für die Pferde- und Kleintiermedizin erforderlich sind, auch

im Hinblick auf die Anpassungsfähigkeit bestimmter Genotyp-Linien dieser Bakterien an unterschiedliche Tierarten und den Menschen.

Zusammenfassend wird durch die vorgestellten Publikationen deutlich, dass die Forschung zu EHSG- Pathogenen und ihrer Bedeutung gerade erst begonnen hat. Durch neue Methoden der molekularen Epidemiologie bieten sich gegenwärtig (2020) ganz neue Möglichkeiten, die adaptiven Veränderungen in bakteriellen Genomen in großen, repräsentativen Studien zu untersuchen. Allein, hierfür ist die Auswahl der Isolate von ganz entscheidenderer Bedeutung. Ein integrierte One Health -Surveillance, wie sie in dieser Schrift skizziert wird, könnte die jeweils aktuelle Bedeutung von multiresistenten EHSG-Pathogenen für die Human- und Veterinärmedizin widerspiegeln. Dies könnte zu einem besseren Verständnis der bakteriellen Adaptationsprozesse an ökologische Nischen insgesamt führen sowie neue und epidemiologisch bedeutsame Entwicklungen zeitnah aufzeigen, wogegen wir heute oftmals nur in der Retrospektive diese Art von Entwicklungen nachzeichnen können.

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1 Introduction

1.1 The broader context of antimicrobial resistance in bacteria

Antimicrobial resistance (AMR) is defined as the development of resistance in microorganisms including bacteria, viruses, fungi and parasites, to an antimicrobial agent to which they originally have been sensitive [1]. Focusing on bacteria only, AMR represents an ecological problem that is characterized by complex interactions involving diverse microbial populations affecting the health and well-being of humans, animals and the environment [2]. It is common knowledge that infections caused by resistant bacteria may require more care as well as alternative and more expensive antibiotics, which may lead to an increase of severe side effects [3] among the patients. Not surprisingly, the same is true for antibiotic treatment options available for animals. The drivers of AMR include, beyond others, antimicrobial use and abuse in human, animal, and environmental sectors and the spread of resistant bacteria and resistance determinants within and between these sectors and around the globe [2,4].

In September 2016, AMR became the fourth health issue after HIV, non-communicable diseases and Ebola to be discussed by the United Nations General Assembly [5]. The collaborative effort of health science professions to attain optimal health for humans, domestic animals, wildlife, plants, and the environment is generally called One Health [4]. The world health organization (WHO) has summarized that the rising threat of antimicrobial resistance requires such a holistic and multi-sectoral One Health approach, because antimicrobials used to treat various infectious diseases in animals may be the same or similar to those used for humans [6]. Accordingly, the first aim of the WHO global action plan on AMR is “to improve awareness and understanding of antimicrobial resistance through effective communication, education and training” [7].

Due to a lack of collaborative and integrated research, in the past the problem of AMR had been characterized by finger-pointing and denial of responsibility in both human and veterinary medicine when discussing the causes for the worldwide increase in AMR [8]. For instance, among the common misconceptions at the time was the demand for an immediate and complete ban for all antimicrobial substances commonly prescribed for human patients’ therapies of infectious diseases for treatment of animals, neglecting the fact that virtually no substances would have been left to treat sick animals at all (figure 2).

Since then, many efforts have been made, both national and international, to increase bi-directional understanding for both human and veterinary medicine by fostering cross-sectional research. These exertions have led to the formation of the National Research Platform for Zoonoses in 2009, which is currently an information and service network for all scientists

active in the field of zoonoses research, including more than 1000 members in Germany, funded by the Federal Ministry of Education and Research (BMBF), the Federal Ministry of Health (BMG), the Federal Ministry of Food and Agriculture (BMEL) and the Federal Ministry of Defence (BMVg).

While various milestones in improving the understanding between the human and veterinary medical sectors have been reached, the recent “colistin case” illustrates the current issues. While colistin (polymyxin E, discovered in 1947) represents an antimicrobial class that has long been used in animals for therapeutic, prophylactic, and in some countries, growth promotion purposes, it was only rarely used to treat humans due to its severe side effects [4, 9]. Colistin only recently gained attention as a last resort antibiotic in critical-ill human patients suffering from infections caused by Gram-negative MDR bacterial [9].

At present, certain antimicrobial classes are reserved exclusively for humans, in particular those used to treat tuberculosis (e.g., isoniazid) or other infections for which animals are typically not treated due to infection control by eradication of the afflicted population [4]. A few others are limited to veterinary use, mainly because of toxicity to humans [4]. Nevertheless, the fact remains that most classes of antibiotics used in veterinary medicine have human analogues. Among the important exceptions are the ionophores (e.g. monensin, narasin, salinomycin, lasalocid), the quinoxalines (e.g. olaquindox), bambermycins (flavophospholipol) and avilamycin [4, 8].

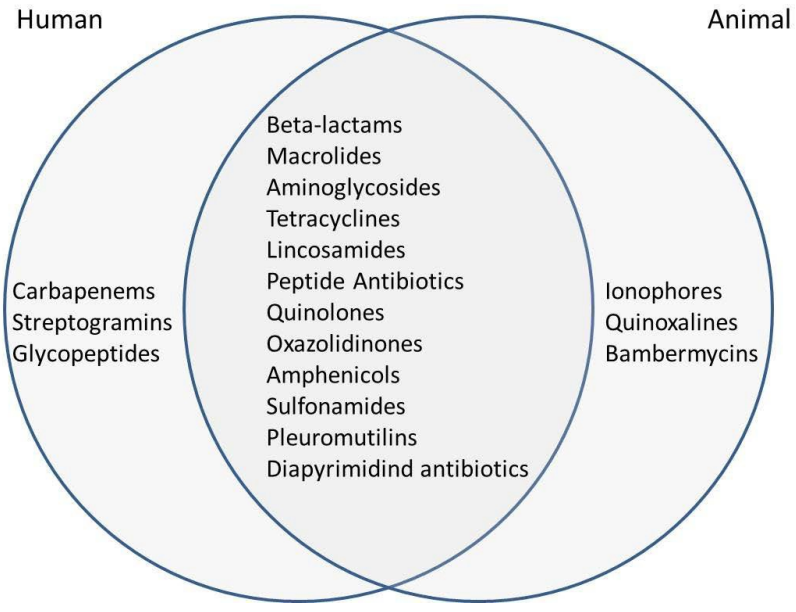

Fig. 2 Antimicrobial substances used in human- and veterinary medicine [4, 6, 8]

Moreover, antibiotics of the shared subset include those classified by WHO as “Highest Priority Critically Import Antimicrobials” for anti-infective therapies in human patients such as

quinolones, third- and fourth -generation cephalosporins, macrolides and polymyxins (http://www.whogis.com/foodsafety/publications/cia2017.pdf.). However, selective pressure induced by utilization of any antibiotic is generally capable of selecting for resistant variants.

As a variety of different antibiotics are currently available, one could conclude that there will always be an option to treat a bacterial infection exhibiting AMR. However, having a closer look at the chemical background of these drugs, only a few antibiotic classes prevail. Figure 3 summarizes the most common targets of antibiotics in bacteria, namely protein biosynthesis (1), folate synthesis (2), cell membrane synthesis (3), DNA or RNA replication machineries (4), cell wall synthesis (5) and modification of target structures (6).

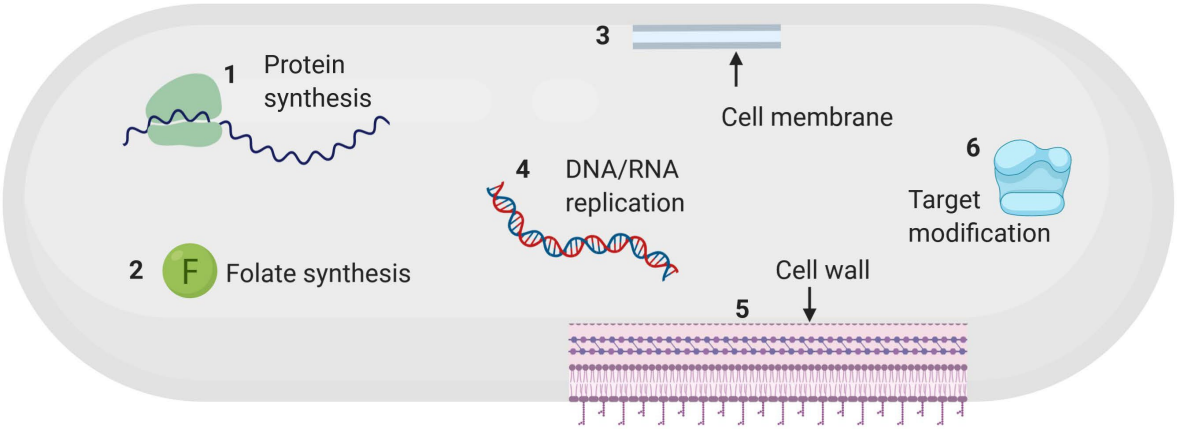

Fig. 3 The most common target structures of antibiotics in bacteria

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Generally, classification of antimicrobial agents into classes is based on their chemical backbone and therefore directly reflects the limited number of target sites (Figure 3) for anti-infective drugs used to impede growth of bacteria (bacteriostatic) or even kill the cells (bactericide).

There is currently little prospect for the development and market launch of novel antibiotics in the near future, especially for the veterinary sector. Consequently, preservation of antimicrobials which are currently available is the main theme behind the prudent use campaign – mediated by antibiotic stewardship (ABS) initiatives in human and animal medicine [10, 11].

1.1.1 Intrinsic and acquired antimicrobial resistance in bacteria

Some bacteria are intrinsically resistant to certain antibiotic classes, independently of previous antibiotic exposure. These resistance mechanisms are not horizontally transferable, but often characteristics of certain bacterial families or species, for instance multidrug efflux pumps, lack of cell wall, enzymes, specific impermeabilities of the cell wall or lack of particular intracellular drug targets [12]. Among the common mechanisms conferring acquired AMR in bacteria are enzyme production, target site alterations, switching of metabolic pathways, modification of outer membrane permeability and induction and employment of efflux pumps [12].

In general, acquired resistance is either a result of spontaneous or provoked mutations of the bacterial genome or the result foreign genetic material uptake. One or more spontaneous mutations in the bacterial genome may decrease sensitivity to a particular anti-infective agent [12].

Mutations in the genome of a living being is one of the major drivers of evolution, it is the cornerstone for the process of natural adaptation and selection [13]. In bacteria, mutations emerge and accumulate rapidly, and significant phenotypic changes often appear to occur in real-time, including those associated with antibiotic resistance [13]. This mutation rate may increase to varying degrees in the presence of the drug in question. Resistant mutants of a bacterial species can occur even when confronted by antibiotic concentrations one hundred times below the actual minimal inhibitory concentration (MIC) of a susceptible population [14]. In addition, mutations also occur in DNA associated with MGEs. This seems important for the continuing evolution of antimicrobial resistance genes (ARGs), mirrored for instance by the existence of more than 100 variants of the Temoneira (TEM) family of beta-lactamases [15].

In addition to mutations affecting the core genome of bacteria, AMR can also be the result of horizontal gene transfer (HGT). Bacterial uptake of RNA or DNA, including antimicrobial resistance genes (ARGs) takes place by transduction mediated by bacteriophages, transformation (uptake of exogenous sequence information) and conjugation (uptake of plasmids). Since HGT among bacteria can occur between different genomic lineages, species, taxa and habitats [16], the evolutionary paths to AMR via the acquisition of ARGs is probably more complex than those involving mutation-based resistance alone [16]. Drivers of horizontal gene transfer (HGT) of ARGs are most commonly anthropogenic forces (figure 4), including the use of herbicides, pesticides, antibiotics and other drugs with antibiotic-like site effects [16-18]. However, it is important to note that in some clinically relevant species, e.g. *Mycobacterium tuberculosis* and *Helicobacter pylori*, mutation is the major force leading to AMR variants [13].

Accumulation of different mechanisms in AMR bacteria enables them to exhibit resistance to more than one class of antimicrobials.

1.1.2 The mobile resistome as an inexhaustible source of bacterial antimicrobial resistance

When Darwin wrote “On the origin of species”, he based his theory of evolution on natural selection [19]. While modern evolution biologists follow different ideas and models, “Darwin would have been astounded to know that some of the best evidence for natural selection resided in his own gastrointestinal tract” [19]. As we know today, microorganisms such as fungi, viruses and bacteria compete within suitable habitats and ecological niches with respect to growth conditions, nutrition supply and space. Therefore, production of molecules to secure an advantageous position compared to rivals within a certain area of settlement is a successful strategy to preserve favorable life circumstances for the progeny. Besides cytostatic agents and a broad range of toxins, microorganisms produce antibiotics to shield their population from intruding and competing populations [20].

Wherever antimicrobials are used, there are often already large reservoirs of AMR bacteria [2, 21]. The term “resistome” comprises all intrinsic and acquired ARGs associated with AMR, referring to resistance associated elements found in both pathogenic and antibiotic-producing bacteria, including cryptic resistance genes as well as precursor genes [22]. In earlier studies, the emergence, spread and accumulation of AMR among bacteria was largely attributed to the transfer of ARGs by HGT (reviewed in [23]). Consequently, revealing the transmission pathways of these genes between bacteria and the forces driving the gene flow was seen as important to understand, predict and control AMR in bacterial pathogens of medical relevance [24]. Based on the discrimination between intrinsic and acquired resistance, ARGs can belong to either the mobile or intrinsic resistome.

DNA isolated from permafrost samples near Dawson City, Canada, revealed that bacteria harbored ARGs conferring resistance to beta-lactams, tetracyclines and glycopeptide antibiotics such as vancomycin as early as 30,000 years ago [25], verifying antibiotic resistance as being a concept of natural evolution. Self-protection goes hand in hand with production of antibiotics, and AMR evolvement enabled particular bacteria to survive their own harmful products. During millions of years, some of these resistance mechanisms encoded on ARGs have gained transferability by hitchhiking with MGEs, enabling transfer within a species, between species and even beyond species barriers [26].

Historically, AMR in bacterial pathogens was relatively rare until the wide-scale usage of antibiotics in the second half of the last century [26]. A more or less indiscriminate use of antimicrobials in the “pioneer years” in health care, livestock, and agriculture provided an evolutionary advantage to resistant variants to dominate the ecosystem [27] (figure 4). MGEs

provide the potential for transmission of ARGs – even beyond bacterial species or family boundaries, thereby facilitating AMR consecutive accumulation. In addition, many ARG-containing bacteria belonging to the common microbiota of mammals can move relatively easily within and between humans, animals and the environment [2].

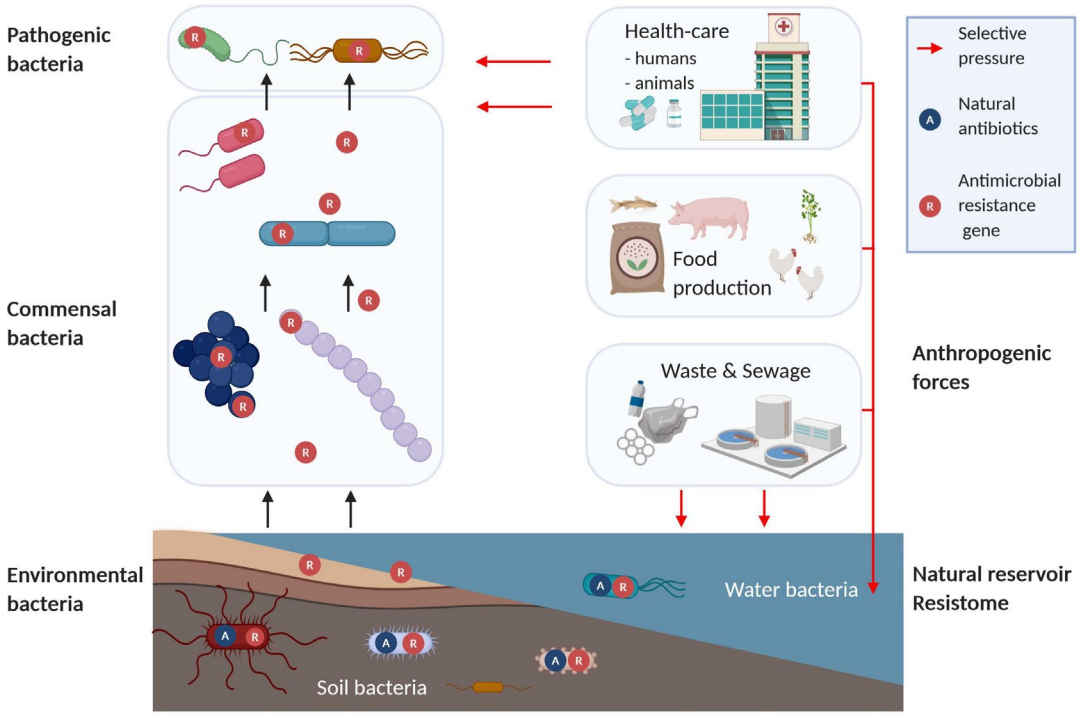

Fig. 4 Illustration of the mobile resistome: evolutionary and ecological relationship of antimicrobial resistance genes (ARGs)

Antibiotic resistance genes (ARGs) are frequently identified in clinical pathogens. However, all pathogenic, commensal as well as environmental bacteria and their mobile genetic elements constitute a reservoir for ARGs (the mobile resistome) from which pathogenic bacteria can acquire resistance via HGT [28]. Therefore, surveillance of AMR not only in pathogenic but also in commensal and environmental bacteria has gained attention (see also general discussion, 4.1.2 and figure 13). Illustration created with BioRender.com, license BW 22.05.2020.

As shown in figure 4, emergence and dissemination of ARGs belonging to the mobile resistome in one sector increases the chances of a "spill over" to a another sector, as has been shown recently for the putative reservoir of the plasmid-bound New Delhi metallo-beta-lactamase 1 (NDM-1) resistance gene conferring resistance to β-lactams including carbapenems in south-east Asian aqua culture [29].

In addition, research on the transfer network of mobile ARGs in bacteria revealed that a multitude of ARGs are commonly transferred between resident bacteria of the human and animal gut and human pathogens [24]. The majority of mobile ARG exchanges were detected in Proteobacteria, Firmicutes, Bacteroidetes, and Actinobacteria. Among these, Proteobacteria

showed the highest activity towards ARG exchange [24]. The top three species displaying the highest numbers of ARG transfer connections were *Escherichia coli*, *Bacteroides fragilis*, and *Staphylococcus aureus*, which each were found to share ARGs with more than 260 other bacterial species. At the species level, *E. coli* and *K. pneumoniae* were found to share the largest number of mobile ARGs (60 individual genes), followed by *E. coli* and *S. enterica* (38 genes), *A. baumannii* and *K. pneumoniae* (38 genes), and *Klebsiella oxytoca* and *K. pneumoniae* (36 genes) [24].

