Aus dem Institut für Mikrobiologie und Tierseuchen

des Fachbereichs Veterinärmedizin

der Freien Universität Berlin

Staphylococcus aureus in companion animals: An infection source for the community?

Inaugural-Dissertation

zur Erlangung des Grades eines

PhD of Biomedical Sciences

an der

Freien Universität Berlin

vorgelegt von

Szilvia Vincze

Tierärztin

aus Budapest

Berlin 2014

Journal-Nr. 3756

Gedruckt mit Genehmigung des Fachbereichs Veterinärmedizin
der Freien Universität Berlin

Dekan:	Univ.-Prof. Dr. Jürgen Zentek
Erster Gutachter:	Univ.-Prof. Dr. Lothar H. Wieler
Zweiter Gutachter:	Univ.-Prof. Dr. Barbara Kohn
Dritter Gutachter:	Univ.-Prof. Dr. Uwe Rösler

Deskriptoren (nach CAB-Thesaurus):
Staphylococcus aureus, methicillin-resistant *Staphylococcus aureus*, cats, dogs, horses, epidemiology, wound infections, zoonoses, genotypic variation, genotypes

Tag der Promotion: 27.02.2015

Bibliografische Information der *Deutschen Nationalbibliothek*
Die Deutsche Nationalbibliothek verzeichnet diese Publikation in der Deutschen Nationalbibliografie; detaillierte bibliografische Daten sind im Internet über <http://dnb.ddb.de> abrufbar.

ISBN: 978-3-86387-578-7
Zugl.: Berlin, Freie Univ., Diss., 2014
Dissertation, Freie Universität Berlin
D 188

Choriner Str. 85 - 10119 Berlin
verlag@menschundbuch.de – www.menschundbuch.de

Figures

Tables

List of Abbreviations

agr	accessory gene regulator
arcC	housekeeping gene: carbamate kinase
aroE	housekeeping gene: shikimate dehydrogenase
BB	Brandenburg
bp	base pairs
BV	Bavaria
BW	Baden-Wuerttemberg
CA-MRSA	community-associated MRSA
CC	clonal complex
CDC	Centers for Disease Control and Prevention
chp	chemotaxis inhibitory protein
DNA	deoxyribonucleic acid
EHSG	extended host spectrum genotype
ET	exfoliative toxin
glpF	housekeeping gene: glycerol kinase
gmk	housekeeping gene: guanylate kinase
H	Hesse
HA-MRSA	healthcare-associated MRSA
hl	hemolysins
IEC	immune evasion cluster
Ig	immunglobulin
IMT	Institute of Microbiology and Epizootics
LA-MRSA	livestock-associated MRSA
LS	Lower Saxony
Luk	leukotoxin
MGE	mobile genetic elements
MLEE	multilocus enzyme electrophoresis
MLST	mulilocus sequence typing
MRSA	methicillin-resistant *S. aureus*
MSCRAMM	microbial surface components recognizing adhesive matrix molecule
MSSA	methicillin-susceptible *S. aureus*
NGS	next generation sequencing
NRW	North Rhine-Westphalia

orfX	open reading frame X
PBP	penicillin-binding protein
PCR	polymerase chain reaction
PFGE	pulsed field gel electrophoresis
pta	housekeeping gene: phosphate acyltransferase
PVL	Panton-Valentine leukocidin
RKI	Robert Koch-Institute
RP	Rhineland-Palatinate
SAg	superantigen
SaPI	*S. aureus* pathogenicity island
$SaPI_{ov}$	ovine *S. aureus* pathogenicity island
sak	staphylokinase
S. aureus	*Staphylococcus aureus*
SAXA	Saxony Anhalt
SCC*mec*	staphylococcal cassette chromosome *mec*
scn	staphylococcal complement inhibitor
SE	staphylococcal enterotoxin
SE*l*	staphylococcal enterotoxin-like toxin
sea	staphylococcal enterotoxin A
SH	Schleswig Holstein
S. hyicus	*Staphylococcus hyicus*
SIG	*Staphylococcus intermedius*-group
SNP	single nucleotide polymorphism
spa	*S. aureus* protein A
ST	sequence type
TH	Thuringia
Tn	transposon
tpi	housekeeping gene: triosephosphate isomerase
WGS	whole genome sequencing
X region	highly variable tandem repeat region
yqil	housekeeping gene: acetyl coenzyme A acyltransferase

1. Introduction

Infections with multi-resistant bacteria are a burden in both human and veterinary medicine. Of special concern are multi-resistant pathogens with a zoonotic potential as they can be transmitted between humans and animals. One prominent example is *Staphylococcus (S.) aureus.* This opportunistic pathogen is able to cause a broad range of different infections in humans and animals. The occurrence and global spread of methicillin-resistant *S. aureus* (MRSA) has resulted in a major threat for human healthcare systems due to limited treatment options. While MRSA infections were mainly restricted to humans in the past, multiple case reports raised the awareness of infections with this pathogen in companion animals as a rising therapeutically challenge in veterinary medicine in the late 90s. Since then, several studies investigated the impact of MRSA infection and colonization in animals with regard to the putative zoonotic potential. These surveys focused in particular on the investigation of MRSA isolates from livestock even though *S.°aureus*-mediated infections were generally also described for various infection sites in companion animals [1]. Nowadays, *S. aureus* seems to be frequently associated with cases of wound infections in dogs, cats and horses. In contrast to livestock, most companion animals live in close contact to their owners, a fact that is of special concern with regard to the possibility of zoonotic transmission of pathogens [2]. Several case reports from the recent past demonstrated the zoonotic potential for MRSA between companion animals and their owners within one household [3-6], raising the question whether those animals might serve as a potential MRSA infection source for humans. In order to address this question, it is especially important to gain more knowledge about the frequency of *S. aureus*-mediated infections in companion animals and the genetic background of these isolates. Only by defining the genetic lineage of MRSA it is possible to identify similar or identical isolates that are shared by different hosts. Therefore, the aim of this study was

i) To identify the frequency of *S. aureus* among wound swabs obtained from dogs, cats and horses.
ii) To determine the MRSA-frequency within these isolates and to investigate the associated phenotypic resistance profiles regarding multi-drug resistance.
iii) To investigate the genetic background of MRSA and methicillin-susceptible *S aureus* (MSSA) to gain more insights into the genetic diversity of *S. aureus* isolates from dogs, cats and horses
iv) The comparison of frequently occurring canine *S. aureus*-lineages with similar strains of human origin.

2. *Staphylococcus aureus*

2.1 Taxonomy and characteristics

Staphylococci are commensals with the ability to colonize the skin as well as sebaceous glands and the mucosa of humans and various animals [7]. The genus *Staphylococcus* belongs to the *Bacillus-Lactobacillus-Streptococcus*-cluster and currently consists of 72 species and subspecies (Leibniz-Institute: German collection of cell cultures and microorganisms, 31.02.2014). The facultative anaerobic, Gram-positive, coccoid-shaped bacteria have a diameter of 0.5-1.5µm. In the microscopic image, they are arranged in pairs or in bunch of grapes which resulted in the naming of these bacteria as staphylococci (staphylé: Greek for grape). Furthermore, staphylococci are non-motile and lack the ability to produce spores. Since 2009, staphylococci belong to the Micrococcaceae family [8] instead of Staphylococcaceae. The nomenclature changes were validated in the „International Journal of Systematic and Evolutionary Microbiology“ in 2010 [9]. During microscopy, staphylococci can appear very similar to streptococci. However, isolates can be distinguished easily by testing the catalase activity, an enzyme that is expressed by staphylococci but missing in streptococci. This enzyme catalyzes the hydrolytic splitting of hydrogen peroxide to water and oxygen [10].