While AMR among bacterial species that reside in complex ecosystems is a natural phenomenon [27] accumulation of mobilized ARGs in bacteria shows an undisputable strongly anthropogenic influenced by artificial substances inducing a selective pressure on microorganisms [20, 22] (figure 4).

1.1.3 Classification of AMR veterinary pathogens

Until now, various definitions have been proposed to classify bacteria of clinical relevance in health care settings exhibiting varying degrees of antimicrobial resistance as multidrug resistant (MDR), extensively drug resistant (XDR) and pandrug resistant (PDR). An expert proposal published in 2012 provided a guideline to classify typical bacteria causing health care-associated infections in human medicine [30]. For each pathogen of clinical relevance, a distinct panel of antimicrobial agents representing different antimicrobial classes is used to define its individual degree of resistance. As a prerequisite, unambiguous species identification and a clear testing result from a standardized antimicrobial susceptibility testing (AST) method following e.g. guidelines of the Clinical & Laboratory Standards Institute (CLSI) or the European Committee on Antimicrobial Susceptibility Testing (EUCAST) is required. When an organism possesses intrinsic resistance to an antimicrobial agent or to the whole class (category) of substances, that agent or category must be removed from the classification list [30].

Considering veterinary pathogens, Schwarz et al. proposed to use the term acquired resistance to three or more antibacterial classes to define MDR in 2010 [31]. Only recently, Sweeney et al. provided explicit definitions for MDR, XDR and PDR among veterinary pathogens associated with bovine respiratory disease such as *Mannheimia haemolytica*, *Pasteurella multocida* and *Histophilus somni*. For porcine respiratory infections due to *Actinobacillus pleuropneumoniae*, *P. multocida* and *Streptococcus suis,* definitions have also been formulated. Moreover, *Staphylococcal* spp. causing skin – and soft tissue infections have been included in the list [32]. Since availability of species- and tissue-specific breakpoints for veterinary pathogens is still (2020) limited [33], additional differentiation criteria for

classification of acquired antimicrobial resistance in veterinary pathogens have yet to be published.

Classification of the “resistance level” for veterinary medicine isolates beyond staphylococci of canine origin [32] were not available for the pathogens reported on in this habilitation thesis. Of note, ESBL-producing *E. coli* as well as MRSA are considered zoonotic pathogens, capable of causing diseases in the animal and human host alike [34]. Here, the term MDR will be used for MRSA since these variants are considered as MDR by CLSI-definition. Moreover, extended-spectrum beta-lactamase producing *E. coli* reported on in this thesis frequently exhibit resistance towards (at least) ≥ 1 agent in ≥ 3 antimicrobial classes, which allows them to be classified as MDR as well [30].

1.2 Health care-associated infections in human and veterinary medicine

The definition for health care-associated infections in this thesis is that given by the WHO: “Health care-associated infections (HAIs) are infections that patients acquire while receiving treatment for medical or surgical conditions and are the most frequent adverse event during care delivery” [35]. Since HAIs initially referred to infections linked with a patients’ admission to a hospital only, the commonly used expression was “nosocomial infections”. However, the commonly used term HAI includes infections developed in various settings where patients obtain health care (e.g. long-term care, family medicine clinics, home care, and ambulatory care) [36].

According to the US Centers for Disease Control (CDC), the health and well-being of the community or state, referred to as public health, depends on detection, combat and prevention of infectious diseases. Therefore, their surveillance is essential [37]. Surveillance has been defined as “the ongoing systematic collection, analysis, and interpretation of health data essential to the planning, implementation, and evaluation of public health practice, closely integrated with the timely dissemination of these data to those who need to know [38]. Primary, there are two distinct strategies for surveillance of infectious diseases: disease-specific surveillance and syndromic surveillance [37].

Generally, the objectives of HAI surveillance include [39]

- assessment of infection incidence rates
- monitoring trends
- detection of outbreaks
- providing early warning and investigation of infection problems
- subsequent planning and intervention to control

- prioritising resource allocation
- examining the impact of interventions
- gaining information on the overall quality of patient care

The European Centre for Disease Prevention and Control (ECDC) runs an “Antimicrobial Resistance and Health care-associated Infections Programme” which is focused on surveillance, response and scientific advice, training and communication [40]. The ECDC estimates that approximately 4 million patients acquire a HAI each year in all EU Member States and that approximately 37,000 deaths directly result from these infections. On their corresponding website, the ECDC states that AMR pathogens and HAIs are among the most serious public health problems, globally and in Europe. The experts point out that the issues of AMR pathogens and HAI overlap widely, but are not synonymous. This can be highlighted by the following example: Data from the European Antimicrobial Resistance Surveillance Network (EARS-Net) 2015 was used to perform a population-level modelling analysis to estimate the burden of infections with AMR bacteria of public health importance. The authors of the study estimated that more than 63% of the infections with AMR bacteria were associated with health care [41]. Overall, the estimated burden of infections with AMR bacteria in the EU and European Economic Area is substantial compared with that of other infectious diseases, and has increased since 2007 [41].

Notably, bacteria harboring resistances towards antimicrobials can be found as part of the microbiota of healthy individuals, in pet animals and in the environment (https://ecdc.europa.eu/en/about-uswho-we-aredisease-programmes/antimicrobial-resistance-and-healthcare-associated-infections). As summarized in an overview provided in table 1, many of general mechanisms and aspects associated with HAI seem quite similar in human and veterinary medicine (patients acquiring infections while receiving treatment for medical or surgical conditions) [42].

Table 1 General aspects of HAI in health care settings providing services for humans, small animals and horses

		Facilities providing health care services for			
Area	**Aspect**	**Humans**	**Small animals**	**Horses**	**References (selection)**
AMR bacteria frequently reported as cause of HAI[1]	MRSA	yes	yes	yes	[43-47]
	MRSP	no	yes	no	[48-51]
	VRE	yes	no	no	[52, 53]
	ESBL-producing Enterobacteriaceae	yes	yes	yes	[54-61]
	Carbapenem-resistant Enterobacteriaceae	yes	no	no	[52, 62-66]
	Carbapenem-resistant *P. aeruginosa*	yes	no	no	[66-70]
	Acinetobacter baumannii	yes	(yes)	(yes)	[57, 68, 71-74]
hospitals	organized in wards	yes	n.a.	n.a.	[10, 75]
	smooth and easy to clean floors	yes	yes	no	[76-80]
	smooth and easy to clean surfaces	yes	yes	no	[76-80]
	quarantine rooms/wards	yes	s	s	[81, 82]
	stable-like patient environment	no	no	yes	[55, 57, 83-86]
	easy-to clean technical devices	yes	yes	no[2]	[80, 87-89]
	easy-to clean articles used for patient care	yes	(yes)	no[2]	[80, 87-89]
personnel	awareness of HAI	yes	n.c.	n.c.	[66, 73, 90-93]
	recurrent training and hygiene education	yes	n.c.	n.c.	[84, 93-96]
	infection control professionals	yes	no	no	[15, 43, 96, 97]
patient	consciousness	yes	no	no	
	vulnerable groups	yes	yes	yes	
	auto-mutilation	n.c.	yes	yes	
type of HAI infection*	surgical site infection	yes	yes	yes	[73, 98-103]
	urinary tract infection	yes	yes	n.a.	[96, 104-107]
	respiratory infection	yes	n.a.	n.a.	[68, 108]
	septicemia	yes	n.a.	(yes)	[109, 110]
reservoir and transmission	permanent and transient microbiota of the patient	yes	yes	yes	[27, 111-113]
	bacteria from other patients or member of staff	yes	yes	yes	[47, 73, 75, 114-117]
	bacteria from the health care environment	yes	yes	yes	[75, 83, 118-123]
surveillance of HAI	implementation at hospital level	yes	no	no	[108, 124-128]
	implementation at the network level	yes	no	no	[108, 124-128]
	overall goal: reduction of HAI and their costs	yes	n.a.	n.a.	[108, 124-128]
surveillance of AMR bacteria	implementation at hospital level	yes	no	no	[1, 108, 129]
	implementation at the network level	yes	no	no	[1, 108, 129]

Abbreviations: AMR, antimicrobial resistant; HAI, health care associated infection; MRSA, methicillin resistant *S. aureus*, ESBL-producing *E. coli*, extended spectrum beta-lactam producing *E. coli*; s, seldom; n.c., not certain

[1]All AMR bacteria mentioned have been identified from samples of companion animal origin, VRE intestinal carriage for instance was reported for dogs [130, 131]. Here, only those AMR bacteria which were frequently reported as being the cause of HAI are indicated. Nonetheless, viruses [132-137] are reported as causes of HAI in human and veterinary medicine, too, while spread of fungi [138, 139] and parasites [140] is mostly reported from human hospitals.

[2] Surfaces and equipment are adjusted to the size and nature of horses and need to be practicable in stables

Currently, numerous reports on HAI are available for companion animal medicine, including facilities providing health care services for small animals [79, 93, 141-145], as well as those for horses [46, 77, 120, 146-154]. In fact, surgical site infections (SSI), urinary tract infections (UTIs), blood stream infections, infectious diarrhea and, to a lesser extent, pneumoniae, seem the most frequently reported HAIs in veterinary settings providing companion animal health care [93, 155, 156]. However, to our knowledge there is a lack of systematic HAI ascertainments or structured surveillance activities in veterinary medicine. Therefore, the factual extent and significance of HAIs cannot be seriously estimated yet. So far, the only evidence of HAIs are studies reporting them [157], including those released from our group (reviewed in **paper1**).

1.3 AMR in bacteria associated with HAIs in human and veterinary medicine

1.3.1 Brief introduction and overview on AMR bacteria commonly associated with HAIs

A recent review indicated that the number of pathogens reported as cause of HAIs in human medicine is limited to 12–17 microorganisms only, which account for up to 87% of the reported cases [36]. This group includes Gram-positive bacteria such as *S. aureus*, Coagulase-negative Staphylococci, *Enterococcus* spp. and Gram-negative Enterobacteria such as *E. coli*, *Klebsiella* spp., *Enterobacter* spp., *Proteus* spp. as well as non-fermenters such as *P. aeruginosa* and *A. baumannii*. The authors estimated that 16-20% of these cases were associated with MDR variants including MRSA, vancomycin-resistant *E. faecium*, extended-spectrum beta-lactamase producing Enterobacteriaceae and carbapenem-resistant Enterobacteria and Gram-negative non-fermenters [36].

Reports on bacteria exhibiting any kind of AMR causing diseases in companion animals has clearly increased since the early 1990s [158, 159], and is expected to increase further [160]. However, accumulation of ARGs in certain bacterial species promoting multidrug or even pan-resistant variants is a much more recent phenomenon (**paper1**). While antimicrobial susceptibility of bacteria causing infectious diseases in companion animal medicine

decreased, frequently used antibiotics such as penicillin (also in combination with streptomycin), sulfonamides and tetracyclines, have been found to show increasing ineffectiveness [57, 161]. In veterinary medicine, microorganisms which have been reported as cause of HAIs include MRS such as MRSA and *Staphylococcus pseudintermedius* (MRSP) as well as (ESBL)-producing Enterobacteriaceae and, to a lesser extent, *Acinetobacter baumannii* [34, 93, 156, 162].

Resistance to beta-lactams is clinically important, since antibiotics belonging to this class are often used as a first-line of treatment for severe and life-threatening infections due to their broad spectrum of activity, convenient pharmacological properties and general low toxicity. Not surprisingly, resistance to beta-lactam-based antibiotics is a common key characteristic of pathogens frequently associated with HAI in human and veterinary medicine, including Gram-positive (MRSA, MRSP) and –negative bacteria (ESBL-producing Enterobacteriaceae, especially *E. coli*) (reviewed in **paper1**). Moreover, beta-lactam resistance is also common among pathogens belonging to EHSG lineages (**paper1**). The following section's intention is to provide a brief introduction in the nature of these opportunistic pathogens, especially those addressed in this habilitation thesis. While its essentials are provided in **paper1** as well, some further aspects mentioned here are intended to introduce some of the later specific topics.

1.3.2 Methicillin resistant staphylococci (MRS)

Staphylococci are common members of the commensal bacteria residing on the skin and mucous membranes of humans and animals [163]. Microbiologists discriminate between coagulase-negative (CNS) and coagulase-positive (CPS) staphylococci, since the latter are considered to be of greater medical importance. The CPS group encompass *e.g*. *S. aureus*, *S. pseudintermedius*, *S. schleiferi* subsp. *coagulans*, *S. hyicus* and some species of minor overall importance. The CPS are generally considered as being able to cause opportunistic infections in humans and animals [163]. The staphylocoagulase produced by CPS is a crucial virulence factor, since the enzyme enables the bacteria to coagulate blood plasma of mammals by inducing the conversion of fibrinogen to its activated form fibrin, fostering a mechanical shield for the bacteria to avoid phagocytosis while providing enhanced adhesion possibilities within the generated clot [163, 164].

Methicillin resistance in staphylococci is conferred by an additional penicillin binding protein (PBP2a), which substitutes the transpeptidase function of the native PBP2 during the crucial process of cell wall building in the presence of beta-lactam antibiotics [165, 168]. The gene encoding methicillin resistance is located on a mobile element called staphylococcal cassette chromosome *mec* (SCC*mec*) [166, 167]. Figure 5 shows the general mechanism of resistance harbored by all MRS.

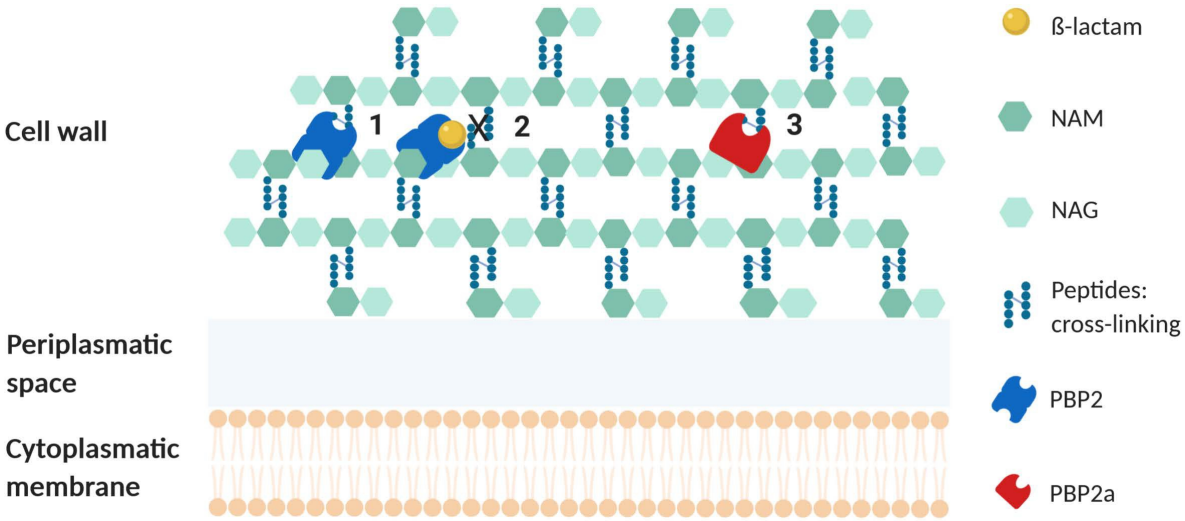

Fig. 5 Mechanism of methicillin resistance in staphylococci

Illustration of the cell wall building processes (biosynthesis of peptidoglycan) in staphylococci in the presence of β-lactam antibiotics. The native penicillin binding protein 2 (**PBP2**) is a bi-functional enzyme. The transglycosylase domain links N-acetyl-muramic acid and N-acetyl-D-glucosamine acid in an 1, 4 ß-glycosidic bond (1). The transpeptidase function of **PBP2**, which is needed to cross-link the neighboring layers of peptidoglycan in a structural solid manner, is inhibited in the presence of β-lactam antibiotics (2). The addition of protein **PBP2a**, encoded by *mec*A in MRS, substitutes the essential cross-linking step maintaining the stability of the peptiodoglycane layers during bacterial growth (3) [168]. Illustration created with BioRender.com, license BW 22.05.2020.

Methicillin resistant *Staphylococus aureus* (MRSA)

The therapeutic challenges due to methicillin resistant *S. aureus (*MRSA) in recent decades is considered a One Health challenge by both human and veterinary health care experts worldwide [34, 169]. Strains expressing resistance to methicillin emerged during the late 1970s, but effective measures to control their spread have been still “under construction” during the 1990s [170].

The particular significance of MRSA for cases of wound infections among dogs, cats and horses was clearly depicted by a broad survey (2010-2012), which is not part of this habilitation thesis [171]. However, results of that study provided representative data about the occurrence and genotypic variation of MRSA from wound swabs of companion animal origin in Germany. In total, 5,229 samples from 1,170 veterinary practices have been evaluated [171]. Swabs from wound infections of dogs revealed an overall identification rate of 5.8% for *S. aureus*, while swabs from cats showed a rate of 12.2% and those from horses 22.8%. High MRSA rates were identified with 62.7%, 46.4% and 41.3% for these *S. aureus* of canine, feline and equine origin, respectively [171]. Further genotyping revealed a comparable distribution of canine and feline MRSA genotypes with CC22 (47.6%; 49.2%) and CC5 (30.2%; 29.2%) as predominant lineages followed by CC398 (13.5%; 7.7%) and CC8 (4.0%; 9.2%). In contrast,

the majority of equine MRSA belonged to CC398 (87.7%) [171]. The latter result indicated that MRSA of equine origin seem to have a different phylogenetic background than those from obtained from diagnostic specimens from dogs and cats, which mirror mostly predominating epidemic human lineages in Germany [171].

Methicillin resistant *Staphylococcus pseudintermedius* (MRSP)

In comparison to MRSA, MRSP are a much younger phenomenon in veterinary medicine [156, 173-175]. When I started as a doctoral student at the Institute of Microbiology and Epizootics at the Freie Universität Berlin in 2002, all CPS isolated from clinical specimens subjected to microbiological diagnostics were screened for the presence of *mec*A using a novel in-house Triplex-PCR validated during my doctoral thesis. As a consequence, the first *mec*A-positive *S. pseudintermedius* case attracted our attention in 2005. Since then, cases of infectious diseases associated with MRSP have been reported with increasing frequency [156, 174, 176], and are now recognized as a serious animal health problem [177-180], in particular with regard to HAI [115], challenging hygiene prevention measures and therapy concepts in veterinary clinical facilities. Like other typical pathogens causing HAI, MRSP are frequently associated with multidrug resistance [51], and MRSP-infected animals are often difficult or unable to be treated with antibiotics [156, 173, 178, 180]. *S. pseudintermedius* can be transmitted to humans, especially to those having close contact with colonized/infected dogs, but which is a rare event [115, 181]. However, once invading the human body, MRSP appears to be as harmful to humans as other CPS [181-186].

1.3.3 ESBL-producing *E. coli*

Escherichia coli is a bacterial species of human and veterinary relevance, including apathogenic commensals, but also opportunistic and obligate pathogens causing severe to life-threatening systemic infections [187]. The gastrointestinal tract is the most common reservoir for MDR Gram-negative bacteria in all mammals [188], and enteral carriage seems to pose a risk for subsequent extra-intestinal infection in hospitalized (human) patients [189].

E. coli and other Gram-negative bacteria produce different kinds of beta-lactamases, a group of enzymes that acts on the β-lactam ring of this class of antibiotics [190]. In the early 1980s, beta-lactamases with extended substrate spectra and altered substrate affinity emerged in human medicine, referred to as Extended-Spectrum beta-Lactamases (ESBL) [191]. Extended-spectrum beta-lactamases (ESBLs) are enzymes that efficiently hydrolyze oxyimino-cephalosporins and monobactams, yet are inhibited by beta-lactamase inhibitors such as clavulanic acid [190]. The Clinical and Laboratory Standards Institute (CLSI) recommends a

phenotypic confirmatory combined-disk test for ESBL production in Enterobacteriaceae. It consists of measuring the growth-inhibitory zones around both cefotaxime (CTX) and ceftazidime (CAZ) disks with or without clavulanic acid (CA) [192] (figure 6).

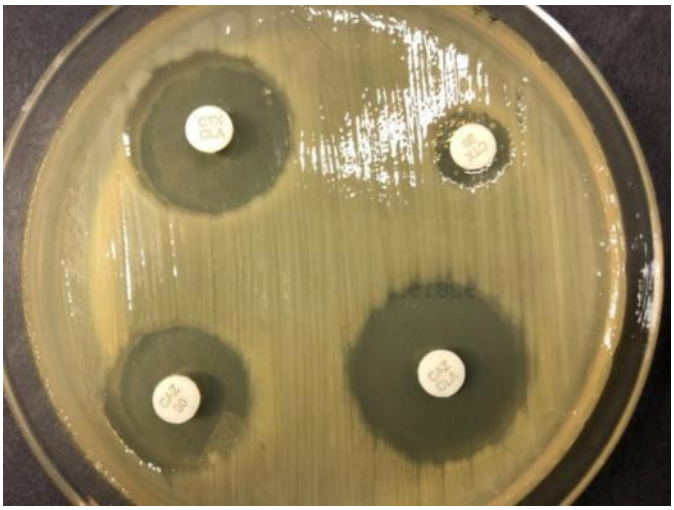

Fig. 6 ESBL confirmatory test according to CLSI guidelines

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The Clinical and Laboratory Standards Institute (CLSI) recommends a phenotypic confirmatory test using disc diffusion for verification of ESBL production in different Gram-negative bacterial species (i.e. *E. coli*, *Klebsiella* spp. and *Proteus* spp.). Considering *E. coli*, the growth-inhibitory zones around both, cefotaxime (CTX) and ceftazidime (CAZ) with or without clavulanate (CA) are measured [192]. A difference of ≥5 mm in the growth-inhibitory zone diameter of either CTX-CA or CAZ-CA is considered a positive result for ESBL production [192].

The spread of ESBL-producing Enterobacteriaceae (ESBL-E) has dramatically increased worldwide, and this “evolving crisis” is currently regarded as one of the most important public health threats [193]. First reports on ESBL-producing *E. coli* isolated from dogs in Spain and Portugal were published as early as 2000 and 2002 [194, 195]. Currently, dogs, cats and horses worldwide seem to be at risk for infections with ESBL-producing *E. coli* (**paper1**), including HAI [143, 196-198]. In addition, the general transferability of plasmids harboring ESBL-encoding genes between Enterobacteriaceae, is of rising concern [199].

ESBL genes were commonly found on mobile genetic elements, namely plasmids, were they are frequently accompanied by genes conferring further antimicrobial biocide and heavy metal resistances [200]. Consequently, Enterobacteriaceae acquiring these plasmids are often MDR. Remarkably, more than 300 natural ESBL variants have been identified since the mid-1980s but in vitro studies suggest that ESBL evolution has certainly not come to an end [191]. Particular substitutions at crucial positions induce changes in both, substrate affinity and specificity, changing a broad spectrum into an extended spectrum [191]. Figure 7 illustrates the differences between a common beta-lactamase and an ESBL activity in Gram-negative bacteria.

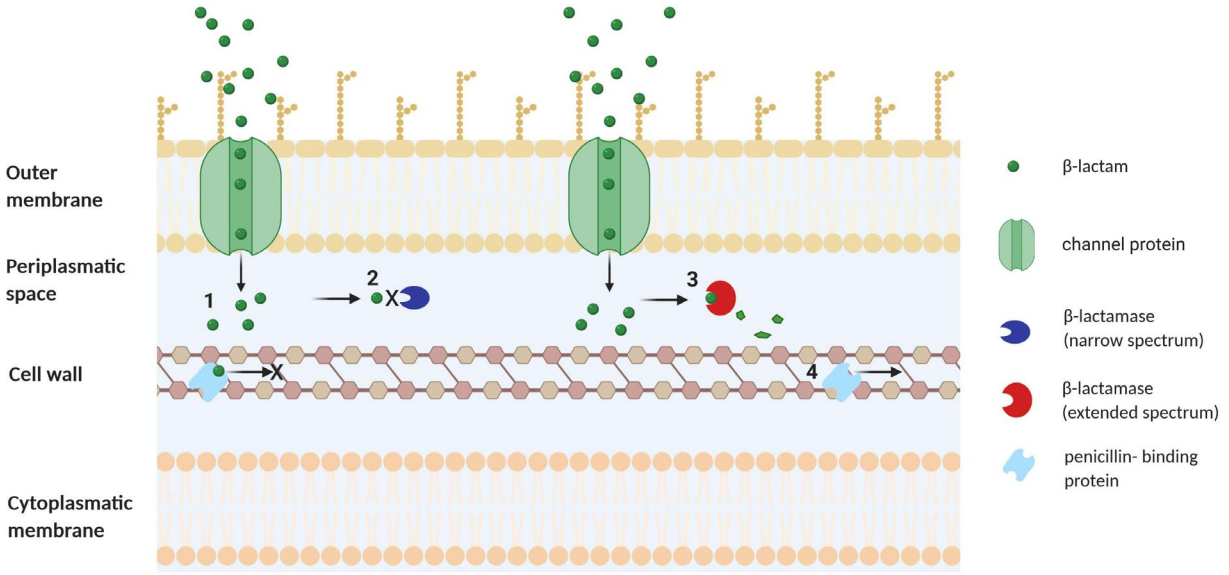


Fig. 7 Differences between narrow-spectrum ß-lactamases and ESBL's

Abbreviations: PBP, Penicillin-binding Protein; A, enzymatic active centrum of a ß-lactamase; ESBL, Extended spectrum ß-lactamase.
1. Diffusion of beta-lactams through the outer membrane and peptidoglycan layer of the cell wall into the periplasmatic space of Gram-negative bacteria.
2. Narrow-spectrum beta-lactamase fails to enzymatically hydrolyze the ß-lactam, allowing the antibiotic to bind its target, penicillin-binding proteins which are enzymes involved in peptidoglycan biosynthesis.
3. Extended-spectrum ß-lactamase with altered affinity of the active centrum and reduced steric hindrance for large molecules towards the active centrum hydrolyzes the antibiotic and takes its biocidal effect away.
4. Activity of penicillin-binding protein is not decreased by β-lactam antibiotic.
Illustration created with BioRender.com, license BW 22.05.2020.

At present, beta-lactamase enzymes such as TEM and SHV (sulphydryl variable) become less prevalent in ESBL-producing *E. coli*, while CTX-M is now the most predominant mechanism in both humans and animals [201].

1.4 Overview on common methods used in molecular epidemiology

As stated in the preliminary note, this sections' intention is to provide a brief introduction in the general idea of molecular epidemiology and the typing methods which have been employed for this habilitation thesis in at least one of the ten papers included. Moreover, it gives a short overview on the development of molecular typing tools during recent years.

Infectious diseases result from direct transmission of microbes, e.g. *via* person-to-person, animal-to-person or *vice-versa*, or indirect e.g. by contaminated food, water or fomites, or by intermediate vectors (either living beings or objects) [202]. Investigating pathogen distribution

and putative relatedness is indispensable for elucidation of the epidemiology of HAI and aiding in the design of rational pathogen control methods [203]. Historically, analysis of bacterial isolates putatively involved in HAI outbreaks has relied on a comparative investigation of particular phenotypic characteristics such as biotype, serotype, bacteriophage or bacteriocin type, and antimicrobial susceptibility profile [203]. Beyond the classical, conventional typing, molecular typing methods have been increasingly introduced in molecular epidemiology (see also: discussion 3.2).

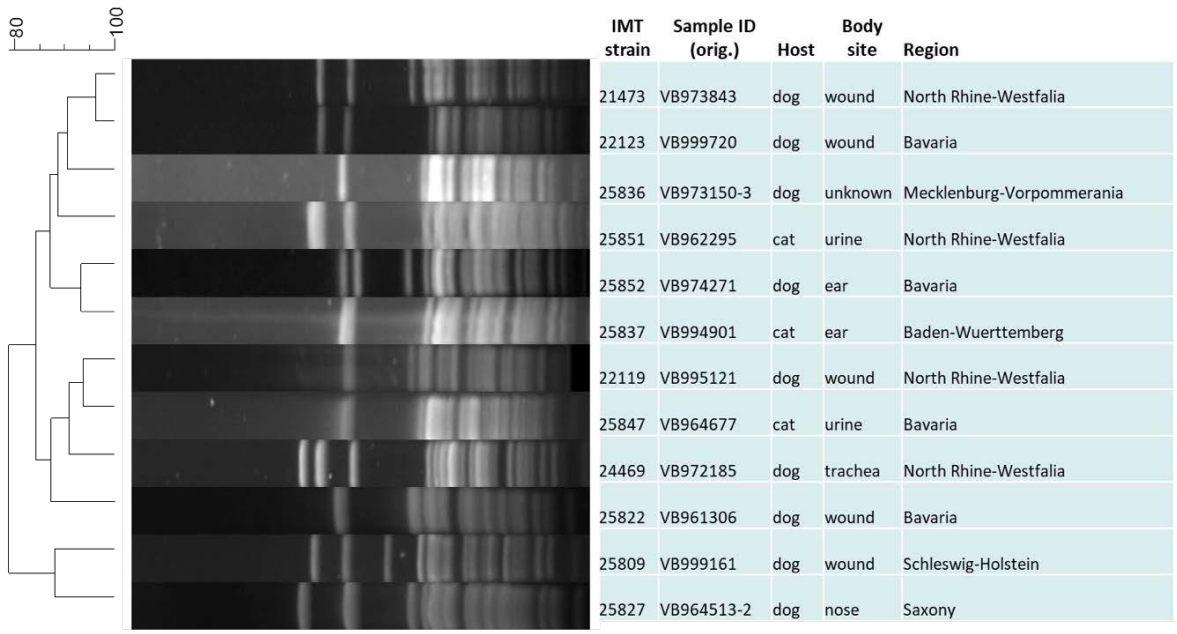
With regard to bacterial species able to cause disease in humans and animals, differentiation between “obligate pathogenic species” and “opportunistic species” is essential. Most of the bacterial species adapted to the hospital environment, regardless if circulating in human and/or veterinary medicine, are considered as opportunistic species, including *E. coli* and *Staphylococcus* spp.. This particular group of bacteria is characterized by being commonly part of the natural microbiota of mammals, in other words a typical resident among the bacteria inhabiting inner and outer mucosal surfaces or the skin. Certain virulence factors such as adherence factors, toxins or enhanced metabolic capabilities turn harmless residents into potential health threats. The species *E. coli* for instance includes harmless gut commensals as well as harmful pathogens able to cause sever (extra-) intestinal diseases, including urinary pathogenic *E. coli* (UPEC), neonatal meningitis *E. coli* (NMEC), enterotoxigenic *E. coli* (ETEC) and enterohemorrhagic *E. coli* (EHEC). Consequently, characterization of the bacterial species including their virulence as well as antimicrobial resistance characteristics is necessary to describe the patho- and resistance type.

Epidemiology is the medical discipline concerned with elucidating the causes of health outcomes and diseases in human and animal populations. In epidemiology, the population under investigation is dichotomized or stratified according to shared characteristics and the consecutive subgroups that are generated become units for comparative analyses [202]. Molecular epidemiology uses the tools of molecular microbiology to generate such subgroup data that can then be analyzed by observational and experimental techniques [202].

In the following section, the most common typing methods used for molecular epidemiological approaches **important for evaluating the results included in this thesis** are discussed. In the era of whole-genome sequencing (WGS), some of the methods (e.g. Pulsed-Field Gel Electrophoresis) appear somewhat outdated, but ten years earlier most of these methods have nonetheless been used to trace transmission of bacteria between individuals and/or within a given areas.

1.4.1 Pulsed-Field Gel Electrophoresis (PFGE)

For more than 30 years, Pulsed-Field Gel Electrophoresis (PFGE) was considered the ‘gold standard’ among molecular typing methods for most of the clinically important bacteria, especially for *E. coli* and *S. aureus* [204, 205]. Until the rise of next generation sequencing techniques providing easy-to-analyze WGS, PFGE was seen as one of the most discriminatory typing methods and in particular was indicated for surveillance and outbreak investigations. PFGE analysis is based on the extraction of DNA from the bacterial species in focus: the total DNA is then cleaved (digested) into fragments of different sizes by an endonuclease specifically chosen for that purpose. The DNA fragments are loaded into the upper chamber of an agarose gel which is then subjected to an electric field. The electric field changes applied to the DNA fragments separates them according to their length and isoelectric characteristics in the agarose. Each lane on the gel represents a bacterial isolate, and the PFGE pattern obtained can be regarded as a “molecular fingerprint”, often referred to as “pulso-type (PT)” (figure 8).



IMT strain	Sample ID (orig.)	Host	Body site	Region
21473	VB973843	dog	wound	North Rhine-Westfalia
22123	VB999720	dog	wound	Bavaria
25836	VB973150-3	dog	unknown	Mecklenburg-Vorpommerania
25851	VB962295	cat	urine	North Rhine-Westfalia
25852	VB974271	dog	ear	Bavaria
25837	VB994901	cat	ear	Baden-Wuerttemberg
22119	VB995121	dog	wound	North Rhine-Westfalia
25847	VB964677	cat	urine	Bavaria
24469	VB972185	dog	trachea	North Rhine-Westfalia
25822	VB961306	dog	wound	Bavaria
25809	VB999161	dog	wound	Schleswig-Holstein
25827	VB964513-2	dog	nose	Saxony

Fig. 8 Example: DNA restriction-pattern based dendrogram of canine MRSP.

A representative processed gel showing the different pulsotypes obtained for canine MRSP together with a dendrogram generated using the fingerprinting software provided by BioNumerics (AppliedMath, Belgium) using a 0.5 tolerance level.

Isolates: Ivonne Stamm, Vet Med Labor GmbH, IDEXX Laboratories, Kornwestheim.

To interpret DNA fragment patterns generated by PFGE and transform them into epidemiologically useful information for typing nosocomial pathogens, the clinical microbiologist must understand how to compare PFGE patterns and how random genetic events can alter them [203]. For comparative analysis of patterns from more than one gel, a

suitable software is needed, since the naked eye has its limits with respect to pattern analysis [203]. The example shown in figure 8 includes a comparative analysis of PFGE pattern using commercial software (Bionumerics, AppliedMath, Belgium). However, differences between individual PFGE protocols and interpretation rules led to difficulties with respect to comparison of results between studies and communication of PFGE data between laboratories. As a consequence, PFGE is generally not suitable for long term epidemiological surveillance and has been mostly outdated by WGS based approaches [56].

1.4.2 Multilocus Sequence Typing (MLST)

Originally, multilocus sequence typing (MLST) was developed to overcome the poor portability of traditional molecular typing approaches [206]. In 1998, the first MLST scheme was developed for typing of *Neisseria meningitides* isolates [207]. Since then, MLST has proven its value as a useful tool for molecular epidemiology, including studies on the molecular evolution of pathogens [205, 206, 208].

MLST is based on partial sequencing (usually: 450–500 bp) of commonly 5 to 11 housekeeping genes followed by a gene-by-gene comparison. For each of the housekeeping genes included in a typing scheme, allelic variants are assigned a certain number, and the consecutive number code (e.g. 15-3-5-7-12-23-16) for each of the allelic variants defines the sequence type (ST). Genes commonly selected for MLST were specifically chosen to provide a robust structure of a 'population framework': Isolates exhibiting similar or identical genotypes are considered as very closely related and presumptive descendants of a recent common ancestor [209].

Sequences that differ at even a single nucleotide are assigned as different alleles and no weighting is given to take into account the number of nucleotide differences between alleles, as we cannot distinguish whether differences at multiple nucleotide sites are a result of multiple point mutations or a single recombination event.

The main advantage of MLST is that all data produced by this approach are unambiguous and electronically portable. Furthermore, the allele sequences and ST profiles are available in online databases (*e.g.* www.mlst.net).

While whole genome sequencing techniques allow researchers to comparatively investigate isolates with respect to their phylogenetic relationship very easily, description of sequence types (ST) and sequence-type complexes (STC) or clonal complexes (CC) are still widely used as the initial information while classifying bacterial isolates, since many of these genetic lineages have been associated with specific epidemiological appearances, virulence-, and resistance factors - or both.

1.4.3 *Spa*-typing technique used for *S. aureus* lineage identification (including MRSA)

A polymorphic variable number of tandem repeats containing region in the gene encoding for the staphylococcal protein A (*spa*) has been widely used to characterize genomic lineages in *S. aureus* (figure 9*)* [210-212]. Since the method showed a high concordance with the MLST results [213], it has been used in molecular epidemiology [213], especially earlier when sequencing of seven genetic loci for MLST was not financially feasible for all research groups. However, since one MLST lineage can include isolates associated with different spa types, denomination of strains is frequently based on resistance type, sequence type, *spa* type and SCC*mec* type (e.g. MRSA ST398-t011-SCC*mec*IV).

Fig. 9 Schematic representation of the *spa* typing procedure

The gene encoding for protein A (*spa*) in staphylococci is characterized by a polymorphic repeat-containing region. This region starts with a particular sequence (here: “S”), usually summarized as “RCAMCAAAA” and ends with a corresponding sequence, summarized as “TAYATGTCGT” (here: ”E”). The repeats in-between commonly consist of 24 bp (range: 21 to 30 bp). The order of the repeats defines the *spa* type (https://www.spaserver.ridom.de/).