As opportunistic pathogens, several staphylococcal species are not only able to colonize humans and animals, but are also capable to infect their hosts during immune suppression or by invading injured tissue. In veterinary medicine, the following species are of special concern: *S. aureus,* species of the *Staphylococcus intermedius*-group (SIG) and *S. hyicus* [11]. Some *S. aureus* strains form grey-white or yellowish colonies. The yellow pigmentation is the result of carotenoid production and led to the choice of the name “aureus” by Rosenbach (aureus: Latin for golden) in 1954 [12].

2.2 Relevance of *S. aureus* in infectious diseases

The natural habitat of *S. aureus* are the skin and mucosa of humans and it is well known that about 20-30% of the human population are permanently and up to 60% are transiently colonized [13]. Besides, *S. aureus* can colonize various animals as well [14]. The opportunistic pathogen has the ability to cause a broad range of different diseases, including localized infections like furuncle, impetigo, carbuncle, keratitis and wound infections as well as life threatening systemic infections like necrotizing pneumonia, septicemia, osteomyelitis and pericarditis [15]. Furthermore, *S. aureus* accounts for one of the most important

nosocomial pathogens in human medicine. Especially methicillin-resistant *S. aureus* (MRSA) harbor not only resistance towards all beta-lactam antibiotics but acquire frequently multi-drug resistance to various antibiotic classes, causing a high amount of infectious diseases in hospitals worldwide. These nosocomial infections are difficult to treat and are a reason for longer hospital stays, higher costs and higher mortality rates [16]. In 1960, the first report of a human MRSA infection was published in Great Britain by Jevons et al. [17]. A screening study, which was conducted to identify the frequency of MRSA-mediated infections in British hospitals, revealed only a moderate increase between 1961 and 1964 [18]. However, in the late sixties MRSA infections were identified in increasing numbers [19]. Nowadays, MRSA account for one of the major nosocomial pathogens in most industrial countries. Since the nineties, MRSA are no longer restricted to healthcare settings but can be found in persons within the community as well [20].

In veterinary medicine, infections caused by *S. aureus* were coincidental findings for a long time. The first report of an MRSA-infected animal counts back to 1972. In that year, MRSA were identified in bulk milk samples and therefore diagnosed as cause of bovine mastitis [21]. Another case report described the occurrence of MRSA infections in two dogs [22]. In both cases humans were considered as infection source. In recent years, increasing numbers of MRSA infections in animals raised the awareness of this pathogen in veterinary medicine. Particularly companion animals like dogs, cats and horses are known to suffer regularly from MRSA-mediated diseases like skin-, soft tissue and surgical site infections [23]. Other diseases like systemic infections, respiratory infections or infections of the urogenital tract, auditory channel, gastrointestinal tract or eye have been reported in lower frequencies as well [1,24,25]. Due to these increasing reports, a couple of research studies were conducted with a focus on infection rates in dogs, cats and horses. Existing data are still rare and difficult to compare because of different study setups and small sample sizes. Nevertheless, increasing reports about MRSA infections indicate either raising infection rates or enhanced awareness for this pathogen in companion animals during the last decade [25,26]. Especially wound infections seem to be increasingly caused by MRSA in these animals [1,27,28].

Since 2005, the occurrence of MRSA particularly associated with colonization was frequently reported for different animal species on livestock farms [29-36]. Nevertheless, clinical infection rates seem to be relatively low in comparison to those reported for companion animals. In 2010, Weese reviewed data concerning the incidence of MRSA infections in cattle and stated an overall low MRSA infection rate for these animals [33]. MRSA infections

in cattle and other ruminants have been reported regularly in the past [29,34-36]. In swine, *S. aureus,* in particular MRSA has been reported as a frequent colonizer. However, infections are rarely reported, suggesting that MRSA infections do not play an important role in pigs at the moment [32,33].

In poultry, *S. aureus* can cause infections like gangrenous dermatitis, septic arthritis and subdermal abscesses [31]. Data concerning the frequency of *S. aureus*-mediated infections in poultry industry are limited but evidence exists that it is an important pathogen in this sector [29,30].

2.3 Resistance mechanisms towards beta-lactam antibiotics

Alexander Fleming discovered penicillin as antimicrobial agent in 1928. Several years after this discovery, the beta-lactam antibiotic was routinely used for therapy of infections and thus, enabled for the first time effective treatment of invasive staphylococcal infections. The bactericidal effect of penicillin is based on the inhibited synthesis of the bacterial cell wall. In case of interaction, penicillin binds to staphylococcal penicillin-binding proteins (PBP) which results in the inactivation of an essential transpeptidase. In general, transpeptidases are necessary to crosslink peptidoglycans during cell wall synthesis, providing structural integrity. The functional loss of this particular enzyme leads to instability of the cell wall during growth so that the cell is not able to withstand the osmotic pressure [37].

In 1945, the expression of beta-lactamases as a mechanism to avoid the bactericidal effect of penicillin was described by Bondi and colleagues [38]. This resistance mechanism is based on hydrolytic splitting of the beta-lactam structure that leads to the functional loss of the antibiotic. So far, four different beta-lactamases have been described in staphylococci (A-D). Three of these enzymes (A-C) are only expressed on high levels after induction with beta-lactams while beta-lactamase D is constitutively expressed. The enzymes can be encoded on a transposon (Tn552) or on plasmids [19].

Only a short time after the introduction of penicillin for antimicrobial therapy in 1941, resistant staphylococci had been reported [39,40]. Already in the late 1950s, susceptibility rates for penicillin in *S. aureus* were not higher than 15% and nowadays most *S. aureus*-isolates harbor the beta-lactamase operon [41].

The semi-synthetic beta-lactam antibiotic methicillin was introduced in 1959 for treatment of penicillin-resistant staphylococci. In contrast to penicillin, methicillin is resistant against beta-lactamases and preserves its bactericidal effects on beta-lactamase producing *S. aureus*

strains. However, *S. aureus* isolates showing resistance against methicillin were reported only a short time after the therapeutical introduction of this antimicrobial agent and those strains were named as MRSA [17]. By definition, MRSA are considered as resistant against all beta-lactam antibiotics including carbapenems and cephalosporins [42]. The resistance mechanism is triggered by the expression of the penicillin-binding protein PBP2a, an enzyme that functions as an additional transpeptidase, which, in contrast to the native PBP2, expresses a low binding affinity for beta-lactams [43].

MRSA harbor the mobile genetic SCC*mec* (staphylococcal cassette chromosome *mec*) element with the PBP2a encoding *mec*A or *mec*C gene. The integration of one of 11 different SCC*mec* types or SCC*mec*-hybrids into the staphylococcal chromosome results in an intrinsic resistance against beta-lactam antibiotics [20]. Until 2010, *mec*A was the only gene known to encode PBP2a but in 2011 *mec*C, a homologue of *mec*A, was described as another PBP2a encoding gene conferring methicillin resistance in *S. aureus* [44]. Depending on the structure of the SCC*mec* element, the PBP2a encoding gene can be either expressed constitutively or in presence of the active regulatory genes *mec*R and *mec*I (part of the *mec* encoding region) within the SCC*mec* element after induction with beta-lactams. Beside beta-lactam resistance, MRSA frequently express resistances towards several other antibiotic classes, leading to multi-drug resistant phenotypes associated with limited therapeutic options [45].

2.4 Virulence-associated factors

S. aureus comprises a variety of different virulence-associated factors. It is possible to distinguish between a) surface-associated virulence factors with the ability to adhere to the host and/or to avoid phagocytosis and b) secreted virulence factors [46].

a) Most surface-associated virulence factors are linked with the staphylococcal cell wall or the outer surface of the cell envelope, including microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) as most important group of adhesins. A common feature of all MSCRAMMs is the existence of identical amino acid motifs (LPXTG motif) in the wall-spanning region. This motif functions as cell wall anchor of surface proteins in *S. aureus* [47]. All MSCRAMMs that have been described so far are able to bind to several different ligands [48]. Apart from adhesins that are bound to the staphylococcal surface, some secreted virulence factors are also associated with the surface. Table 1 gives an overview of important MSCRAMMs and secreted surface proteins for *S. aureus*.

b) The other class of virulence-associated factors includes four main groups of proteins: leukotoxins, hemolysins, superantigens and exfoliative toxins.

Hemolysins are membrane-damaging exotoxins that are able to destroy erythrocytes and several other mammalian cells. For *S. aureus*, four different hemolysins have been described: hemolysin α, hemolysin β, hemolysin δ and hemolysin γ, named chronologically in order of their first description. Hemolysin α and hemolysin δ are pore-forming toxins. Hemolysin β shows activity as sphingomyelinase [49]. Since Hemolysin γ (Hlg) is a two-component toxin, it has been classified as leukotoxin and will be discussed in the leukotoxin section.

Leukotoxins (Luk) can target monocytes, macrophages and neutrophils. The bi-component virulence factors consist of two proteins from different classes (class F and class S), which are not toxic on their own. Both proteins are required for pore formation. Until now, five different class F (HlgB, LukF-PV (Panton Valentine), LukD, LukF'-PV, and LukG) as well as six different class S subunits (HlgA, HlgC, LukS- PV, LukE, LukM, and LukH) have been described, each of them forming a pore-structure after pairing of two specific proteins. The only known exception is the hemolysin γ gene cluster, where HlgB (F subunit) can build a complex with either HlgA or HlgC (both: S subunits) [50].

Three different exfoliative toxins have been described in *S. aureus* (exfoliative toxin (ET)A, ETB, ETD). All proteins lead to desquamation due to cleavage of desmoglein 1, a keratinocyte cell-to-cell adhesion molecule, resulting in severe skin diseases like scalded skin syndrome and bullous impetigo [50].

S. aureus encodes various superantigens (SAgs) that have been first named as staphylococcal enterotoxins based on their ability to cause food poisoning after oral uptake. However, given the fact that all SAgs induce T-cell stimulation but not all of them cause food poisoning, a new nomenclature was established in 2004 to distinguish between SAgs with (named as staphylococcal enterotoxins (SEs)) and without emetic toxicity (named as staphylococcal enterotoxin-like toxins (SE*l*s)) [51]. 23 different SAgs have been described so far, including several SEs and SEIs like the staphylococcal toxic shock syndrome toxin (an overview of all SEs and SEIs is given in table 1) [50].

group	virulence-associated factors	function
hemolysins (Hl)	hemolysin α	pore formation in erythrocytes and various other mammalian cells
	hemolysin ß	cleavage of sphingomyelin
	hemolysin δ	membrane permeabilization of erythrocytes and various other mammalian cells
leukotoxins (Luk)	PVL (Panton-Valentine leukocidin)	pore formation in PMNs (polymorphonuclear leukocytes)
	LukDE	
	LukF´M	
	LukGH	
	Hemolysin γ	pore formation in erythrocytes and various other mammalian cells
exfoliative toxins (ET)	ETA	cleavage of desmoglein 1
	ETB	
	ETD	
superantigenic toxins (SAgs)	staphylococcal enterotoxins (SE) SEA-SEE, SEG-SEJ, SER-SET	activation of T-lymphocytes: creation of a cytokine storm
	staphylococcal enterotoxin-like toxins SE*I*K-SE*I*Q, SEIU-SEIX	
	toxic shock syndrome toxin	
	surface-associated virulence factors	**main functions**
MSCRAMMs (microbial surface components recognizing adhesive matrix molecules) selected proteins	clumping factor A/B	binding to fibrinogen
	fibronectin binding protein A/B	binding to fibronectin
	elastin binding protein	binding to elastin
	collagen adhesin	binding to collagen
	extracellular matrix protein	binding to fibronectin, fibrinogen and vitronectin
	iron regulated surface determinants A-H	adhesion to and aggregation of platelets
secreted surface proteins (selection)	extracellular complement binding protein	complement binding
	coagulase	plasma coagulation

Table 1: Overview of important virulence-associated factors divided according to a) surface-associated virulence factors and b) virulence-associated factors. The table displays a selection of important staphylococcal virulence factors.

2.5 Epidemiology

The introduction of penicillin for antibiotic therapy of staphylococcal infections marked a new era with the possibility to treat severe and life-threatening *S. aureus*-infections [37]. The routine use of penicillin for staphylococcal infections resulted in a selection pressure for penicillin-resistant *S. aureus,* which spread rapidly and became pandemic in both hospitals and the community [41]. This development has been described as “the first wave of

resistance" by Chambers and deLeo [52]. "The second wave" was initiated by the introduction of methicillin. Again driven by selection pressure, the use of this semi-synthetic beta-lactam antibiotic led to the spread of MRSA. It is known that several independent uptakes of SCC*mec* elements harboring the methicillin resistance encoding gene resulted in the development of distinct MRSA genotypes [53]. In the beginning, MRSA were restricted to hospitals and human healthcare settings and therefore denominated as healthcare-associated (HA) MRSA. HA-MRSA became one of the most important nosocomial pathogens in human medicine with several successful lineages that spread within hospitals, countries or even internationally [53]. It was not before the late 1990s that MRSA were also regularly described in the community. At that time, these community-associated (CA) MRSA were distinguished from HA-MRSA by their genetic profile [54]. At present, various different definitions are available to distinguish between HA- and CA-MRSA. In 2000, the U.S. Centers for Disease Control and Prevention (CDC) published a definition based on defined risk factors for the development of HA-MRSA. Thus, CA-MRSA included all MRSA infections from patients without exposure to risk factors for HA-MRSA infections [55]. Other attempts to differentiate between HA- and CA-MRSA were based on a genotypic differentiation like the SCC*mec* type (CA-MRSA harbor smaller SCC*mec* types IV or V), a lower number of antibiotic resistances beside beta-lactams (described for CA-MRSA) or the occurrence of the virulence factor Panton-Valentine leukocidin (PVL) (frequent carriage in CA-MRSA) [52]. However, these lines are increasingly blurred and differentiation between classical HA- and CA-MRSA is becoming more and more difficult.