1.4.4 SCC*mec* typing

The gene encoding for the penicillin binding protein (PBP2a) which mediates methicillin resistance in staphylococci is part of the *mec* complex, consisting of a methicillin resistance encoding *mec* homologue (commonly allelic variants of *mec*A or mecC) usually accompanied by intact or truncated versions of the regulatory genes *mec*I (repressor) and mecR1 (sensor inducer) [214, 215] (**paper5**). The *mec* complex can be part of a larger, potential mobile element called staphylococcal cassette chromosome *mec* (SCC*mec*). These SCC*mec* elements also contain subtypes of site-specific large serine recombinases, either *ccr*AB or *ccr*C, of the resolvase/invertase family as well as further flanking (“J”) regions (figure 10) [214, 215] (**paper5**).

The characteristic composition of the *mec* complex together with the recombinase subtype defines the SCC*mec* type. An example for the general variability of SCC*mec* elements is given in figure 10. So far, 13 different SCC*mec* types have been acknowledged by the International Working Group on the Classification of Staphylococcal Cassette Chromosome [216].

Typing of the SCC*mec* element, which might be suitable for many epidemic strains, clearly has its limits. Here, the genomic region of interest is shown for two distinct MRSP isolates KM1381 [217] and S1 (unpublished) and an MRSA [218].

Since the SCC*mec* type is defined by the variation within the *mec* complex (here: *mec*A, *mec*I and *mec*R1) and the allelic variants of the recombinase genes (here *ccr*A, *ccr*B), mosaic structures are generally considered as non-typeable.

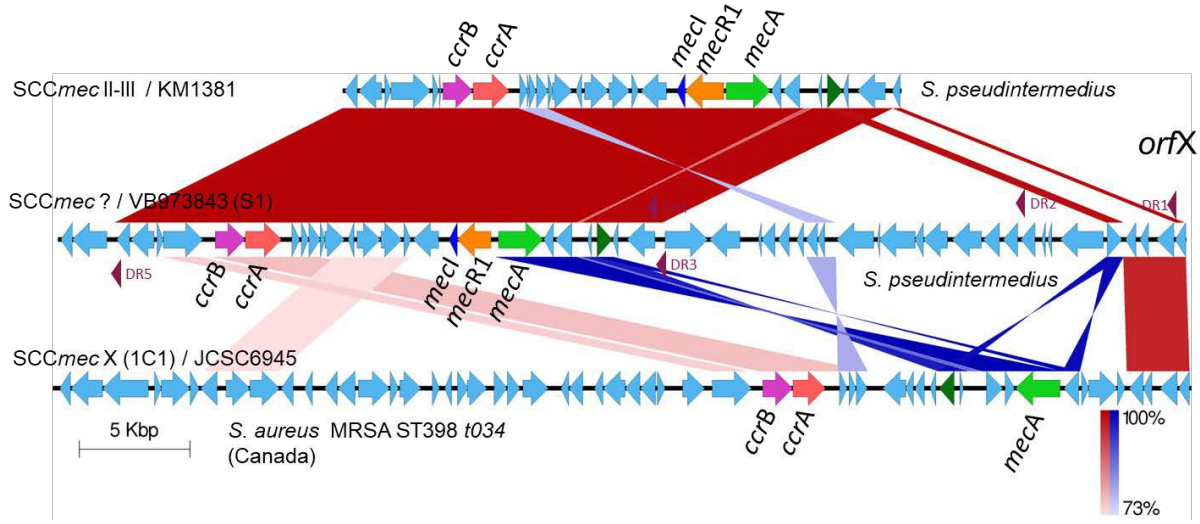


Fig. 10 Example: SCC*mec* elements harbored by MRSP and MRSA.
Isolate S1: Ivonne Stamm, Vet Med Labor GmbH, IDEXX Laboratories, Kornwestheim.

Since the region downstream of *orf*X (now: *rlm*H) is a hot spot for recombination events (**paper5**), composite SCC*mec* elements such as the element present in strain KM1381 (SCC*mec*II-III) are difficult to identify using PCR-based methods. WGS sequence analysis currently allows in-depth comparison of this region, revealing the “secrets” of formerly non-typeable SCC*mec* elements.

1.4.5 Whole genome sequence analyses (WGS)

Whole genome sequencing (WGS) allows comparison of the genetic differences between organisms down to a resolution of a single base pair [219]. In recent years, the increased accessibility of benchtop sequencers using next generation sequencing (NGS) technologies allows high-throughput of bacterial whole genome sequencing (WGS) [219]. WGS is a powerful tool for epidemiological investigations since it provides an increased resolution of the current situation under investigation due to its ability for in-depth genomic characterization of bacterial isolates [219]. Genomes of isolates of possibly epidemiologically related cases can be examined for their virulence potential and compared with one another to determine their relatedness at the genomic level [220]. Depending on the scientific question, molecular typing methods to discriminate bacterial strains based on their whole genome can either be based on allelic

differences within the genes forming the coregenome (cgMLST) or the pangenome (wgMLST) or they can be based on the SNPs occurring over the whole genome, including non-coding regions. Advantages of allele-based methods are that they are stable, reference free, and an internationally curated nomenclature scheme exists that can be accessed via databases and so allow global epidemiology and other analyses. The advantage of the detection of single SNPs over the whole genome is the theoretical higher resolution of the discrimination of very closely related bacterial strains.

A detailed comparison of WGS data representing a collection of isolates from different sources reveals single nucleotide changes which, together with detailed epidemiologic information, can be used to track transmission routes as well as the possible source of an outbreak [202].

Common applications of WGS in microbiology include isolate characterization, AMR profiling, and establishing the sources of recurrent infections and between-patient transmissions [118]. WGS-informed isolate characterization could be of particular significance for bacteria with large accessory genomes, which encompass many of the clinically most problematic bacteria, where much of the relevant genetic diversity is driven by differences in the variable accessory genome on the chromosome or mobile genetic elements [118]. However, a harmonized framework with guidelines for the validation of WGS workflows currently does not exist (2019), despite several recent case studies highlighting the urgent need thereof [221].

2 Research issue

2.1 Multidrug resistant bacteria challenging veterinary infection control

2.1.1 Introduction, comprehensive overview and historical aspects of MDR bacteria in companion animal medicine

*The objective of **paper1** was not only to offer an overview on hospital-associated infections (HAI) in veterinary medicine published so far, but to provide an insight including a short historical perspective, significant factors influencing HAI in veterinary medicine and a comprehensive overview on the most important commonly associated multidrug resistant (MDR) bacteria. Moreover, it provides possible explanations for the lack of data with respect to HAI in companion animal medicine and describes initiatives which might help to improve biosecurity for humans and animals in close contact with hospitalized dogs, cats and horses. Since **paper1** provides a nearly complete landscape of the research area covered by this thesis, it was selected as both a starting point and serves as a summary.*

While long regarded as epidemic to the veterinary hospital environment [102], HAI represent an important but mostly unresolved issue in companion animal medicine (**paper1**). Data about HAI associated with multidrug resistant (MDR) pathogens in companion animal medicine remains limited, but the problem has gained increasing awareness [93, 101, 222]. Here, the most frequently reported HAI are surgical site infections (SSI) and wound infections (**paper1**), but urinary tract infections and central line-associated bloodstream infections have been reported as well [34, 93, 95]. While working on **paper1**, questions arose concerning the earliest reports about HAI in veterinary medicine. We found a detailed report describing the spread of drug resistant staphylococci in a United States veterinary university clinic, including transmission of resistant *S. aureus* between students, veterinary staff and animal patients [223]. More than 40 years later, a similar cluster of skin infections in workers who had contact with a MRSA-infected neonatal foal was reported [116]. Such exemplary studies together with our own previous study results [144] indicated that veterinary personnel is constantly at risk of acquiring diseases from animal patients or at least becoming colonized with MDR bacteria, and further, that this phenomenon is not a recent development. As a result, open questions concerning work place safety and infection control in companion animal medicine have been addressed on a large scale in **paper1**.

A critical review of the current literature concerning HAI in veterinary medicine revealed that MRS such as MRSA and MRSP, ESBL-producing *E. coli* and MDR *Salmonella* serovars are frequently associated with outbreak scenarios worldwide (**paper1**).

Clinical outbreak events reported for MRSA and MRSP, ESBL-producing *E. coli* and MDR *Salmonella* Serovars indicate the necessity of infection control strategies for protecting animal patients at risk as well as veterinary personnel [90, 95, 96, 147, 224, 225]. Moreover, the often close bond between humans and their companion animals provides multiple opportunities for an exchange of microorganisms, including MDR pathogens (**paper1**, **paper6**) [222, 226, 227].

Since the late 1990s, reports on MRSA outbreaks in veterinary settings for horses, dogs and cats have been published. Further reports on MRSA in companion animal medicine have addressed the animal hospital environment, colonized animal patients, human-to-animal transmissions, the pathogen's molecular characteristics, and risk factors for colonization- and infection (reviewed and summarized in: **paper1**).

MRSP are the second most reported MDR staphylococcal species associated with outbreaks in veterinary clinics for dogs and cats [100, 115]. While occurrence and distribution of MRSP was intensively reported in the past [51, 179, 228, 229], data on epidemiological outbreak research is comparatively limited (**paper1**). Similar to reports on MRSA, hospitalization was identified as an important risk factor for MRSP infections in independent studies [50, 177], mirroring the importance of veterinary environments as a source for MRSP infections in animal patients (**paper1**). Considering antibiotics available for companion animals, antibiotic susceptibility testing (AST) profiles of MRSP isolated from severe clinical cases often reveal limited or even absent chemotherapeutical options [145, 230, 231].

With regard to Gram-negative bacteria, a worldwide emergence of infections with ESBL-*E. coli* in dogs, cats and horses including local spread is obvious [143, 196-198]. Here, the general transferability of plasmids harboring ESBL-encoding genes within the Enterobacteriaceae is of increasing concern, illustrated by a study showing transfer of a CTX-M1 harboring plasmid from a clinical *E. coli* isolate to *Salmonella in vitro* [199]. Summarized in **paper1**, MDR *Salmonella* serovars associated with HAI in companion animal medicine are a major threat to veterinary facilities worldwide, including veterinary staff and other contact persons [232]. Complete shut-downs of clinics, severe to life-threatening infections of hospitalized horses, cases of zoonotic transmission, financial losses and loss of reputation have been reported for such outbreaks [155]. In addition, MDR variants such as the high-fluoroquinolone (ciprofloxacin) resistant *Salmonella* serovar Kentucky ST198 spreading was reported for horse clinics as well [233]. Of note, reports on outbreaks of *Salmonella* within veterinary clinics have been rare in Europe (**paper1**). In Germany, horses are regarded as livestock, until designated otherwise by the animals' owner.

In general, monitoring of *Salmonella* in animals and foods in Germany is based on the Regulations (EC)No. 2160/2003 and 2073/2005. Implementation of these regulations, and the

realisation of the European directive 92/117/EWG into national laws, respectively, might have indirectly influenced the chance of companion animals, including horses, to become colonized or infected by *Salmonella*, in terms of a side effect.

On the other hand, reports on the occurrence or even outbreaks due to (MDR) *Salmonella* serovars involving companion animals, veterinary staff and animal owners (mostly outside from Europe) has clearly increased for horses [83, 234-239] as well as for dogs [240-245] and cats [104, 244, 246]. In the United States and Canada, a novel initiative aims to enhance the One Health idea by monitoring trends of antimicrobial resistance in animal pathogens using whole genome sequencing, including *Salmonella* serovars [247].

2.1.2 Introduction rates of MDR indicator pathogens in a horse clinic

*Pathogens frequently associated with multidrug resistant (MDR) phenotypes, including extended-spectrum beta-lactamase (*ESBL*)-producing Enterobacteriaceae (*ESBL-*E), Acinetobacter baumannii and MRSA have been identified as a threat for horse clinics in recent years [155]. As a premise for a targeted hygiene management update scheduled for the Pferdeklinik Freie Universität Berlin, definite values on current rates of MDR colonized horses entering the clinic were needed. As a consequence, the objectives of* ***paper2*** *and* ***paper10*** *were to determine reliable introduction rates for indicator pathogens frequently associated with HAI in horse clinics, including* ESBL*-producing Enterobacteriaceae (****paper2****) and MRSA (****paper10****), accompanied by insights into their respective genomic background and antibiotic resistance profiles.*

In order to ease understanding of the conclusions of the publications, results of ***paper10*** *concerning MRSA introduction rates and genomic background will be presented together with* ***paper2*** *in the following section, while mobile genetic elements influencing niche adaptation capabilities of equine MRSA are addressed in section 3.5.*

In brief, screening samples reported here were taken directly at hospital admission from two groups of horses admitted for health care services at the Pferdeklinik of the Freie Universität Berlin. Figure 11 provides a comprehensive graphical abstract of the screening procedure accomplished by a doctoral student of the Pferdeklinik (KSK) (**paper2**).

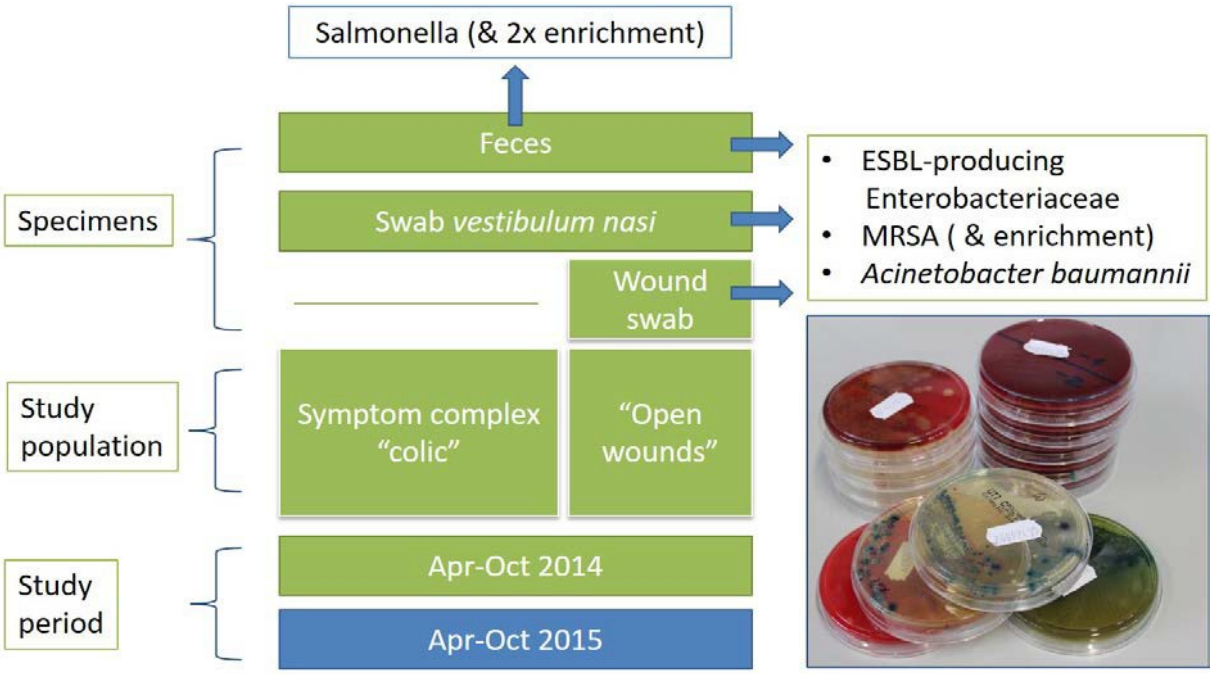


Fig. 11 Screening procedure for MDR carriage among horses at hospital admission

A total of 341 equine patients were screened for carriage of zoonotic indicator pathogens (here: MRSA, ESBL-producing Enterobacteriaceae, Salmonella and *A. baumannii*) at hospital admission. Horses showing clinical signs associated with colic (n = 233) or open wounds (n = 108) were selected for microbiological examination of nostril swabs, fecal samples and wound swabs taken. The latter was taken only if the horse was admitted to the "open wound" group (figure 11).

The screening results showed notable carriage rates for MDR pathogens in equine patients at hospital admission: Out of a total of 340 validated (= meeting the criteria for study inclusion) nostril swabs, 3.5% were found positive for MRSA, with a slightly higher rate (4.3%) among the "colic" group in comparison with the "open wound" group (1.9%), but these differences were not statistically significant (p = 0.253, chi-square-test) (**paper10**). The detection rates for ESBL-producing Enterobacteriaceae among the nostril swabs were 3.4% among the colic patients and 0.9% for the horses in the open wound group, with an average rate of 2.6% (9/340) considering both indications (**paper2**).

Four wound swabs taken from horses of the latter group (n = 108) were MRSA-positive (3.7%) (**paper2**).

Strikingly, 10.7% (34 of 318) of the validated fecal specimens were positive for ESBL-producing Enterobacteriaceae (94%: ESBL-producing *E. coli*), with recorded rates of 10.5% for the colic and 11% for the open wound group. *A. baumannii* was rarely detected (0.9%) (**paper2**) and the isolation rate for MRSA from fecal samples was 0.6% (**paper10**). All fecal samples investigated were negative for *Salmonella*, both directly and after two enrichment steps (**paper2**).

AST results for all 45 ESBL-*E. coli* revealed that 92.7% of the isolates were phenotypically resistant to three or more classes of antimicrobials (**paper2**). For all MRSA (n= 18), antimicrobial resistances other than beta-lactams encompassed resistance to aminoglycosides (gentamicin, kanamycin) and tetracycline, while 66.7% of the isolates showed additional resistance to fluoroquinolones (enrofloxacin, marbofloxacin) and 61% to the combination trimethoprim-sulfonamide (**paper10**).

For all 41 ESBL-producing *E. coli* isolated, a broad heterogeneity was revealed using pulsed-field gel electrophoresis (PFGE) pattern and whole genome sequencing (WGS) analysis. However, a predominance of sequence type complex (STC)10 and STC1250 was observed, including several novel STs. The most common genes associated with ESBL-production were identified as *bla*CTX-M-1 (31/41; 75.6%) and *bla*SHV-12 (24.4%) (**paper2**).

The results of this study revealed that even before hospitalization, a notable number of horses were colonized with MDR bacteria, including ESBL-producing *E. coli* (ESBL-*E. coli*), which challenged the local hygiene management system and work-place safety of veterinary staff in horse clinics. PFGE pattern analyses revealed a very limited clonal relatedness of the isolates (see also figure 2 in **paper2**).

MLST revealed 22 different STs associated with the equine ESBL-*E. coli*, including eight novel STs (ST7434 to ST7441, figure 2 and table 1 in **paper2**). Considering phylogenetic lineages, 13 isolates (31.7%) where associated with sequence type complex (STC) 1250 (ST1250, ST826, ST4164, ST7434, ST7437, ST7439), eight (19.5%) with STC10 (ST10, ST1683) and a further three with ST224. A phylogenetic tree generated based on the maximum common genome confirmed the overall broad range of phylogenetic backgrounds consistent with the PFGE analysis. While the isolates identified as ST1250 and ST10 or single locus variants of those STs formed the two dominating clusters in the genome comparison, the other genomes showed a rather diverse distribution with no similarity to the two clusters or between themselves (**paper2**).