Reports about MRSA infections in animals increased along with the description of CA-MRSA in humans. Formerly, MRSA infections in animals were only rarely described and accidental transmission from humans to animals was considered as reason for these sporadic events [21] [22]. The increasing detection rate of MRSA-mediated infections in animals since the late 90s raised the awareness for this pathogen in veterinary medicine. Epidemiological inquiries where conducted to acquire information about the relevance of MRSA as a pathogen and furthermore as colonizer in animals. First results showed the need for distinction between different animal hosts. While some animals like poultry and ruminants seemed to be frequently colonized and / or infected with specific MRSA-lineages [30,56-61], MRSA infections from small animals were shown to be predominantly caused by well-known human lineages with respect to the investigated geographic region [24,62,63]. Nevertheless, research focused predominantly on MRSA in livestock. Lineages that were firstly identified in livestock and absent or rarely present in human medicine were described as genotypes with restriction to certain hosts from livestock and therefore denominated as livestock-associated

(LA) MRSA. The best studied LA-MRSA belong to clonal complex (CC)398 and have been first identified in swine and humans in close contact to these animals [64] (a definition for clonal complex is given in chapter 2.7.1). Further screening studies identified swine as a reservoir for CC398-MRSA and therefore revealed for the first time animals as an important infection source for humans [65]. Besides, CC398-MRSA could be isolated from bovine, equine and poultry samples [66-69] as well as from small animals like dogs [70].

Even though it is known that MRSA can colonize and infect small animals and horses, there are not much data available about the impact of this opportunistic pathogen in these animals. Colonization studies showed rates of 0-4% in small animals as well as 0-9% in horses [33]. In general, companion animals seem to be transiently colonized and show the ability to get decolonized when not exposed to a re-colonization source [23]. As described in section 2.2, existing prevalence data for MRSA infections in companion animals like dogs, cats and horses give evidence for increasing rates, particularly in case of wound infections. While predominant lineages from dogs and cats mirror HA-MRSA observed in human medicine within the investigated geographic region (like CC22-MRSA in Germany [63]), horses seemed to be predominantly infected with CC8-MRSA in the past [33]. Only recently, CC398-MRSA were reported regularly as colonizer and pathogen in horses as well [67,68].

The categorization of MRSA as HA-, CA- and LA-MRSA in the literature displays the understanding of niche (HA-MRSA) respectively host adaptation (LA-MRSA) of this pathogen in the past. While categorization of identified lineages to one of these three groups was easily performed only a short time after introduction of the terms ongoing characterization of isolates from different hosts and ecological niches provided evidence for the capacity of MRSA to infect several hosts in various habitats. Today it is accepted, that transmission can occur between different hosts, including companion animals and humans, but it is still unknown how often these events take place. However, a risk factor analysis conducted by Loeffler et al. determined the keeping of MRSA-infected companion animals as a risk factor for MRSA-colonization in humans [62], indicating transmission between animals and their owners as a regular occurring event. In summary, the lack of knowledge about the frequency of MRSA infections and epidemiological changes in companion animals bears risks for both animals and humans.

2.6 Aspects of host specificity in *S. aureus*

Although host specific lineages as well as genotypes with a broad host spectrum were first discussed by Devriese et al. in 1984 [71,72], it was not before the 2000s that research

focused on this area due to increasing reports of infections caused by MRSA in animals. Comparisons of strains from animal and human origin based on genotyping methods identified cases where an association between genetic lineages and host species could be drawn. In particular *S. aureus* causing infections in ruminants and poultry seem to be adapted to certain host species [30,56-61].

As a pathogen of bovine mastitis, epidemiological background information was needed to gain more knowledge about infection- and possible transmission routes between cattle and humans. Genotypic characterization of strains from cases of bovine mastitis in comparison with isolates obtained from human infections showed different genetic patterns according to their genetic profiles by use of different genotyping methods, including pulsed field gel electrophoresis (PFGE) and multilocus enzyme electrophoresis (MLEE) [73-75]. Further, multilocus sequence typing (MLST) identified clonal complexes (CCs) with an association to ruminant infections, whereby differentiations between bovine [56-61] and small ruminant clusters were possible as well [61]. Phenotypic and genetic characteristics which may be involved in host tropism of *S. aureus* strains in general are still poorly understood, but first evidence exists that variation in the core genome as well as in the variable genome plays an important role in the process of host adaptation. The raveling possibility to compare whole genomes from strains of different host origin using next generation sequencing (NGS) techniques enables researchers to gain insights into the genomic diversity.

Comparative whole genome analysis including one bovine mastitis ST151-*S. aureus* (ET3-1) and several human *S. aureus* genomes, revealed differences like a high allelic variation among virulence- and surface-associated genes involved in host colonization, toxin production, iron metabolism, antibiotic resistance, and gene regulation. Further, a number of well-known *S. aureus* virulence factors such as protein A and clumping factor A occur as pseudogenes in ET3-1. Thus, the functional loss of certain genes associated with the core genome due to gene decay may serve as one step promoting the adaptation process to another host. However, in total the core and core variable genome of isolate ET3-1 closely resembles that of the fully sequenced *S. aureus* isolates from humans with respect to gene content and organization (maximum common genome; MCG) [46]. In contrast to a relatively stable core genome, the comparison identified a high variation with respect to mobile genetic elements (MGEs). *S. aureus* genomes of ruminant origin comprised different MGEs like *S. aureus* pathogenicity islands (SaPI), phages and plasmids. In summary, this study was able to provide evidence that bovine *S. aureus* strains (ST151) had emerged from human evolutionary precursors due to a combination of acquisition of MGEs and gene decay [76].

Results of another survey with the focus on CC133-*S. aureus*, a lineage that seems to be predominantly associated with isolates obtained from small ruminants, were in accordance with the previously mentioned study and were additionally able to link the genetic findings to phenotypic differences. Thus, it was shown that most CC133-MRSA harbored newly identified MGEs like phages as well as staphylococcal pathogenicity islands (SaPI) and that strains containing $SaPI_{ov}$ (SaPI from an ovine isolate) have the ability to coagulate ruminant plasma in contrast to $SaPI_{ov}$-deletion mutants' ore strains naturally lacking the pathogenicity island [61]. Another example of host adaptation has been described for sequence type (ST)5-*S. aureus* in poultry. In this survey, Lowder et al. were able to identify the source of host switching as a single host jump from humans to poultry. Further acquisition of MGEs from avian specific lineages and the inactivation of several proteins with importance in human infections led to the occurrence of a poultry-specific sub-lineage. The described modifications of the ST5-*S. aureus* resulted in enhanced virulence towards poultry [30].

As a typical colonizer of the human nose and a usual pathogen in human medicine, it is of special interest to identify putative human specific elements in *S. aureus*. One important example is given by the immune evasion cluster (IEC), which comprises various combinations of the following proteins and corresponding genes: the staphylococcal complement inhibitor (*scn*), the plasminogen activator staphylokinase (*sak*), the chemotaxis inhibitory protein (*chp*), and the superantigen staphylococcal enterotoxin A (*sea*). The IEC can be detected in the majority of human strains [77,78], while *S. aureus* isolated from animals (especially isolates of livestock origin) seem to lack this cluster [36,77,79]. Despite this description, the mechanisms for the IEC that could contribute to host specific colonization and / or infection are still poorly understood. For CC97-*S. aureus*, a ruminant specific lineage, Spoor et al. were able to document independent host jumps of isolates from this lineage from livestock to humans. Both of these host jumps were accompanied by the integration of the IEC-encoding phage [80]. An additional example of a host jump from humans to animals is given by Price and colleagues, who investigated 89 CC398-*S. aureus* from various host species originating from 19 different countries on four different continents. Results of this broad comparative whole genome analysis support the hypothesis that CC398-MRSA originated as CC398-MSSA in humans. The host jump from humans to animals was accompanied by the acquisition of methicillin- and tetracycline resistance and the loss of phage-encoded virulence genes like for example the IEC [81]. Nowadays, CC398-MRSA are an infection source for various mammalians and a comparative survey conducted by Ballhausen and colleagues revealed a huge heterogeneity regarding virulence characteristics, thus defining several subpopulations within this specific lineage [82].

Both examples, the transmission of CC97-*S. aureus* from cattle to humans as well as the host jump from humans to livestock for CC398-*S.°aureus* illustrate the zoonotic potential of this opportunistic pathogen. In addition, both examples highlight the need to expand investigations on MSSA to obtain a complete picture about epidemiological pathways used by *S. aureus*-lineages and possible genomic and functional alterations that might be involved in these adaptation processes.

However, the majority of known *S. aureus* genotypes seem to lack certain host specific factors [3-6,63,69,83,84]. These lineages with the ability to infect a broad spectrum of hosts have been denominated as extended host spectrum genotypes (EHSG) [69]. Especially MRSA-lineages that have been identified from samples of small animal infections seem to mirror common human genotypes [63,83,84]. Several reports document the occurrence of similar EHSG-MRSA in pets and their owners in the same household, suggesting cross-species transmission either from humans to their pets or vice versa [3-6]. Nevertheless, information is scarce about the frequency and impact of these transmission events as well as the relevance of methicillin-susceptible *S. aureus* (MSSA).

2.7 Molecular typing tools

Various different molecular typing methods have been developed in order to gain information about the genetic background of *S. aureus*. The common objective of all these methods is to create a basis for comparative analysis of different strains allowing drawing conclusions regarding epidemiological pathways and the genetic relationship. Especially the rapid spread of MRSA showed the need to develop appropriate methods to investigate possible transmission routes, for example within hospitals. Each of these typing methods combines advantages and disadvantages. However, it is of special importance that adequate molecular typing methods provide a high discriminatory power, but are nonetheless able to distinguish between distinct lineages based on the identified genetic pattern.

For a sound understanding and evaluation of genotyping methods it is important to understand the genome structure and organization of *S. aureus* in general. So far (August 2014), 4,176 *S. aureus* genome assemblies are available with a constantly increasing number of published genomes. The genomes are variable in size ranging between 2.73 and 3.08 Megabases (Mb) and encode between 2595 and 2998 genes (http://www.ncbi.nlm.nih.gov/genome/?term=staphylococcus+aureus).

The genome is organized on three levels: the core genome, the core variable genome and mobile genetic elements [85]. The core genome is present in all *S. aureus* and is highly conserved. Most baseline and essential molecular mechanism are encoded on genes within this group. The core variable genome consists mostly of virulence-associated genes (including adhesins) and enables a differentiation of genetic lineages based on core variable gene profiles. As a third level, mobile genetic elements promote diversification within clonal complexes and thus define sub-lineages within a distinct clonal complex. Interestingly, there is a limited transferability of MGEs within different lineages [85]. The restriction modification system encoded by the genes *hsd*M (modification) /S (specificity) /R (restriction) is a key defense mechanism protecting the DNA of individual cells from the uptake of foreign DNA. *HsdS* gene variants of each lineage promote this restriction by modification and digestion of DNA at different sites. Thus, successful MGE-transfer takes place more frequently in isolates belonging to the same genetic lineage.

2.7.1 Multilocus sequence typing (MLST)

Multilocus sequence typing (MLST) has long been regarded as gold standard with respect to genotypic characterization of various bacteria. This method was first established for *Neisseria meningitidis* in 1999 [86]. Until now, MLST databases are available for more than 27 different microorganisms (for example: www.mlst.net). The principle is based on the sequence analysis of highly conserved regions (450-500 base pairs (bp)) from (mostly: seven) well-defined housekeeping genes. Each variant from these investigated loci defines a unique allele. The use of an internet-based platform (www.mlst.net) allows the identification of the specific allele number for each sequenced allele. All seven allele numbers form a specific allelic profile defining a distinct sequence type (ST). For *S. aureus*, the MLST scheme is based on the investigation of alleles from the following housekeeping genes: *arc*C, *aro*E, *glp*F, *gmk*, *pta*, *tpi* and *yqi*L (see figure 1) [87]. Currently, the database for *S. aureus* on www.mlst.net comprises data on 4704 isolates, including 2972 sequence types (08/2014). MLST enables genotypic characterization based on sequence typing of alleles from highly conserved housekeeping genes. Based on slowly accumulating gene modifications, it provides information about evolutionary changes and global spread of *S. aureus* (including MRSA) [52]. The method provides unambiguous results that are easy to compare between different laboratories. In conjunction with the establishment of MLST, specific terms were defined for better comparability of results. In this context, a clone was defined based on the sequence type so that in terms of MLST clones are defined as *S. aureus* sharing the same ST. This definition of MLST clones is very useful to investigate the evolutionary history of

bacteria but it is also misleading with respect to the term “clone” since clones have been defined as isolates of bacteria with indistinguishable genotypes in the past [88].

However, for bacteria it is very difficult to define clones based on the second definition since high mutation and recombination rates as well as the uptake or loss of mobile genetic elements can lead (depending on the species) to a highly variable bacterial genome. The permanent diversification of the ancestral genome results in a cluster of increasingly diverse genotypes. Nonetheless, the MLST typing scheme provides an efficient method to identify genetic lineages sharing the same common ancestor by investigation of loci from seven highly conserved housekeeping genes. For better comparability with the literature, the term clone within this work will be used referring solely to the ST scheme [86]. Based on this definition, (MLST) clones include isolates with the same sequence type but are not necessarily genetically identical. Further, isolates with changes in one or two alleles are grouped into one complex [87]. This complex is defined as clonal complex (CC). Each CC harbors isolates from one specific lineage which have been very likely evolved from a common progenitor lineage [51]. To categorize strains with different STs into clonal complexes provides a good tool to reconstruct putative evolutionary pathways and to gain information about the global spread. The term “clonal complex” was successfully established for *S. aureus* and several other bacteria worldwide so that it will be used accordingly in this thesis as well, based on the provided definition.