Interestingly, these finding are in concordance with the data of Apostolakos *et al*. (2017) [114], who reported STC1250 and STC10 among the dominating backgrounds for the ESBL-*E. coli* from intestinal colonized horses in an equine clinic in the Netherlands (**paper2**). Consequently, further research is warranted to reveal factors fostering successful spread of certain ESBL-*E. coli* in or between horse clinics (**paper2**). Notably, the ESBL-Enterobacteriaceae detection rate of horses at hospital admission reported by our study seems surprisingly similar to those reported from the human medical sector (reviewed in [248]).

The 18 MRSA from that screening study were also submitted for whole-genome sequencing (WGS) using Illumina MiSeq 300 bp paired-end sequencing. In contrast to the results obtained

for the ESBL-*E. coli*, molecular typing (details in **paper10**) revealed that the MRSA isolates were most closely related and belonged to the so-called livestock-associated lineage predominating in Europe (ST398, *spa* type t011). All isolates harbored a complete SCC*mec*IV element and a staphylococcal pathogenicity island originally described for *S. aureus* of bovine origin (SaPIbov5). However, separation of two distinct clusters within the genotype lineage was possible based on the core genome phylogeny (figure 1 in **paper10**).

Detection rates for MRSA in horses at hospital admission have shown large variations in former studies commonly ranging from 2.9 to 10.9% [249, 250]. While the colic group yielded more MRSA-positive isolates than the open wound group (4.3 vs. 1.5%), the average detection rate was 3.5%. Some horses have recurrent episodes of colic, so prior veterinary care or even hospital stays might have influenced the actual detection rate reported in **paper10**. However, since the studies´ main goal was to provide first and reliable data on indicator pathogen introduction rates, deeper epidemiological questions and retrospective investigation of risk factors associated with colonized horses was beyond the scope of the study. In Germany, the reported rates for MRSA colonization of human patients at hospital admission are commonly around 1.6% and 2.2% [251, 252].

2.2 Diagnostic and characterization of MRS of animal origin

Antimicrobials belonging to the group based on the beta-lactam ring commonly provide a broad antibiotic spectrum, are easy to administer and considered safe for the patient, a clearly outstanding combination of beneficial attributes for antibiotics in general. Consequently, bacterial resistance to beta-lactams is one of the most important issues in both, Gram-positive and -negative bacteria, which are both addressed in terms of MRS and ESBL-*producing Enterobacteriaceae within this thesis.*

Rapid techniques for pathogen identification are needed to determine whether the staphylococcal species isolated from the patient should be judged as a harmless/commensal or if the bacterium possesses the ability to cause a severe disease. In addition, reliable species identification is also indispensable with respect to a valid interpretation of AST results. Until 2010, differentiation of Staphylococci belonging to the S. intermedius group (SIG) using matrix-assisted laser desorption ionization - time of flight mass spectrometry (MALDI-TOF MS) *was challenging due to a lack of valid reference spectra in the databases available. Thus, the objective of* ***paper3*** *was to establish validated spectra associated with the different members of SIG to improve fast and reliable diagnostics using MALDI-TOF, including methicillin resistant variants.*

MRSA from bovine and human origin were the first isolates described harboring the novel "mecA" variant [253, 254] in 2011. Results published in ***paper4*** *indicated the occurrence of*

these variants (now: mecC) are not limited to samples obtained from either humans or ruminants, but from companion animals as well. However, questions concerning the origin of this novel variant arose. Consequently, the objective of ***paper5*** *was to investigate the genetic surroundings of mecC and the corresponding genomic integration site in different staphylococcal species, shedding light on the genetic composition of the transferable elements associated with it.*

In 2005, gene-based approaches revealed that CPS phenotypically identified as *S. intermedius* belong to three closely related but nonetheless distinct species, namely *S. intermedius*, *S. delphini* and *S. pseudintermedius*, referred to as the *Staphylococcus intermedius*-group (SIG) [255-257]. Results from that particular research suggested that most canine isolates previously identified as *S. intermedius* should have been classified as *S. pseudintermedius* [258]. Classical biochemical differentiation between the distinct SIG members is complex and has often led to unreliable or insufficient results with regard to species identification [258, 259]. The first reliable method to discriminate *S. pseudintermedius* from other SIG and *S. aureus* by demonstrating a specific *Mbo*I restriction site within the housekeeping gene *pta* published in 2009 [258]. However, that particular approach was highly time-consuming and not suitable for "high throughput" laboratories (**paper3**). Furthermore, a sudden rise in reports on methicillin resistant *S. pseudintermedius* (MRSP), which were reported as being frequently associated with a multidrug- or even a pan-resistant phenotype, has enhanced the need for a reliable and fast SIG distinction [34, 51, 173, 178]. Since unambiguous species diagnosis is also a key factor for a reliable interpretation of AST results, a rapid identification method was urgently needed. Therefore, matrix-assisted laser desorption ionization - time of flight mass spectrometry (MALDI-TOF MS)-based SIG-identification with Bruker Microflex LT in combination with Biotyper 3.0 software (Bruker Daltonics, Bremen, Germany) was evaluated using (i), the original database content and (ii), the database after extension with distinct hierarchical clustered reference spectra representing 51 distinct SIG (**paper3**). We have selected 200 isolates to compare both database performances.

As a result, 17 isolates initially diagnosed as *S. intermedius* with the current content of the Bruker database were identified as *S. pseudintermedius* by applying the in-house reference spectra extended version (**paper3**). Furthermore, a significant improvement (average rise of log score value: 0.24) of the SIG identification score values was achieved, underscoring that further sequence-based refinement of the Bruker database content allowed improvement of MALDI- TOF MS-based identification (**paper3**). The general Bruker database was updated with the respective reference spectra generated in this study, and which is still (2019) in use.

A further diagnostic challenge for veterinary microbiological diagnostics arose with reports about MRSA carrying a novel *mec*A homologue (*mec*A_{LGA251}) of a predicted amino acid identity of only 62% with other *mec*A allotypes which possibly remain undetected by conventional PCR approaches in 2010 [260, 261]. Since previous reports described MRSA harboring *mec*A_{LGA251} only from human or ruminant hosts, we decided to conduct a screening study on isolates displaying an MRSA phenotype of companion animal origin which failed to give a positive PCR result for *mec*A (**paper4**). In this study, MRSA from companion animal origin routinely isolated from diagnostic specimens submitted for diagnostic purposes by veterinarians to either Vet Med Labor GmbH (now: IDEXX Laboratories Ludwigsburg, Germany) or the Institute of Microbiology and Epizootics (IMT; Freie Universität Berlin, Germany) have been investigated. Ten MRSA of companion animal origin failed to produce a positive signal for *mec*A during the routinely employed PCR [165] for confirmation of *mec*A-mediated methicillin resistance. These isolates were screened for the *mec*A homologue now known as *mec*C by PCR [261], and the amplicons obtained were sent for sequencing to LGC Genomics GmbH (Berlin, Germany). The occurrence of the *mec*A-homologue (*mec*C) was verified for two isolates from dogs, 7 from cats, and one from a guinea pig, and all PCR amplicons demonstrated 100% identity with the DNA sequence of *mec*A_{LGA251} (NCBI FR821779.1) (**paper4**). The strains represent a broad geographic origin (five different federal states of Germany) and occurred in different infected body sites. All strains were identified as being MRSA by the VITEK®2 system (based on growth in the presence of 6 µg/ml cefoxitin according to the manufacturer's VITEK®2 Advanced Expert System), although they showed rather low or moderately high oxacillin MICs between 0.5 and ≤4 µg/ml (more detailed information table 1 in **paper4**).

Our findings of MRSA harboring *mec*C in different companion animal species verified that the presence of the *mec*A-homologue in MRSA is not exclusively associated with isolates from human or ruminant origin in Germany and supports the hypothesis that some, if not all MRSA harboring *mec*C, are able to cause infections among a broad range of different hosts (**paper4**). This result was important for veterinary microbiological diagnostics, as MRSA harboring *mec*A_{LGA251} would putatively have been misdiagnosed as methicillin sensitive during the verification process by routine PCR targeting *mec*A only [261].

Since the *mec*C gene was previously seen as a "novel gene" mediating methicillin resistance in 2011, a comparative analysis of its adjacent genetic loci in non-clinical *Staphylococcus* isolates of animal origin was conducted in order to shed light on the phylogenetic relationship of this region which is present in different staphylococcal species (**paper5**). According to the current view, the *mec* complex formation is a result of the integration of a (putative

chromosomal) *mec* allotype in an intact beta-lactamase encoding operon consisting of *bla*Z-(*mec*A)-*bla*R1-*bla*I followed by the loss of the native beta-lactamase-encoding *bla*Z over time [254, 262] (**paper5**). Thus, the *mec* complex harboring *mec*C with its "ancestral" *bla*Z still being present is an interesting phenomenon from an evolutionary perspective: This structure is either more conserved than comparable progenitor forms of other *mec* complexes harboring *mec*A allotypes and/or benefits from the potential antimicrobial activity of *bla*Z (**paper5**).

We have analyzed the entire genomic region downstream of the chromosomal integration site (*att*) for the Staphyloccocal chromosomal cassettes (SCC) carrying *mec*C in different coagulase-negative staphylococcal species, which is a known hot spot for integration of mobile genetic elements, especially SCC (**paper5**). Initially, the nucleotide sequence between the rRNA-methyltransferase (*orfX*)-like gene and the tRNA dihydrouridine synthase B (*orfY*)-like gene in a *mec*C-positive *S. stepanovic* (IMT28705, GenBank accession no. KR732654) and the *mec*C-negative reference strain (CCM7717, GenBank accession no. KR732653) was investigated: The comparison revealed that *S. stepanovicii* IMT28705 harbors a *mec*C gene which shared 99.2% nucleotide (and 98.5% amino acid) sequence identity with the *mec*C prototype in MRSA_LGA251. A schematic representation of a typical SCCmec XI element is shown in figure 12.

The regulator genes originally directing expression of *bla*Z, namely *bla*I (repressor) and *bla*R1 (inducer), have evolved to *mec*R1 and *mec*I of *mec*A in MRS [263, 264]. Notably, there is a "cross-talk" between the regulatory genes of the *mec* complex and other beta-lactamase operons which might be present in a particular strain, leading to different levels of resistance expression while confronted to beta-lactams [265].

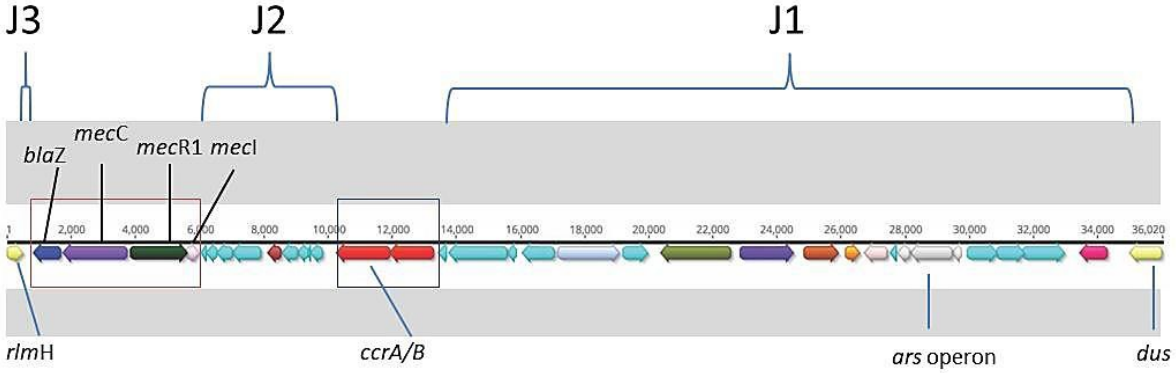


Fig. 12 Schematic representation of an SCC*mec* XI element harboring *mec*C

Genomic arrangement of the "hot spot" of recombination (**paper5**) downstream of *rlm*H ((pseudouridine-N3-)-methyltransferase; RlmH) until dihydrouridine synthase (*dus*). Red square, *mec* complex consisting of genes encoding the beta-lactamase BlaZ, methicillin resistance (*mec*C), and expression modulator genes (*mec*R1 and *mec*I). Blue square, recombinase genes (*ccr*A,*ccr*B). J1-J3, "junkyard regions", including further resistance genes (arsen resistance operon, grey) and insertion sequences (red arrow). SCC*mec* type is defined by *mec* complex and recombinase genes [266].

At the chromosomal insertion site (*rlm*H) of SCC*mec*, five terminal amino acids were encoded by 15 bp, followed by a stop codon sequence (TGA). Insertion of SCC*mec* alters this site to *att*R1 or direct repeat (DR)1 (more details in: **paper5**). DRs like these are analogues to those reported for other SCC*mec* elements in MRSA [214, 267], indicating a broad and general exchangeability of genomic regions flanked by these widely distributed DR sequences downstream of *rlm*H, at least among staphylococci (**paper5**).

2.3 Molecular- and infection epidemiology of MDR bacteria in companion animal medicine and beyond

Settled at the interface of molecular biology and epidemiology, the tools and methods used in molecular epidemiology offer unique opportunities to study a collection of bacterial isolates comparatively at the molecular level. Together with metadata on spatial and temporal circumstances and events, the relatedness and most likely spreading route or even the original source or reservoir for the spread might be identified.

The following subchapters address questions concerning the spread of *E. coli*, including ESBL-producing variants, in a horse clinic (**paper6**) in order to reveal their putative role as a nosocomial pathogen in horse medicine as early as 2012; the risk factors associated with MRSA infections among dogs, cats and horses (**paper7**); and the behavioral patterns of dog owners which might influence transmission of coagulase-positive staphylococcal species, including MRS, between them and their dogs (**paper8***).*

2.3.1 Hospital-associated transmission of *E. coli* in a horse clinic

In recent years, veterinary clinics were identified as "hot spots" with respect to HAI associated with MDR opportunistic pathogens, including E. coli. While there was sufficient data available on the occurrence, spread and impact of colonized personnel of MRSA in clinics providing health care for dogs, cats and horses from our group [15, 103, 144, 171, 268-270] as well as from other research groups [86, 271-275], ESBL*-producing Enterobacteriaceae was not regarded as endemic to the horse clinic environment in 2011/2012. Therefore, the aim of* ***paper6*** *was to investigate a putative spatio-temporal relationship of E. coli isolated from different sites of three horses suffering from surgical site and other HAIs by use of methods providing insights into molecular epidemiology and metadata analysis.*

The retrospective study presented in **paper6** included a follow-up on three horses (Horse A, B and C) with clinical signs of surgical site infections (SSI) after initial colic surgery. *E. coli* isolated from microbiological specimens received from these three horses within 22 days (= "sampling period") were further investigated with respect to their phylogenetic relatedness in order to reveal a putative spread between the equine patients.

Hospital admission for horse A was 12 days prior to the start of the sampling period (total hospital stay: six months), while horse B was hospitalized six days prior to the sampling time (hospital stay: one month). Horse C was admitted to the equine clinic at the third day of the sampling period (total stay: 11 days). More details are provided in table 1 of **paper6**. Initially, horse A was subjected to laparotomy and suffered from a subsequent paralytic ileus. The horse was then again subjected to surgery, developed a SSI together with a generalized disease state including septic joint infection (osteomyelitis).

Similarly, Horse B had colic surgery as well as re-laparotomy because of complications (SSI and anastomosis-site-leakage) followed by peritonitis. Horse C developed SSI after initial colic surgery and was also re-operated, whereby obstruction at the anastomosis site was diagnosed (**paper6**).

Since a hospital-associated spread of distinct *E. coli* lineages was assumed based on the initial AST results received for the *E. coli* isolates of equine origin during routine microbiological diagnostics, a room hygiene evaluation was carried out on day 14 including five rooms (surgery ward, surgery preparation room, pharmacy room, surgery associated storage room, knock down box) using wetted cotton swabs (**paper6**).

In total, 13 *E. coli*, 10 isolated from infected sites of the three horses and further three from the environmental screening, were subjected to PFGE typing, MLST and PCRs for ESBL-gene detection. As a result, isolates obtained from the three hospitalized horses and from two further environmental sources (**paper6;** figure 1) were assigned to two major pulsotypes (PT), referred to as PT A (including A-1, one band difference) and PT B as well as to two singletons (PT C and D) (**paper6**; figure 1 and table 2). Identical PTs were judged as *E. coli* belonging to the same clone.

The PT B was shared by six *E. coli* originating from different infected sites of each of the three horses and the fixation rope (surgery room). Due to the clear spatiotemporal relationship of these isolates, a hospital-associated spread was assumed (**paper6**, table 2). ESBL-producing *E. coli* associated with PT A were obtained from horses A (SSI and tracheal wash) and C (SSI) while the closely related PT A-1 was shown by isolates of horse A (septic arthritis /joint puncture) and horse B (SSI) (**paper6**, figure 1, tables 1 and 2.). Samples taken from horse B were positive for both, the ESBL-producing *E. coli* associated with PT A-1 and the ESBL-negative clone PT B at the SSI, while the peritoneal fluid was found positive for PT C (IMT26118), a further multidrug resistant ESBL-producing *E. coli* (table 2). One additional ESBL-producing *E. coli* was isolated solely from the floor of a knock down box (PT D; IMT26576) (**paper6**).

AST results obtained for the 13 isolates matched with their respective PT. Regardless of identification as ESBL-producing *E. coli* or not, all strains exhibited phenotypic resistance towards ampicillin, tetracycline, piperacilline, gentamicin, enrofloxacin, marbofloxacin, and trimethoprim-sulfamethoxazole combinations (**paper6**).

Multilocus sequence typing revealed that isolates assigned to PT A belonged to sequence type 10 (ST10), those associated with PT B to ST354 and the singletons belonged to ST224 (IMT26576) and ST1011 (IMT26118), respectively (**paper6**). Interestingly, isolates belonging to ST10 ST224 were identified among the ESBL-producing *E. coli* isolated from horses at hospital admission in 2018 (**paper2**).