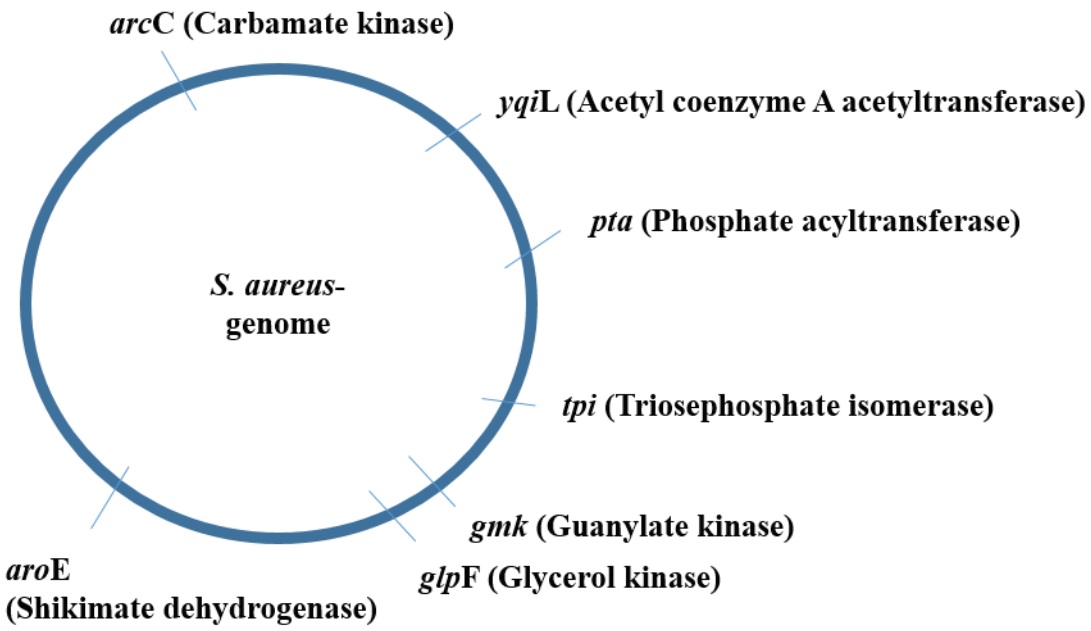


Figure 1: Localization of the housekeeping genes used for MLST of *S. aureus*. Figure modified from [89].

2.7.2 *Spa* typing

Spa typing is a useful typing method for characterization of *S. aureus* based on sequence analysis of a single gene locus from the Protein A-encoding gene (*spa*). The locus includes a highly variable tandem repeat region ("X region") that consists of multiple repeats with each repeat showing a length of 24bp (with exceptions of 21 to 30bp) [90]. Variability of this region is based on point mutations, deletions, insertions or duplications. Amplification of this repeat region [91] combined with sequence analysis by use of the database provided by www.ridom.de allows a differentiation of distinct *spa* types [92]. For most *spa* types that consist of more than three similar repeats there is a good correlation between grouping based on *spa* typing as well as based on MLST. Compared to MLST, this method provides a less expensive option to gain information about the genetic background of *S. aureus* isolates. However, since the genetic change of a *spa* type is not necessarily linked to a change of the housekeeping genes from MLST but evolves independently *spa* typing does not provide information about the sequence type (ST) of the investigated *S. aureus* [93].

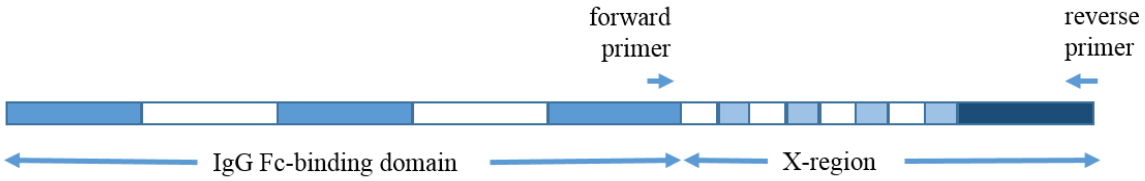


Figure 2: Structure of the X region from the *spa* gene, modified from [90].

2.7.3 Pulsed field gel electrophoresis

Pulsed field gel electrophoresis (PFGE) enables the comparison of DNA fragment pattern after digestion of DNA with a restriction enzyme. S*ma*I is mainly used as restriction enzyme for comparative analysis of *S. aureus* isolates. Due to methylation of the *Sma*I-restriction site in CC398-*S. aureus*, *Apa*I is the restriction enzyme of choice for PFGE analysis of this genetic lineage [94]. PFGE is frequently utilized for individual investigations of local outbreaks and can be used to identify nosocomial transmission routes, if epidemiological data are available. In comparison to the results of MLST, this technique allows to detect more frequently occurring genomic changes [52]. Thus, by use of PFGE it is possible to display the diversity of *S. aureus* that share the same sequence type and therefore to proof that clones based on the common definition according to the MLST scheme are not necessarily

genetically identical. In the past, this method was specifically used to identify globally important MRSA-lineages [95,96]. The main disadvantage of PFGE is the poor comparability of the results between different laboratories. To minimize this problem, several laboratories collaborated and developed a standardized protocol [45]. Despite these attempts for harmonization, comparison of PFGE pattern remains very difficult, at least between different laboratories.

2.7.4 DNA microarray hybridization

DNA microarray hybridization is a useful tool that gives access to information about the presence of various predefined target genes. The DNA microarray chip from Alere (Identibac *S. aureus* genotyping ®) for example provides information about 334 different gene loci including among others the capsule-, *agr*- and SCC*mec* type as well as important genes for adhesion, virulence and metabolic pathways. For this purpose, oligonucleotides of the target genes are spotted on a microarray chip. The DNA from the isolate of interest undergoes linear PCR (polymerase chain reaction), using 128 different forward primers simultaneously. The subsequent hybridization of the PCR product on the microarray chip gives information about the presence of the mapped gene loci. Furthermore, the specific pattern of each isolate provides additional information about the affiliation to known specific lineages. This is possible due to different lineage specific pattern. Hence, DNA microarray hybridization displays another method (besides MLST and *spa* typing) to gain knowledge about the specific lineage of the investigated strain [97].

2.7.5 Whole genome sequencing

Sequenced genomes provide information about sequence types, *spa* types and the presence of gene loci of interest and therefore combine information from MLST, *spa* typing and DNA microarray hybridization. Further, whole genome sequencing (WGS) by use of the next generation sequencing (NGS) technology gives access to multiple useful tools, including investigation of single nucleotide polymorphisms (SNPs) for strains belonging to a certain lineage or to analyze the diversity of mobile genetic elements. In addition, it enables the detection of so far unknown structures within genomes. WGS data reveal new insights into the population structure of *S. aureus* [98] and offer the possibility to gain more knowledge about the evolution of bacteria and their possible transmission routes [99].

2.7.6 Comparison of molecular typing tools for genotypic characterization of *S. aureus*

As a highly structured organism, *S. aureus* populations possess a stable core genome showing only moderate variations about 1.5% [100]. Hence, MLST is very suitable for identification and characterization of lineages by investigation of point mutations in loci of seven highly conserved housekeeping genes. By use of this method it is possible to investigate the (global) spread and distribution of important genotypes. The genotypic characterization based on the investigation of one locus of the highly variable tandem repeat region of the *spa* gene proved to be another successful tool for genotypic characterization of *S. aureus*. Beside the investigation of SNPs *spa* typing takes also recombination events of the X region into account. Since recombination occurs frequently within this region this typing method provides more (*spa*) types than MLST by investigation of seven highly conserved loci. Although *spa* typing and MLST are highly concordant and allow a similar classification to distinct genotypes, there are also several cases of misclassifications described by use of *spa* typing which are very likely caused by recombination events [101]. Thus, *spa* typing is a useful method to gain information about the genotype of investigated isolates but is not sufficient to replace MLST. By providing information about the core and core variable genome DNA microarray analysis provides various information about the genetic background of each investigated isolate and therefore is a useful method for genotypic characterization. However, it needs to be considered that DNA microarray provides exclusively information about known gene structures.

Continually falling prices for WGS lead to increasing whole genome comparisons and show the advantages of various possible tools like for example SNP analysis and the screening for mobile genetic elements. However, various studies in this field confirm the robustness of MLST as a basic genotyping tool for the categorization of lineages [81,102,103]. While WGS allows drawing a genomic portrait of each isolate and to perform a detailed comparative analysis, MLST should be regarded as a fundamental classification for genetic lineages. Whether and to what extend *spa* typing will be used in future to characterize *S. aureus* is not clear since current studies based on whole genome data allow a better classification of sub-lineages based on methods with a higher resolution than *spa* typing. However, since *spa* typing is a well-established classification method since more than ten years, current studies based on whole genome data still include information about the *spa* types [81,102,104,105].

PFGE analysis was considered as gold standard for investigation of outbreaks or transmission in the past since macrorestriction pattern provide more information about the genotype than MLST and *spa* typing [3,4,106,107]. Holmes and colleagues were able to show that SNP analysis based on whole genome data has the same discriminatory power in comparison to the current reference standard PFGE [103]. Furthermore, there are already studies available investigating nosocomial outbreaks and transmission routes by use of WGS [104,108]. In contrast to PFGE, results from WGS can be easily compared between different laboratories and furthermore WGS provides the opportunity to gain additional genetic information for each isolate. Therefore, the current development leads to the suggestion, that WGS will replace PFGE as gold standard for investigation of nosocomial outbreaks as well as transmission events.

3. Publications

3.1 Publication 1

Molecular analysis of human and canine *Staphylococcus aureus* strains reveals distinct extended-host-spectrum genotypes (EHSG) independent of their methicillin resistance

Published in the journal „Applied and environmental microbiology“ January 2013; Volume 79(2); pages 655-662.

Doi: 10.1128/AEM.02704-12.

Received: 06.09.2012

Accepted: 07.11.2012

Published ahead of print: 16.11.2012

3.2 Publication 2

Alarming Proportions of Methicillin-Resistant *Staphylococcus aureus* (MRSA) in Wound Samples from Companion Animals, Germany 2010–2012

Published in the journal „PLOS ONE" January 2014; online journal.

Doi: 10.1371/journal.pone.0085656

Received: 22.08.2013

Accepted: 29.11.2013

Published: 20.01.2014

OPEN ACCESS Freely available online

PLOS ONE

Alarming Proportions of Methicillin-Resistant *Staphylococcus aureus* (MRSA) in Wound Samples from Companion Animals, Germany 2010–2012

Szilvia Vincze[1]*, Ivonne Stamm[2], Peter A. Kopp[2], Julia Hermes[3], Cornelia Adlhoch[3], Torsten Semmler[1], Lothar H. Wieler[1], Antina Lübke-Becker[1], Birgit Walther[1]

1 Institute of Microbiology and Epizootics, Veterinary Faculty, Free University Berlin, Germany, **2** Vet Med Labor GmbH, Division of IDEXX Laboratories, Ludwigsburg, Germany, **3** Department of Infectious Diseases Epidemiology, Robert Koch Institute, Berlin, Germany

Abstract

Staphylococcus (*S.*) *aureus* is an important cause of wound infections in companion animals, and infections with methicillin-resistant *S. aureus* (MRSA) are of particular concern due to limited treatment options and their zoonotic potential. However, comparable epidemiological data on MRSA infections in dogs, cats and horses is scarce, also limiting the knowledge about possible links to MRSA isolates from human populations. To gain more knowledge about the occurrence and genotypic variation of MRSA among wound swabs of companion animal origin in Germany we performed a survey (2010–2012) including 5,229 samples from 1,170 veterinary practices. *S. aureus* was identified in 201 (5.8%) canine, 140 (12.2%) feline and 138 (22.8%) equine swabs from a total of 3,479 canine, 1,146 feline and 604 equine wounds, respectively. High MRSA rates were identified with 62.7%, 46.4% and 41.3% in *S. aureus* of canine, feline and equine origin, respectively. Further genotyping including *spa* typing and multilocus sequence typing (MLST) revealed a comparable distribution of *spa* types among canine and feline MRSA 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%). Our data highlight the importance of *S. aureus* and MRSA as a cause of wound infections, particularly in cats and horses in Germany. While "human-associated" MRSA lineages were most common in dogs and cats, a remarkable number of CC398-MRSA was detected in horses, indicating a replacement of CC8-MRSA as the predominant lineage within horses in Germany. These data enforce further longitudinal epidemiological approaches to examine the diversity and temporal relatedness of MRSA populations in humans and animals to assess probable sources of MRSA infections. This would enable a sound risk assessment and establishment of intervention strategies to limit the additional spread of MRSA.

Citation: Vincze S, Stamm I, Kopp PA, Hermes J, Adlhoch C, et al. (2014) Alarming Proportions of Methicillin-Resistant *Staphylococcus aureus* (MRSA) in Wound Samples from Companion Animals, Germany 2010–2012. PLoS ONE 9(1): e85656. doi:10.1371/journal.pone.0085656

Editor: Franklin D. Lowy, Columbia University, College of Physicians and Surgeons, United States of America

Received August 22, 2013; **Accepted** November 29, 2013; **Published** January 20, 2014

Funding: This work was supported by the Federal Ministry of Education and Research (BMBF) and is part of the MedVet-Staph project (01KI1014A). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: Ivonne Stamm and Peter A. Kopp are employees of IDEXX Vet Med Labor GmbH (Ludwigsburg). The authors' collaboration partners from VetMed Labor GmbH Ludwigsburg provided the investigated samples for this study. The authors confirm that this does not alter their adherence to all the PLOS ONE policies on sharing data and materials.

* E-mail: szilvia.vincze@fu-berlin.de

Introduction

Staphylococcus (S.) aureus is an important pathogen in human and veterinary medicine that is capable of causing purulent and toxin-mediated infections. An uptake of the *mec*A gene, which is encoded on the Staphylococcal cassette chromosome *mec*, enables the expression of an additional penicillin-binding protein (PBP)2a which substitutes an essential cross-linking step of the native PBP2 in the presence of ß-lactams, leading to resistance against antibiotics of this group [1]. In addition, methicillin-resistant *S. aureus* (MRSA) are often resistant against further antimicrobial classes [2].

MRSA raised attention in companion animal medicine during the last decades, especially with regard to transmission from humans to dogs and cats and vice versa [3–5]. Further, MRSA are known as important nosocomial pathogens in both human and veterinary medicine [6].

Data concerning the proportion and impact of *S. aureus* and MRSA infections in clinical samples of companion animals are variable but give evidence of an increased infection rate in horses [7] as well as dogs and cats [8] during the last years. Wound infections in particular are described to be frequently caused by *S. aureus* [9,10].

Genotypic characterization of MRSA from several infection sites of dogs and cats revealed the predominant occurrence of well-known regional hospital associated (HA-) MRSA [5,11–13]. Within Germany, clonal complex (CC) 22-MRSA was reported as the most common lineage in small animals in 2003 and 2004 [9,13]. Only recently, CC398-MRSA was reported as a cause of canine infections in Germany, as well [14,15].

In contrast, CC8-MRSA were mainly identified in equine infections, leading to suggestions of adaptation of this lineage to horses [16]. In the recent past, studies revealed the occurrence of LA-MRSA CC398 in horses, as well [7,16–18].

Suspected transmission events between humans and small animals or horses were reported several times in the past [3–5,19] and strains of these lineages with the ability to infect different hosts were denominated as Extended Host Spectrum Genotypes (EHSGs) [17].

The predominance of infections with regularly occurring regional human lineages in companion animals raised the question whether dogs and cats might be an important source of human infection and colonization [20]. Therefore, continuous *S. aureus*-screening in specimens of companion animal origin with further molecular characterization is important to determine changes in frequency or possible adaptation of specific lineages to different hosts.