2.3.2 Risk factors associated with MRSA wound infections in companion animals

*While risk factors for HAI in human patients have been investigated for decades [75], similar analyses for companion animals remain limited and meta-analysis are not yet available (reviewed in: **paper1**). Consequently, the aim of **paper7** was to investigate factors associated with a prime example for HAI, MRSA infections, in companion animal medicine.*

Based on our study results on the occurrence and dissemination of MRSA among companion animals [171, 276], questions arose concerning the risk factors which might be associated with these MRSA infections. Since MRSA-lineages isolated from infected companion animals often mirror typical human epidemic strains circulating in the same region [277], successful strategies to combat MRSA need strong and coordinated efforts from both the human and the veterinary fields according to the One Health approach (see also 1.1). The aim of **paper7** was therefore to investigate potential risk factors related to MRSA infections in dogs, cats and horses. Hence, a case-control study was conducted by our group in 2014, including data on 106 MRSA-infected animal patients as cases and 102 MSSA-infected animals as controls, originating from 155 different veterinary settings within Germany (**paper7**). Demographic data on animal patients, patient history and administration of antibiotics as well as practice / clinic specific parameters were assessed as putative risk factors. Multivariable logistic regression identified the following variables as risk factors for MRSA infection compared to MSSA infection: number of employees working at the veterinary setting ($n>10$: $p<0.001$), antibiotic treatment prior to sampling (systemic: $p=0.002$; local: $p=0.049$, both: $p=0.011$) and surgical site infection ($p<0.001$) (**paper7**). *Spa* typing was performed for all MRSA, revealing predominantly clonal complexes well-known for hospital-associated lineages spreading in human health care settings in Germany (CC5 and CC22) for isolates of dog and cat origin (**paper7**). The so-called livestock associated lineage in Europe, CC398-MRSA, dominated among equine isolates, a phylogenetic background that was previously described for equine clinical settings [275, 278-281]. The identified risk factors and genotyping results have been in accordance with numerous study

outcomes from the field of human medicine and indicated problems with hospital-associated spread of MRSA in veterinary medicine, especially within clinics providing extensive health care services for companion animals.

2.3.3 Behavioral aspects influencing transmission of Staphylococci between dog owners and their dogs

Transmission of opportunistic and antimicrobial resistant bacteria in animal clinics leads to a rise of HAI in animal patients, especially if a significant proportion of the actual personnel is colonized with the respective "outbreak strain" [86, 115, 154, 275]. *Within our research group we considered the importance of companion animals colonized with multidrug resistant (MDR) bacteria, especially Staphylococcus spp., outside of veterinary clinics, where they might contribute to the colonization of animal owners. Consequently, the aim of* ***paper8*** *was to investigate the occurrences of Staphylococcus spp. of clinical importance, including AMR variants, among dog owners and their dogs together with their interactive behavioral patterns.*

The majority of the opportunistic and resistant bacteria causing HAI in veterinary medicine are generally transmissible to humans (**paper1 & paper7),** [154, 282-288]. Humans in close contact with animals colonized or infected with resistant bacteria may also become colonized [286, 289, 290]. While colonization might be transient and needs to be strictly distinguished from a clinical infection, unfavorable circumstances such as invasive procedures, immune-incompetence or open wounds may put the colonized person or other vulnerable persons of a household at risk for infection [284]. Previous studies have shown that veterinarians are in general more often colonized with MDR bacteria such as MRSA [117, 280, 291, 292], and high colonization rates among veterinary staff have been mirrored in increased infection rates among hospitalized companion animals [144].

Since the relationship between dogs and their owners has changed, and dogs have moved from being working animals to family members in post-industrial countries, we hypothesized that zoonotic transmission of opportunistic pathogens like coagulase-positive staphylococci (CPS) is likely to occur between dogs and their owners (**paper8**). In order to investigate the different aspects of human-to-dog relationships promoting interspecies transfer of *Staphylococcus* sp., nasal swabs and questionnaires were offered to dog owners and their dogs at a dog show in 2009 (**paper8**). In that study, 18.5% of the human and 1.8% of the canine swabs were found positive for *S. aureus* belonging to a broad range of different genetic lineages. Cultures of nasal swabs obtained from fifteen dogs (13.9%) and six owners (5.6%) showed growth of *S. pseudintermedius*, including a *mec*A-positive isolate (MRSP) from a human participant (**paper8**). While *S. pseudintermedius* clearly possess the potential to cause severe

diseases not only in dogs but also in humans [182, 183, 185], positive nasal swabs have only been reported sporadically (**paper8**).

Further typing procedures including PFGE and MLST revealed that swabs from one dog/owner pair exhibited indistinguishable *S. pseudintermedius* isolates belonging to ST33, indicating a direct transmission. The survey which accompanied the nasal sampling procedure reported in **paper8** revealed that 88.9% of the dog owners allowed at least one dog within their home, while 68.5% allowed the dog(s) to rest on the sofa, 39.8% allowed their dogs to rest on the bed, 93.5% let them lick their hands and 52.8% let them lick their face. Bivariate analysis of putative risk factors revealed that dog owners who keep more than two dogs have a significantly higher chance of being colonized with *S. pseudintermedius* than those who keep 1-2 dogs ($p<0.05$) (**paper8**). We concluded that transmission of *Staphylococci* sp. between dog owners and their dogs is possible, an observation which has since been verified in multiple studies [115, 178, 292, 293].

A recent US cross-sectional study investigated 150 children with MRSA infections and their household contacts and pets between 2012 and 2015. Of 132 pets (dogs and cats), 14% were colonized with MRSA. Pets whose primary caretaker was MRSA-colonized were more likely to be MRSA-colonized than pets whose primary caretaker was not MRSA-colonized (50% vs. 4%, $p < 0.001$) [294]. The authors concluded that household environments and pet dogs and cats serve as reservoirs of MRSA [294].

2.4 Current aspects of niche adaptation: equine MRSA serve as a prime example

*The objective of **paper9** was to comparatively investigate the genomic background of equine and human MRSA to shed light on their commonalities and potential for interspecies, especially zoonotic, transmission. Years later, a comparative genomic analysis of equine MRSA revealed the presence of different mobile genetic elements with individual capabilities to foster adaptation to different host species, including humans (**paper10**). In addition, a circle will be closed concerning molecular epidemiology of MRSA from horses. While the initial publication (**paper9**) provided the first evidence for a close phylogenetic relationship of MRSA isolated from samples of human patients and horses in 2009, **paper10** (2018) provided evidence for the concurrent presence of mobile genetic elements harboring factors involved in fostering adaptation not only to the human host, but to the equine and bovine host as well (**paper10**). The latter results can therefore be regarded as molecular evidence for the existence of extended-host spectrum genotype (EHSG) lineages first described in **paper9**. In addition to determining MRSA colonization rates for horses admitted to a large university*

clinic, ***paper10*** *aimed to determine the occurrence of mobile genetic elements facilitating survival in the early stages of invasive infection in different host species, including humans and horses, in MRSA carried by equine patients admitted to a large horse clinic.*

Until 2007, MRSA infections of horses had been reported from Asia, North-America, Australia and Europe, and the rising importance of MRSA as a cause of HAI in horses became obvious [149, 295-297]. At that time, little was known about the phylogenetic background or putative genetic relationship of equine MRSA and epidemic MRSA circulating in human medicine. Therefore, the aim of **paper9** was to elucidate the genetic background and molecular characteristics of equine MRSA strains. In total, 19 equine MRSA collected between 2003 and 2007 in different federal states of Germany were rigorously investigated together with a representative collection of six human epidemic MRSA (EMRSA) provided by Prof. W. Witte (Robert Koch Institute, Wernigerode branch, Germany). Further information regarding geographical distribution, date of isolation and body site of specimen origin is given in figure 1 of **paper9**. Retrospectively, it is interesting that MRSA belonging to the clonal complex (CC) 8 were overwhelmingly predominant among that isolate collection as well as in horses worldwide [149, 211, 298, 299]. Later publications, including our own work [171], described a sudden shift of genotypes associated with equine MRSA from CC8 to CC398, the latter epidemic lineage known as typical European "livestock MRSA" [44, 199, 272, 275, 300]. In the isolate collection of horse origin thoroughly investigated in **paper9**, only one isolate was assigned to the MRSA-CC398, which is currently considered as an epidemic lineage [43].

The human EMRSA belonging to CC8 displayed indistinguishable molecular typing results compared with equine CC8 isolates using MLST, PFGE, and a commercial microarray hybridization array that included probes for 185 distinct genes and 300 alleles, including species-specific controls, accessory gene regulator (*agr*) alleles, genes encoding virulence factors and microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) (**paper7**). The microarray hybridization results of human and equine MRSA isolates were in accordance with results of MLST and PFGE analysis: CC8 strains of equine origin closely resembled those of human origin, especially the Hannover EMRSA strain [301] (**paper7:** see figures 2 and 3; supplemental data 1 and 2). In contrast to the majority of human Hannover EMRSA strains, all equine MRSA lacked the virulence genes for staphylokinase (*sak*) and enterotoxin A (*sea*), which are commonly associated with phages harboring the immune evasion cluster regarded as human-specific (see also: 4.5).

The extensive molecular pathogen profiling of MRSA from human and equine origin presented in **paper9** indicated that certain genetic lineages appeared to have been able to colonize and infect a broad range of host species, and were referred to as extended host spectrum genotypes (EHSG) in **paper9.**

Results of that work strengthened our working hypothesis on the existence of certain genotype lineages which adapt to a broader host spectrum more easily than others [34, 276]. Moreover, we hypothesized that EHSG-MRSA might harbor a broad range of virulence factors contributing to its success in different host species.

This hypothesis was at least partially verified by the results of **paper10**. All MRSA isolated during the screening procedure summarized in 3.2 were found to be closely related and belonged to sequence type (ST) 398_t011 with up to four additional antimicrobial resistances besides methicillin resistance.

Moreover, a β-hemolysin (*hlb*) converting ΦSa3 bacteriophage encoding the human-specific Immune Evasion Cluster (IEC) was present in 72% of the isolates, and 22% of the isolates harbored an equid-specific leukotoxin encoded by an additional temperate phage (Saeq1), which was recently described [302] (**paper10**).

In this study, three different homologs of the staphyloccoal complement inhibitor (SCIN) encoding gene (*scn*) were identified, one associated with the IEC (*scn*), one with the pathogenicity island SaPIbov5 (*scn*bov), and another with the equine phage Saeq1 (*scn*eq), on three MGEs of a completely different nature and composition. As a consequence, 17/18 MRSA-ST398 of equine origin harbored two different SCIN-encoding variants (**paper10**).

SCIN inactivates the C3 convertase complex, which is a crucial step for the activation of the alternative pathway of the complement system. In addition, inhibition of C3 activation is considered one of the most important immune evasion strategies of *S. aureus* [303] (**paper10)**. A recent study on the biological effects of the equine variant of SCIN encoded by *scn*eq revealed its ability to block activation of the equine complement system, hence interfering with phagocytosis [304].

Strikingly, eqSCIN was identified as a SCIN variant that functions in a much broader range of hosts, including horses, humans, and pigs [304] (**paper10**). In addition, our data might indicate a greater importance for contemporary carriage of allelic variants encoding Staphylococcal complement inhibitor (SCIN) for successful *S. aureus* niche- and host-adaptation than previously thought, since complement activation is pivotal for *S. aureus* killing [305] (**paper10**).

All MRSA ST398 were positive for SAPI*bov*5, meaning that these isolates harbor two allelic variants encoding the von Willebrand binding protein, a potent activator of blood prothrombin [306]. While one variant is encoded by the core genome near the gene encoding for staphylocoagulase (*coa*), the SaPIbov5-encoded *vwb* variant showed specific activity toward equine and ruminant plasma [307]. The presence of two, different vWbp-encoding genes might indicate a broadening in this genetic background of its flexibility with respect to host range (**paper10**).

However, the functional role of the chromosomally-encoded *vwb* reported here, as well as the interplay and regulation of both variants requires further investigation (**paper10**).

A study from 2016 investigating WGS data of LA-MRSA belonging to clonal complex 398 revealed some evidence of phylogeographic patterns for the majority of European isolates: These isolates clustered together and formed a unique lineage compared with the non-European isolates [308]. The largest European sub-lineage, denominated as EU t011, was further separated into two branches: One group harboring SCC*mec*IV and the other SCC*mec*V [308]. Interestingly, transferable resistance genes detected in our study (**paper10**) were similar to the profiles reported by Sharma *et al*. for sub-lineage EU t011 SCC*mec*IV, including *bla*Z, *mec*A, tetM, *aac*(A)-*aph*(D), *dfr*K, and *str*A [308]. In addition, this sub-lineage was associated with MRSA strains from a broad range of host species, including horses, cattle and pigs. In contrast to our study results (**paper10**), none of the ST-398 isolates investigated by Sharma *et al*. carried any human-associated virulence genes [308], a fact that clearly supports the idea of ongoing adaptive changes within this host generalist lineage (**paper10**).

In **paper10** we have described MRSA-ST398 of equine origin which were found to harbor different mobile genetic elements encoding variants of immune evasion factors and toxins previously shown to contribute to *S. aureus* invasive diseases in specific host species or ecologic niches. We suggested these combinations most likely contribute to the adaptation capabilities of MRSA belonging to ST398 with respect to epidemic spread across different habitats and hosts, and may therefore confer a host “generalist” phenotype, as initially proposed in **paper9**.

3 General discussion and outlook

3.1 Importance of AMR bacteria and HAI surveillance in companion animal medicine for veterinary medicine and beyond

4.1.1 General remarks on the research topic

When work on the topic covered by this habilitation thesis began in 2007, the research field “AMR bacteria and HAI in companion animal medicine” was still in its infancy, at least in Germany. As a result, the declared goal was to establish a “primary area under the curve” for veterinary medicine by means of facilitating both understanding and knowledge transfer concerning resistant and zoonotic AMR bacteria frequently causing HAI in facilities providing health care services to companion animals. Accordingly, scientific papers aimed at an international, scientific audience alone seemed insufficient to gain the attention needed to create substantial awareness among veterinarians working directly with companion animals. Consequently, together with my colleagues from different scientific backgrounds, a number of easy-to-access articles mostly written in German have been published in popular journals and magazines to inform, update and educate veterinarians and physicians at all stages of their carrier about the nature of HAIs, targeted hygiene improvements and zoonotic aspects in veterinary medicine [15, 21, 88, 89, 155, 270, 309-312].

The data presented and discussed in this habitation thesis showed that i) companion animals can be colonized and infected with AMR bacteria, ii) AMR and MDR bacteria occur and spread in facilities providing health care services for these animals, iii) most of the MDR bacteria reported by us and other researchers are easily transferable to humans, iv) many ARGs harboured by these bacteria are similar irrespectively of the host they originate from, v) AMR bacteria are able to broaden their host range (extended host spectrum genotypes) by acquisition of factors encoded on mobile genetic elements and, vi) representative data on either AMR bacteria or HAI are currently missing in veterinary medicine.

In 2013, P.S. Morley authored a review including the statement that veterinarians “have ignored, denied, and accepted the risks of health care-associated infections in veterinary settings, despite common occurrence and obvious importance” [222]. Since then, the continuing challenges caused by AMR bacteria and their spread in companion animal health care facilities have gained global attention (reviewed in **paper1**), but efforts to counteract this development in terms of implementation of suitable, continuing surveillance systems on a local, national or even European scale for AMR bacteria, HAI or even both remain scarce.

3.1.1 Does the emergence of HAIs and AMR bacteria in companion animal medicine affect human health affairs?

Due to their importance in food production and nutrition of the human population, animal health, animal welfare and animal disease control in food-producing animals such as pigs, ruminants or poultry in Germany and numerous other industrialized nations are monitored, regulated by numerous laws and tightly controlled by the government. Companion animal medicine, on the other hand, is foremost a completely private matter, as long as animal welfare, pharmaceutical regulations or other public interests such as danger prevention are not affected. Consequently, no primary public interest is devoted to issues concerning health care provided for dogs, cats, horses and other pets, and visiting a veterinarian remains a personal subject. Horses, which are primarily considered as livestock animals, have no significant role in this context since there is no industrial livestock sector for these animals in Germany. Nevertheless, there are some important aspects on the occurrence of AMR bacteria and HAIs in companion animal medicine that clearly affect public health interests, in particular the following areas:

Work place biosecurity

Prevention and protection of employees from biological hazards in companion animal medicine and associated working areas is a public health matter.

Since its first publication in 2017, the “Technische Regeln für den Umgang mit biologischen Arbeitsstoffen in der Veterinärmedizin (TRB 260)” provide an up-to-date, comprehensive and true-to-life guideline for suitable infection prevention – and control programs in veterinary practices and clinics [313]. Many of the insights gained by the work presented in this thesis have been directly used to develop the chapters of the TRBA recommendations on transferable and zoonotic bacteria, especially those tackling AMR and zoonotic pathogens. While the TRBA260 is intentionally focused on the biosecurity of the personnel working in veterinary medicine and associated fields, direct and indirect transmission of these pathogens between animals within veterinary settings was also considered as an important aspect to increase work place safety for the employees. Therefore, it includes extensive hygiene recommendations and concrete examples to prevent accumulation and spread of AMR bacteria locally and between human and animal individuals. This is a clear improvement, since many previous basic infection control concepts in small-animal medicine have simply been copied from the human sector [314].

1. Maintaining the effectiveness of antibiotics for human and veterinary medicine

As explained in detail in chapter 1, the active substance classes of commonly used antibiotics are the same in human and veterinary medicine. An editorial from DI Rendele and SW Page pointed out that “it is reasonable to assume that antimicrobial use in companion animals is dwarfed by use in the livestock sector; however, the antimicrobial agents used are often more closely related to those used in human medicine, and AMR risks should not be ignored as the close relationship between companion animals and human subjects presents an opportunity for the two-way transfer of bacteria (commensal and pathogen) or genetic determinants of resistance with associated potential for morbidity and mortality on both sides” [315].

In Germany, a global summary of data on the consumption of antimicrobials (assessed via sales reports) and the spread of antimicrobial resistance in human and veterinary medicine is provided by the GERMAP working group, which summarizes data from different national surveillance programs (https://www.p-e-g.org/files/content/Ueber%20uns/GERMAP/). However, detailed data on the consumption of antibiotics or AMR bacteria for different animal species or working areas (“companion animal medicine”) are not yet annually available.

While important research was accomplished with respect to a more detailed and informative assessment and evaluation of antibiotic consumption (including definition of suitable indicators) in different livestock animals (including horses) [316-319], there is no continuing and representative surveillance system established yet, especially for antimicrobials used to treat dogs and cats.

In addition, there is a lack of antibiotic consumption assessment in companion animal medicine, especially with respect to the local level. Despite some improvements towards implementation of guidelines for rationalized antibiotic use [320] and first attempts to implement antibiotic stewardship programmes [4, 11, 93, 321], the area still lacks concentrated and continuing efforts in companion animal medicine (**paper1**).

To maintain the effectiveness of well-tolerated antibiotics for human and animal patients in cases of bacterial infection, monitoring antimicrobial resistance rates in important indicator pathogens would disclose their real frequencies in companion animals. A representative MDR bacteria collection would allow investigation of their phylogenetic relationship to isolates from other areas such as human health care, farm animals and environment and challenge the often repeated theory of a simple and occasionally “spill over” from the human to at least the small animal sector.

2. **Putative reservoir function of veterinary clinics for easily transferable and zoonotic MDR bacteria**

It would appear likely that AMR bacteria such as MRSA, ESBL-producing Enterobacteriaceae and MDR *Salmonella* as well as their often highly mobile ARGs can move beyond companion animal medicine into the general community. According to the literature, AMR bacteria are often resident, not only in human, but also in veterinary hospitals [114, 122, 282, 322, 323]. Moreover, animals either colonized or infected with AMR bacteria after being discharged from hospitals, present at least possibilities for further transmission and spread in the community [324]. Indeed, case reports about long-term colonization with either MRSA [144, 325-327] or ESBL-*E. coli* [328] are available for dogs, cats and horses.

As stated above, global antimicrobial consumption data are available through the GERMAP reports (https://www.p-e-g.org/files/content/Ueber%20uns/GERMAP/). With regard to the European Union, a summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2017/2018 was published in 2020, revealing that *Salmonella* and *Campylobacter* from different host origins are becoming increasingly resistant to ciprofloxacin, one of the antibiotics of choice for treating infections caused by these bacteria [329]. Considering data about bacteria of companion animal origin, the report is limited to only reports on local spread of MDR bacteria, foremost from middle and northern European countries [329].

3.1.2 An integrated view: surveillance of antimicrobial consumption and AMR

Only recently, has the current need for implementation of a One Health oriented surveillance system to target health hazards that involves humans, animals and their environment [330, 331] been discussed by governmental organizations and stakeholders [332]. The collaboration across institutions and disciplines operating within the different sectors to plan, coordinate, and implement the surveillance process have been defined as the main characteristics of a One Health surveillance system [332, 333]. To fulfil that concept, a structural framework of a centralized and integrated system for surveillance of antibiotic usage together with AMR data from humans, food, livestock, wild and companion animals and the environment was proposed by Queenan *et al*. [332].

In brief, the integrated antimicrobial resistance surveillance included sampling of commensals from human patients and pathogens from clinical cases in hospitals and community care settings. For livestock animals, clinical cases subjected to microbiological diagnostics are available, and commensals from slaughterhouses. For wildlife and companion animals, bacteria from clinical cases are accessible (**paper1**), but a strategy for collecting commensals

from these animals, other than exceptional research reports, has not been defined yet [332]. The proposed structure is completed with the surveillance of AMR in zoonotic bacteria and commensals isolated from food products. A centralized program needs to be set-up by teams with experience across the sectors, and beginning with definitions of standards for data collection [332].

For antimicrobial consumption surveillance, collecting data in hospitals and community settings was proposed, and data on animals can be gathered on the species level, *e.g.* from livestock and companion animals. This centralized approach depends on communication improvement between disciplines and sectors, and social scientist and behaviour change experts might participate in the development of recommendations [332].

An integrated antimicrobial resistance surveillance of clinical and commensal bacteria, the environment, human and veterinary medicine and food together with antimicrobial consumption surveillance is exemplarily illustrated and summarized in figure 13, which was developed based on an idea presented by [332].

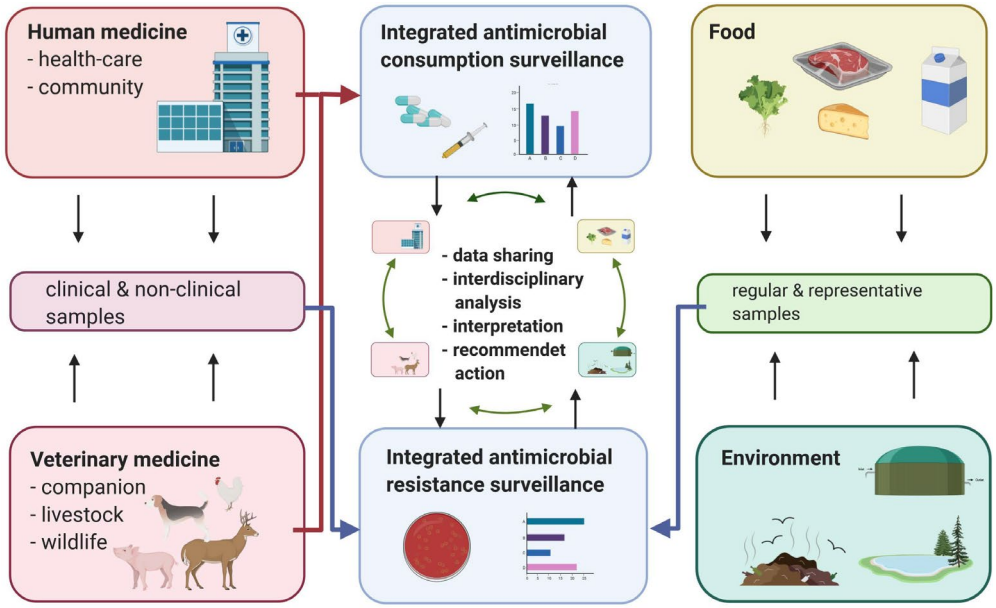


Fig. 13 Illustration presenting an interconnected and One Health surveillance framework

The framework presented is concentrated on integrated and interdisciplinary antimicrobial resistance and antimicrobial consumption surveillance (based on an idea from) [332].
Illustration created with BioRender.com, license BW 22.05.2020.

This “ideal” proposed framework would fulfil many tasks with respect to AMR- and antimicrobial consumption surveillance in human and veterinary sectors and the environment

including a straightforward attempt at “early warning” by including commensals in the sampling routine.

3.1.3 Surveillance of HAI in companion animal medicine

Currently, surveillance activities in health care settings for small animals (dogs, cats, other pets) and horse are scarce in Germany. Since representative data is not yet available, a primary set-up for surveillance activities towards HAI in clinics for small animals and horses might include baseline markers for clinics considered for participation (*e.g*. number of patients/year, number of elective interventions/year, number of employees), a clear definition of at least one frequently occurring HAI (*e.g*. surgical site infection), and the kind and extent of additional data needed (*e.g*. animal species, type of surgery, pathogen identification and AST data from microbiological diagnostics). According to our previous study on risk factors for MRSA infections in dogs, cats and horses, a surgical ward with at least 10 employees could be sufficient additional inclusion criteria [45]. In clinics for small animals and horses, a targeted, site-oriented surveillance of surgical site infections might be the most promising attempt to reveal local problems and provide baseline data for subsequent networks because:

1.) Most surgeons and animal owners see SSI commonly as a failure of veterinary health care, and series of SSIs have been recognized and often reported in informal ways (*e.g*. on social platforms) by animal owners, veterinary staff or both. This commonly results in rumours which influence the reputation of the clinic in question. According to the literature on HAI in human hospitals, there is a baseline rate of HAIs which cannot be prevented by proper hygiene management. However, due to a lack of representative studies or even surveillance efforts, data on the existence of similar rate in small animal or horse medicine is currently not available.
2.) Low SSI rates are beneficial for quality control reports and external audits, which is a major issue, especially in horse medicine.
3.) Samples of SSI cases were often subjected to microbiological diagnostics and results were therefore easily available for a systematic review.

It is reasonable to suggest that particularly university hospitals providing health care for small animals and horses could be the centre of an initiative for German-wide HAI surveillance in companion animal medicine, since these clinics do not belong to the private economy sector.

In 2016, a review on surveillance systems in human medicine described 262 systems implemented worldwide for monitoring infections. Primarily, there are two distinct strategies for surveillance of infectious diseases: the disease-specific surveillance and syndromic surveillance [37]. The disease-specific variety is surveillance of specific pathogens, diseases, or syndromes in a target population [37]. It is the most traditional form of surveillance, based

on sentinel structures reporting clinical cases or data reported from clinical laboratories. Surveillance of a broad range of pathogens is possible and data can be used to track both, local and supra-local developments. Moreover, it is a beneficial tool to assess the impact of changes and improvements with respect to preventive measures. Limitations are the unavoidable data standardization processes and the crisp target definition(s) needed before starting the surveillance at all [37].

Syndromic surveillance, on the other hand, relies on collection of non-specific health indicators which are usually collected and used for another primary reason. This system was evaluated for estimation of (baseline) rates for HAIs in small animal referral hospitals with critical care wards in Colorado, USA [101]. Syndromic surveillance is rapid and easy to implement - however it does not commonly provide laboratory data or even pathogens.

Surveillance of SSI using disease-specific surveillance might be the most promising attempt to start surveillance activities in companion animal medicine (**paper1**). SSI monitoring requires active, patient-based, prospective surveillance procedures [334]. While one might assume that SSI is an easy criterion to judge illness in dogs, cats and horses, a clear case definition is needed to avoid bias due to unreliable inclusion criteria. The human medicine sector provides strict definitions for SSI diagnosis and categorization. Differentiation between superficial incisional SSI, deep incisional SSI and organ/space SSI is defined and recommended by the CDC [334]. The “Krankenhaus-Infektions-Surveillance System” hosted by the national reference centrum for hygiene in Germany provides a similar clear fact sheet for SSI diagnostic and categorization [335].

The current trend towards implementation of automated systems in microbiological diagnostics and antimicrobial resistance testing, laboratory report transmission and animal patient data management in veterinary medicine, lowers the personal efforts needed for data collection, evaluation and transfer, including microbiological results.

3.1.4 Prevention of HAI in veterinary medicine

Spread of transmissible bacteria in healthcare settings depends on hygiene barriers locally implemented to prevent them, and consequently, incidence rates of HAI mirror the efficiency of local infection control efforts (**paper1**).

The overall goal of all infection control programs to prevent HAI is not to completely reduce the natural community of microorganisms by undirected (over-) use of disinfection agents and antibiotics at every given surface and body, but to prevent transmission of pathogens capable of causing disease [21, 310]. While targeted infection prevention, hygiene and workplace biosafety in veterinary medicine is not the focus topic of this habilitation thesis. However,

promoting a constant improvement of prevention measures against spread of zoonotic, opportunistic and drug resistant bacteria within practices, clinics and between individuals of “all species” was the goal of the first “consiliar lab for nosocomial infections” in veterinary medicine in Germany. This effort has recently (2019) culminated in establishment of the official “Konsiliarlabor für Methicillin-resistente Staphylokokken in der tierärztlichen Praxis und Klinik (kleine Haustiere und Pferde)”, lead by Prof. S. Schwarz, and the “Konsiliarlabor für ESBL-bildende Enterobacteriaceae in der tierärztlichen Praxis und Klinik (kleine Haustiere und Pferde)” led by Dr. A. Lübke-Becker, both situated at the "Zentrum für Infektionsmedizin, Institut für Mikrobiologie und Tierseuchen, Fachbereich Veterinärmedizin der Freien Universität Berlin”, and approved by the Deutsche Veterinärmedizinische Gesellschaft e.V. (DVG).

Figure 14 summarizes the key factors propagated by hygiene experts and consiliar labs in companion animal medicine to enhance infection control and biosafety for both, humans in contact and animal patients [94, 155, 336-342].

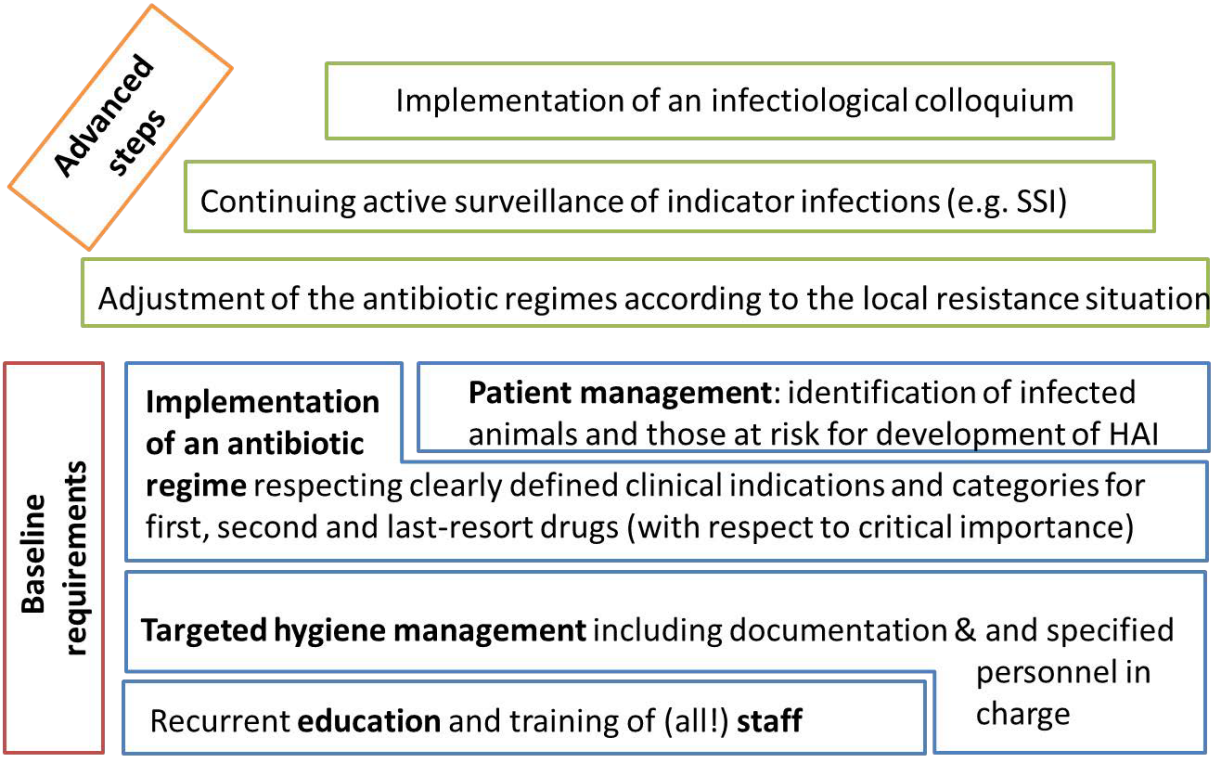


Fig. 14 Summary of key factors propagated by hygiene expert to enhance hygiene in veterinary clinics

While there is scientific data available with respect to the impact of surface contamination and – disinfection [76, 343-346], most reports stress the importance of hand hygiene [94, 96, 336, 338, 339, 341, 344, 347], with continuing education of all employees in a veterinary health

care setting as one of the most important “building blocks” of MDR spread prevention [96, 336, 338, 339, 344, 347]. Consequently, this aspect should remain a foundation for any hygiene management concept [94, 155, 336-339, 341, 348-350].

3.2 Next generation in molecular epidemiological investigations and diagnostics

The use of matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry (MS) for routine microbiological diagnostics has profoundly changed the conventional pathogen identification process in the laboratory, and is progressively replacing biochemical identification methods [351]. For opportunistic pathogens that may be difficult to identify on the sole basis of biochemical tests, such as Staphylococci belonging to the Intermedius group, the MALDI-TOF technology provides rapid and reliable results - but only if the reference spectra database provides the accurate reference [351] (**paper3**). The limits of the possibilities of this technology have not yet been reached. The use of MALDI-TOF as an ultra-fast tool in molecular outbreak investigation, recently shown for MRSA-CC398 in human medicine [352], is likely to offer improvements for molecular epidemiological questions in veterinary medicine as well.

Of note, direct identification of MRSA using MALDI-TOF remains problematic, and depends on factors harbored by the SCC*mec* element in the vicinity of the *mec* complex such as the gene encoding for an addition phenol-soluble modulin (PSM-*mec*) by the SCC*mec* II, III, and VIII [353]. Based on our work presented in **paper4** and **paper5,** the major challenges for differentiation of MRS harboring *mec*A-D are obvious: While the Penicillin-binding protein PBP2a meets its structural homologues in terms of the other PBP proteins (1-4) in MRS providing limited differences for spectra analysis, the SCC*mec* element is a hotspot for recombination (**paper5**), and therefore any target used as a primary indicator for all types of MRS have failed. However, increasing the peptide spectra resolution by technological progress might allow overcoming the current limitations with respect to MRS diagnostic.

AST of pathogens allows the most accurate decision on treatment possibilities for bacterial pathogens and prevents over- and misuse of antibiotics. While all AST methods offer qualitative assessments based on susceptible, intermediate, or resistant categories, certain methods specify qualitative and effective antibiotic dosage (*e.g.*, minimum inhibitory concentration) and formulate a profile of empirical therapy for the proper management of individual patients’ health [354].

A dramatic expansion and technological development in molecular epidemiology has been achieved in recent years [355], mirrored at least partly in the methods used for the publications

considered in this habilitation thesis. Starting with PCR detection of virulence-and resistance genes to verify the species and virulence- and resistance markers together with band-based typing methods such as PFGE for investigation of putative epidemiological clusters or even direct pathogen transmission events in **paper6**, **paper8** and **paper9**, analysis of WGS data is now an indispensable part of the work presented here, as reflected in **paper2**, **paper3**, **paper5** and **paper10**. In the near future, fully automated bioinformatics systems for the analysis of MDR bacteria will be used for molecular epidemiological investigations, as it has been recently proposed for MRSA [356]. However, it might require time until this technological development is widely used in veterinary medicine.

Novel and powerful bioinformatic tools allowing genome wide association studies of the core- and accessory genome are currently used to identify factors promoting niche- and host-adaptation in several important and zoonotic pathogens, but representability and metadata availability of the strain collections under investigation is sometimes questionable, lowering the potential usefulness. An integrated surveillance framework following the One Health idea (figure13) would provide reliable, representative genomes and metadata of pathogens which also mirror the present situation, not only “ancient” developments.

3.3 A broader view: MDR introduction rates into horse clinics

Our recent insights into introduction rates of MDR bacteria, especially MRSA (3.5%, **paper10**) and ESBL-producing *E. coli* (up to 10%, **paper2**) into horse clinics was supported by further studies [61, 114, 151, 196, 357]. A recent study compared ESBL-*E.coli* detection rate changes for hospitalized horses in an equine referral hospital (United Kingdom) over the course of a decade. In 2008, 28.7% of the fecal samples (n= 457, 103 horses) investigated were positive for ESBL-*E.coli*, whereas 50% of the samples from the cohort investigated in 2017 were found positive (n=314, 74 horses) [358]. The authors also noted an increase of AMR to frequently used antibiotics used in veterinary medicine [358], once more illustrating the challenges for veterinary clinics.

A fundamental question has not yet been answered: How do horses become colonized with ESBL-producing bacteria and/or MRSA? Even “healthy” racehorses in Japan seem to be frequently colonized with ESBL-*E. coli* (9.8%) [404]. *E. coli* harboring colistin resistance and ESBL/AmpC genes can be frequently isolated from wildlife exposed to human waste [18]. Consequently, one might hypothesize that the close proximity to urban surroundings might influence the carriage rate of MDR bacteria in horses, but even feral horses with no contact to domestic animals and minimal contact to people were, though rarely, colonized with drug-resistant *E. coli*, occasionally even with ESBL-producing variants [359]. In recent years,

interspecies transmissions of ESBL-*E. coli* [360, 361] has been observed, with wild birds appearing to be of importance with respect to the global dissemination of ESBL-producing Enterobacteriaceae [362-366] (all 2019). While wild birds might live in close proximities to horses, even feral horses, where do the wild birds become colonized with MDR bacteria? Recently, surface waters and sewage systems have gained attention as the possible reservoir for MDR bacteria - places commonly frequented by wild birds (more information on the subject is provided by [367]). To complete the picture, seafood aquacultures were found to be literally "containment basins" for ESBL-producing bacteria, especially in Asia [368-371]. Even MRSA have been isolated from cage-cultured fish [372]. Consequently, contaminating the environment with MDR bacteria enhances the frequency of colonization of wild as well as domestic animals, as has been shown for raccoons in the proximity of pig farms in the US [373], and dogs fed with raw meat in the Netherlands [328]. Limiting the spread of MDR bacteria or even resistance determinants among domestic animals, wildlife, plants, and the environment is a difficult, multifactorial task for future generations. Scientists from all areas mentioned are needed to tackle these challenges illustrated in figures 4 and 13, a collaborative approach reflecting the idea of the One health concept [4].

3.4 The role of MDR pathogens belonging to extended host spectrum genotype lineages for companion animal medicine requires further research

3.4.1 ESBL-*E. coli* belonging to extended host spectrum genotype lineages in horses

Genetic variation in an infectious disease pathogen can be driven by ecological niche dissimilarities arising from different host species and different geographical locations [374]. In **paper2,** MLST typing revealed 22 different sequence types, including eight novel STs. While ESBL-*E. coli* of human and animal origin belonging to ST224, ST1586 and ST539 were reported earlier [114, 122, 375], others (*e.g*. sequence type complex (STC)10 and ST1730) were identified in samples from wild birds and other animals [401, 402] (**paper2, paper6**). ESBL-*E. coli* of equine origin appear frequently associated with STC1250 and STC10 (**paper2**, **paper6**). Interestingly, these findings concur with a publication by Apostolakos et al. (2017), who also reported STC1250 and STC10 as dominating lineages of ESBL-*E. coli* from colonized horses [114]. Since STC10 is the dominant commensal genotype lineage of *E. coli*, these results may reflect genomic plasticity of this particular genetic background with respect to niche adaption [376] (**paper2)**. In addition, independent occurrence and spread of successful lineages in a certain environment indicates that not only transmission of factors promoting host adaptation by HGT are important for niche adaption, but also the overall capacity of the

genomic backbone (core genome) to adapt to novel environmental conditions which defines extended host spectrum genotypes (EHSG).

A recent review on population genomics of bacterial host adaptation provides in-depth insights in the evolutionary aspects of host- and niche-adaption strategies and the corresponding genomic changes [377, 378]. It is currently clear that bacterial niche- and host adaption is a complex research field which is still in its beginnings, since monocausal theories are less and less capable of accounting for the successful establishment of certain EHSG-lineages in new environments.

3.4.2 Niche adaptation of MRSA: An unraveling story still to be told

Questions concerning host specificity of certain *S. aureus* lineages date back to the early 1970's. At that time, certain ecovares had been defined based on phenotypical characterizations. Accordingly, isolates of human origin were regarded as biovar *hominis*, those from cattle as biovar *bovis*, from sheep as biovar *ovis* and those from poultry as biovar *gallinae* [379-385]. Later, different biovars were found in samples of the same animal species [386-388], and more discriminative microbiological typing methods were developed, promoting more detailed investigations concerning host specificity and niche adaption abilities of certain *S. aureus* lineages. Antimicrobial resistance (AMR) and hospital-associated infections (HAI) are closely related issues relevant for human and veterinary medicine likewise. The work presented in this habilitation thesis is, however, driven by a further aspect, the capability of bacteria to adapt to a new environment, especially in terms of a new host or an enhanced host spectrum, as proposed in **paper9** in terms of extended host spectrum genotypes (EHSG).

Mechanisms enabling opportunistic bacteria to cross species barriers, infect a new host or enhance their viability in the environment are still poorly understood. Beyond antimicrobial resistance as a selective advantage, there might by other changes involved in (niche) adaptation processes in MRSA as well as MSSA, encompassing, among others, alterations in metabolic pathways [389], escaping early host defense mechanisms (**paper10**), biofilm formation [390], enhanced iron acquisition [391], pseudogenes [392, 393] or altered genomic promoter structures [394]. In addition, production of cytotoxins [395, 396] and other virulence factors as well as their regulatory circuits might have a strong influence on host adaptation [395, 397] (**paper10**).

Since evidence for a role in adaptation is provided when genomic changes occur independently in divergent lineages [378], tracing of common motifs which might be beneficial for the widespread dissemination of EHSG across different *S. aureus* genotype lineages is a leading question in **paper7**, **paper9** and **paper10,** reflecting 10 years of work (2009-2019). A combination of genomic changes, often a single SNP or even an additional virulence factor

such as SCIN harbored by a mobile genetic element, can contribute to the epidemiological success of certain lineages. From the perspective of a pathogen, steady switching between adaptations to a (novel) human or animal or environmental setting is unlikely. More plausible is a host range extension, which can be highlighted by our recent finding that staphylococcal complement inhibitors (SCIN) with different activity ranges towards ruminant, equine and human plasma located on completely different MGEs where harbored by equine MRSA-ST398 (**paper9**). The existence of the different SCIN proteins indicates adaptation mechanisms evolved for coping with first-line immune defense mechanisms of different hosts, while their accumulation in MRSA-ST398 isolates verifies the existence of EHSG, genomic lineages with the capacity to accumulate such mechanisms better than others. In addition, broadening the host range by acquiring escape strategies to survive primary innate immune defenses in human- and non-human mammals seems a quite promising bacterial response to prosper in a in a health care sector characterized by selection forces (antibiotics, biocides, other pharmaceuticals) and frequent close contact between animals and personnel taking care of them. According to the research presented here and elsewhere, the MRSA-ST398 lineage is currently the most successful operator in that particular niche, especially in horse clinics [148, 272, 278, 308, 398].

On the other hand, epidemic MRSA strains circulating in human hospitals do not necessarily need additional virulence factors for broadening their host range. However, the predominant European epidemic hospital lineage belonging to ST22 was frequently reported in small animal hospitals [49, 91, 144, 295, 399, 400]. More research on the subject, especially on MGEs carrying genes whose products encode immune-evasion activities, could provide insights into the still ongoing spread of this lineage in small animal clinics.

Another question concerns MRSA which have been reported from environmental sources, including wild animals. Our recent work on *mec*C-MRSA revealed that alterations of the accessory gene regulator (*agr*) system of *S. aureus* seems common among lineages not primarily considered as epidemic to human hospitals, but emerging in wild and domestic animals as well as in the environment [403]. Our results might indicate that *agr* variations enhance viability and therefore niche adaptation capacities of *S. aureus* non-hospital-associated epidemic lineages [403].

Identification of genetic signatures fostering host (or niche) adaptation in combination with functional analyses can reveal the biological mechanisms associated with a successful switch to new host species and environments, including critical host–pathogen interactions that may represent novel therapeutic targets [378]. Clearly, further research in this area is warranted.

According to my personal opinion, the most important reason hindering objective niche or even host adaption research is the commonly narrow spectrum of isolate origins included in many of the studies published so far. Comparing apples with oranges will always yield differences between them, but does not allow conclusions to extend to pears. Broadening the content of the "fruit basket" (*i.e.*, isolates representing different environmental origins and host species) allows investigations of the complete picture considering host- or niche adaptation processes. A more scientific example highlights the problem: MRSA-ST398 were long regarded as pig-specific; then, after several reports from other livestock animals, it was re-designated as livestock-associated [44, 199, 272, 275, 300]. When it became clear that in other parts of the world other lineages were predominating in the livestock industry (e.g. MRSA-CC5 in Asia), we now refer to these isolates as the "European livestock associated lineage", a term which still neglects their overwhelming importance for MRSA-infected horses and, to a lesser extent, dogs and cats [171].

In summary, only a solid, broad selection of isolates representing different origins, *e.g.* generated by an integrated surveillance system (figure 13) would allow us to judge the importance of particular EHSG lineages within the One Health framework and reflect recent shifts and emergences in molecular epidemiology of zoonotic MDR pathogens.

4 Danksagung

Frei interpretiert nach dem bekannten Zitat des französischen Arztes und Wissenschaftlers Claude Bernard „Le microbe n'est rien, le terrain c'est tout!“ ist auch der einzelne wissenschaftlich tätige Mensch nichts ohne sein Umfeld, dies gilt insbesondere im Hinblick auf eine kumulative Habilitationsschrift wie diese.

Zunächst gilt mein ganz besonderer Dank Prof. Lothar H. Wieler, der frühzeitig die zahlreichen Herausforderungen durch multiresistente Infektionserreger für veterinärmedizinische Praxen und Kliniken erkannte und mir freundlicherweise das Thema dieser Arbeit überlassen hat. Die langjährige zugewandte und stets konstruktive Unterstützung sowie der kritische Diskurs wichtiger Fragestellungen haben mir sehr geholfen, aus dem jeweiligen „Ist-Zustand“ immer noch etwas Besseres zu generieren.

Bei Frau Dr. Antina Lübke-Becker möchte ich mich für die exzellenten Lehrstunden zu neutraler Perspektive und Gelassenheit im fachlichen Kontext bedanken. Meine ganz besondere Wertschätzung gilt zudem der mir angediehenen herausragenden fachlichen, praktischen und stets innovationsfreundlichen Ausbildung in der Diagnostik von Bakterien und deren Resistenzen sowie der molekularen Epidemiologie. Diese fachlichen Kompetenzen waren die Grundlagen für alle im Rahmen dieser Arbeit vorgestellten Publikationen. Die kollegiale Zusammenarbeit mit ihr war und ist mir stets eine besondere Freude. Selten trifft man im Leben Idealisten, die wie sie unermüdlich danach streben, die Welt wirklich aktiv zu verbessern - vielen Dank auch dafür.

Bei Dr. Torsten Semmler, heute RKI, und seiner Arbeitsgruppe bedanke ich mich ganz besonders für die langjährige, produktive und freundschaftliche Zusammenarbeit. Mit seiner exzellenten und dennoch unprätentiösen bioinformatischen Expertise hat er einen wesentlichen Beitrag zu zahlreichen der hier vorgestellten Arbeiten geleistet.

Ich bedanke mich ausdrücklich bei Prof. Stefan Schwarz, dem derzeitigen Leiter des Instituts für Mikrobiologie und Tierseuchen (IMT) der Freien Universität Berlin, für die freundliche Unterstützung meiner Forschungsprojekte und die Möglichkeit, die Räume und Labore des Instituts zu nutzen. Mein außerordentlicher Dank gilt ferner allen meinen ehemaligen Kollegen vom IMT, insbesondere Christel Simon, Angela Schellin, Andrea Schmidt, Monika Feldhan und Aline Poppe, für die allumfassende Unterstützung nicht nur im Labor, sondern weit darüber hinaus bei allen hier vorgestellten Studienergebnissen. Bei Dr. Astrid Bethe bedanke

ich mich für ihre langjährige, freundliche und kollegiale Unterstützung - wann auch immer ich diese angefragt habe. Herzlich bedanken möchte ich mich auch für die außergewöhnlich gute administrative Unterstützung im Hinblick auf die Organisation von Lehre und Forschung bei Heike Görs und Susanne Hönniger. Bei Dr. Karsten Tedin bedanke ich mich ausdrücklich, weil er immer Zeit gefunden hat, mich an seiner wissenschaftlichen Brillanz und außerordentlichen literarischen Sprachkompetenz teilhaben zu lassen. Ich hoffe sehr, dass mein größtes Versäumnis in meiner Zeit am IMT, namentlich ein absolut mangelhaftes Interesse am NF-kappaB-Signalweg, durch ein gemeinsames Forschungsprojekt irgendwann ausgeglichen werden kann.

Bei den zahlreichen Kollegen am Fachbereich Veterinärmedizin der Freien Universität Berlin, die an den hier vorgestellten Arbeiten beteiligt waren, bedanke ich mich außerdem, ganz besonders bei den klinisch tätigen Kolleginnen Prof. Heidrun Gehlen sowie Prof. Barbara Kohn.

Der größte und innigste Dank gebührt jedoch meiner ganz wunderbaren Familie, besonders Sascha, Lina und Greta Eversmeier, meiner Mutter Brigitte Walther und meinem Bruder Michael Walther, für ihr immenses Verständnis und ihre Hilfe in all diesen Jahren.
Vielen lieben Dank, Sascha, dafür dass Du mich mit Deinem großen Herz, Deiner Ruhe, Stärke, Weitsicht, Nachsicht und Kreativität unglaublich unterstützt hast und bis heute meine Welt immer wieder neu erweiterst.

[Unterschrift nicht Teil der Onlineversion]

Berlin, den 28.09.2020

5 Publications and contribution of individual authors

Many people contributed to the work (**paper1-10**) presented in this habilitation thesis. A complete list of all co-authors and their individual contribution to conceptualization, data curation, formal analysis, funding acquisition, investigation, project administration, supervision, validation, original draft writing, draft reviewing and -editing is commonly provided by the papers mentioned below. However, I declare the individual contribution of the most important co-workers and myself with respect to study ideas and/or design, practical work, data analysis, original draft writing, draft editing and -reviewing for all cases where this information is not clearly stated in the original publication.

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paper1

Walther, B., K. Tedin, and A. Lübke-Becker, Multidrug-resistant opportunistic pathogens challenging veterinary infection control. Vet Microbiol, 2017. 200: p. 71-78.

https://doi.org/10.1016/j.vetmic.2016.05.017

Author contributions

Idea and Design: Walther

Data analysis: Walther

Practical Work: not appropriate

Writing (original draft): Walther

Draft review and editing: Lübke-Becker, Tedin

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paper2

Walther, B., Klein, K.S., Barton, A.K., Semmler, T., Huber, C., Wolf, S.A., Tedin, K., Merle, R., Mitrach, F., Guenther, S., Lübke-Becker, A., Gehlen, H., 2018. Extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* and *Acinetobacter baumannii* among horses entering a veterinary teaching hospital: The contemporary "Trojan Horse". PLoS One 13, e0191873. https://doi.org/10.1371/journal.pone.0191873

Author contributions as declared in **paper2**

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paper3

Murugaiyan*, J., Walther*, B., Stamm, I., Abou-Elnaga, Y., Brüggemann-Schwarze, S., Vincze, S., Wieler, L.H., Lübke-Becker, A., Semmler, T., Roesler, U., 2014.

Species differentiation within the *Staphylococcus intermedius* group (SIG) using a refined MALDI-TOF MS database. https://doi.org/10.1111/1469-0691.12662

Author contributions

Idea and Design: Walther, Murugaiyan

Practical Work: Murugaiyan, Walther

Review and editing (draft): Rösler

Data analysis: Murugaiyan, Semmler, Walther

Writing (original draft): Murugaiyan, Walther (equally)

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paper4

Walther, B., Wieler, L.H., Vincze, Sz., Antão, E.-M., Brandenburg, A., Stamm, I., Kopp, P.A., Kohn, B., Semmler, T., Lübke-Becker, A., 2012b. MRSA variant in companion animals. Emerg Infect Dis 18, 2017-2020. https://doi.org/10.3201/eid1812.120238

Idea and Design: Walther, Lübke-Becker

Practical Work: Stamm, Walther

Review and editing (draft): Lübke-Becker, Wieler

Data analysis: Walther, Semmler

Writing (original draft): Walther

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paper5

Semmler, T., Harrison, E.M., Lübke-Becker, A., Ulrich, R. G., Wieler, L. H., Guenther, S., Stamm, I., Hanssen, A. M., Holmes, M. A., Vincze, Sz., Walther, B. 2016. A Look into the Melting Pot: The *mec*C-Harboring Region Is a Recombination Hot Spot in *Staphylococcus stepanovicii*." PLoS One 11(1): e0147150.

Author contributions

as declared in **paperV** https://doi.org/10.1371/journal.pone.0147150

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paper6

Walther B, Lübke-Becker A, Stamm I, Gehlen H, Barton AK, Janssen T, Wieler LH, Guenther S. Suspected nosocomial infections with multidrug resistant *E. coli*, including extended-spectrum beta-lactamase (ESBL)-producing strains, in an equine clinic. Berl Muench Tieraerztl Wochenschr. 2014, 127(11-12):421-427. https://doi.org/10.2376/0005-9366-127-421

Idea and Design: Walther

Data analysis: Walther, Lübke-Becker, Günther

Practical Work: Walther, Günther

Writing (original draft): Walther

Review and editing (draft): Lübke-Becker, Wieler, Günther

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paper7

Vincze S., Brandenburg, A.G., Espelage W., Stamm I., Wieler L. H., Kopp P. A., Lübke-Becker A., Walther B., 2014. Risk factors for MRSA infection in companion animals: Results from a case-control study within Germany. Intl J Medical Microbiol 10/2014; https://doi.org/10.1016/j.ijmm.2014.07.007

Idea and Design: Walther, Vincze

Data analysis: Vincze, Walther, Lübke-Becker

Practical Work: Brandenburg, Vincze, Stamm

Writing (original draft): Vincze, Walther

Review and editing (draft): Walther, Lübke-Becker, Wieler

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paper8

Walther, B., Hermes, J., Cuny, C., Wieler, L.H., Vincze, S., Abou Elnaga, Y., Stamm, I., Kopp, P.A., Kohn, B., Witte, W., Jansen, A., Conraths, F.J., Semmler, T., Eckmanns, T., Lübke-Becker, A., 2012a. Sharing more than friendship--nasal colonization with coagulase-positive staphylococci (CPS) and co-habitation aspects of dogs and their owners. PLoS One 7, e35197

Author contributions

as declared in **paperVIII** https://doi.org/10.1371/journal.pone.0035197

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paper9

Walther, B., Monecke, S., Ruscher, C., Friedrich, A.W., Ehricht, R., Slickers, P., Soba, A., Wleklinski, C.G., Wieler, L.H., Lübke-Becker, A., 2009a. Comparative molecular analysis substantiates a zoonotic potential of equine Methicillin- resistant *Staphylococcus aureus* (MRSA). J Clin Microbiol. 47, 704-710. https://doi.org/10.1128/JCM.01626-08

Idea and Design: Walther, Lübke-Becker

Data analysis: Walther, Monecke

Practical Work: Walther

Writing (original draft): Walther, Lübke-Becker, Wieler

Review and editing (Draft): Lübke-Becker, Monecke, Wieler

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paper10

Walther B., Klein K.S., Barton A.K., Semmler T., Huber C., Merle R., Tedin K., Mitrach F., Lübke-Becker A., Gehlen H. Equine Methicillin-Resistant Sequence Type 398 *Staphylococcus aureus* (MRSA) Harbor Mobile Genetic Elements Promoting Host Adaptation. Front Microbiol 2018, 9:2516. https://doi.org/10.3389/fmicb.2018.02516

Author contributions

as declared in **paper10**

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