Hence, we conducted a Germany-wide survey to investigate the frequency of *S. aureus* among samples from clinical wound infections of dog, cat and horse origin. The MRSA-proportion was identified for *S. aureus*-positive samples. Each phenotypically methicillin-resistant *S. aureus* was verified by PCR detection of either the *mec*A or *mec*C gene and characterised by *spa* typing as well as screening for the Panton-Valentine leukocidin (PVL).

Materials and Methods

Sampling and Case Definition

We included all swabs from dogs, cats and horses suffering from wound infections, which were sent from 1,170 veterinarians within Germany to Vet Med Labor GmbH (Ludwigsburg, Germany) for bacteriological analysis between November 2010 and March 2012. Vet Med Labor GmbH is one of the largest veterinary microbiological laboratories, covering most areas within Germany. The samples originated from either veterinarian clinicians or practitioners working at mixed or specialized practices in 1,018 different areas with regard to the postal code throughout Germany. TargetMap (www.targetmap.com) was used to visualize the geographic origin of wound samples (figure 1). *S. aureus* positive wound swabs were phenotypically investigated towards methicillin-resistance based on susceptibility testing for oxacillin and cefoxitin (VITEK®2, bioMérieux, Germany). All MRSA were sent to the Institute of Microbiology and Epizootics, FU Berlin for genotypic characterization.

Screening Procedure and Bacterial Characterization

All wound samples were streaked onto tryptic soy agar plates supplemented with 5% sheep blood (Becton Dickinson, Heidelberg, Germany) and the plates were investigated after 24 and 48 hours. Colonies suspected to be staphylococci were identified using the bioMérieux VITEK®2 system (Germany) according to the manufacturers instructions. Automated antimicrobial susceptibility testing of *S. aureus* was performed using the bioMerieux VITEK®2 system (Germany) according to the manufacturer's instructions, including penicillin, ampicillin-sulbactam, oxacillin, cefoxitin, gentamicin, kanamycin, enrofloxacin, marbofloxacin, erythromycin, clindamycin, tetracycline, nitrofurantoin, chloramphenicol and trimethoprim- sulfamethoxazole, following the approved standards of the Clinical and Laboratory Standards Institute (CLSI) [21,22].

MRSA isolates were stored in glycerol stocks at −80°C at the Institute of Microbiology and Epizootics (IMT). Species verification and detection of the *mec*A or *mec*C gene by PCR was performed for all MRSA as described before [23,24]. Further, 121 MRSA were characterized by *spa* typing [25]. Associations of *spa* types with corresponding clonal complexes (CC) were determined according to Ridom (http://spaserver.ridom.de/).

Multilocus sequence typing (MLST) was performed for one to seven representatives of each detected *spa* type as described elsewhere [26].

Detection of the genes encoding the Panton-Valentine leukocidin (PVL)-factor was carried out as described before [27].

Results

Within a 17-month sampling period, veterinarians from 15 German Federal States sent 3,479 swabs declared as "wound swabs" from dogs, 1,146 samples from cats and 604 samples from horses to Vet Med Labor GmbH (Ludwigsburg, Germany) (table 1). *S. aureus* was identified in 5.8% of canine, 12.2% of feline and 22.8% of equine samples, respectively. MRSA accounted for 62.7% of canine, 46.4% of feline and 41.3% of equine *S. aureus* isolates. In total, 121 MRSA strains obtained from dogs, 63 from cats and all isolates from horses were further characterized by *spa* typing while five of the canine and two feline MRSA were not available for the typing procedure. A detailed

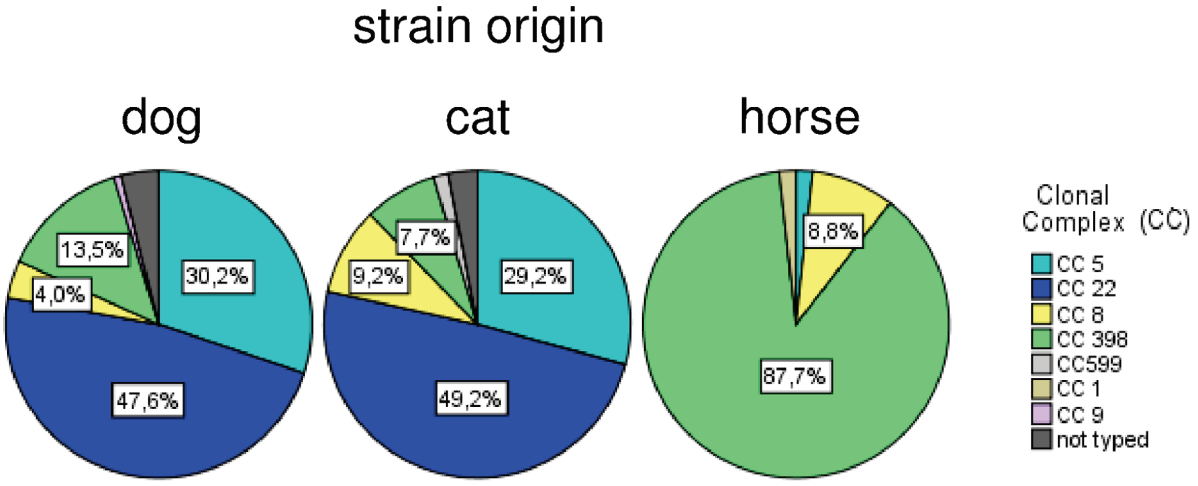


Figure 1. Overview of lineage-diversity among MRSA from dogs, cats and horses.
doi:10.1371/journal.pone.0085656.g002

Table 1. *S. aureus* proportion in wound samples from companion animals.

	total	%	dog	%	cat	%	horse	%
wound swabs	5,229	100	3,479	66.5	1,146	21.9	604	11.6
S. aureus	479	100	201	5.8	140	12.2	138	22.8
MSSA	231	48.2	75	2.2	75	6.5	81	13.4
MRSA	248	51.8	126	3.6	65	5.7	57	9.4

doi:10.1371/journal.pone.0085656.t001

overview of all detected *spa* types and corresponding CCs is given in table 2. Most of the canine isolates belonged to clonal complexes CC22 (47.6%; n = 60) and CC5 (30.2%; n = 38), followed by CC398 (13.5%; n = 17), CC8 (4.0%; n = 5) and CC9 (0.8%; n = 1). Comparable results were obtained for feline strains with CC22 (49.2%; n = 32), CC5 (29.2%; n = 19), CC398 (7.7%; n = 5) and CC8 (9.2%; n = 6). In addition, a CC599-MRSA was identified in one feline sample. The majority of MRSA isolates of equine origin possessed *spa* types associated with CC398 (87.7%; n = 50). The remaining equine isolates belonged to CC8 (8.8%; n = 5) as well as CC5 and CC1 (each: one isolate) (figure 2).

MLST analysis, performed for at least one representative isolate of each distinct *spa* type, revealed common sequence types (ST) for isolates of CC1 (ST1), CC5 (ST5, ST225), CC8 (ST8, ST254), CC9 (ST9) and CC398 (ST398). Representatives of CC22 showed sequence types ST22, ST1117 as well as two new sequence types (ST2743 and ST2745). The feline CC599-MRSA belonged to ST599. All MRSA were *mec*A-positive except for the ST599-MRSA, which yielded a positive signal for *mec*C [28]. Screening

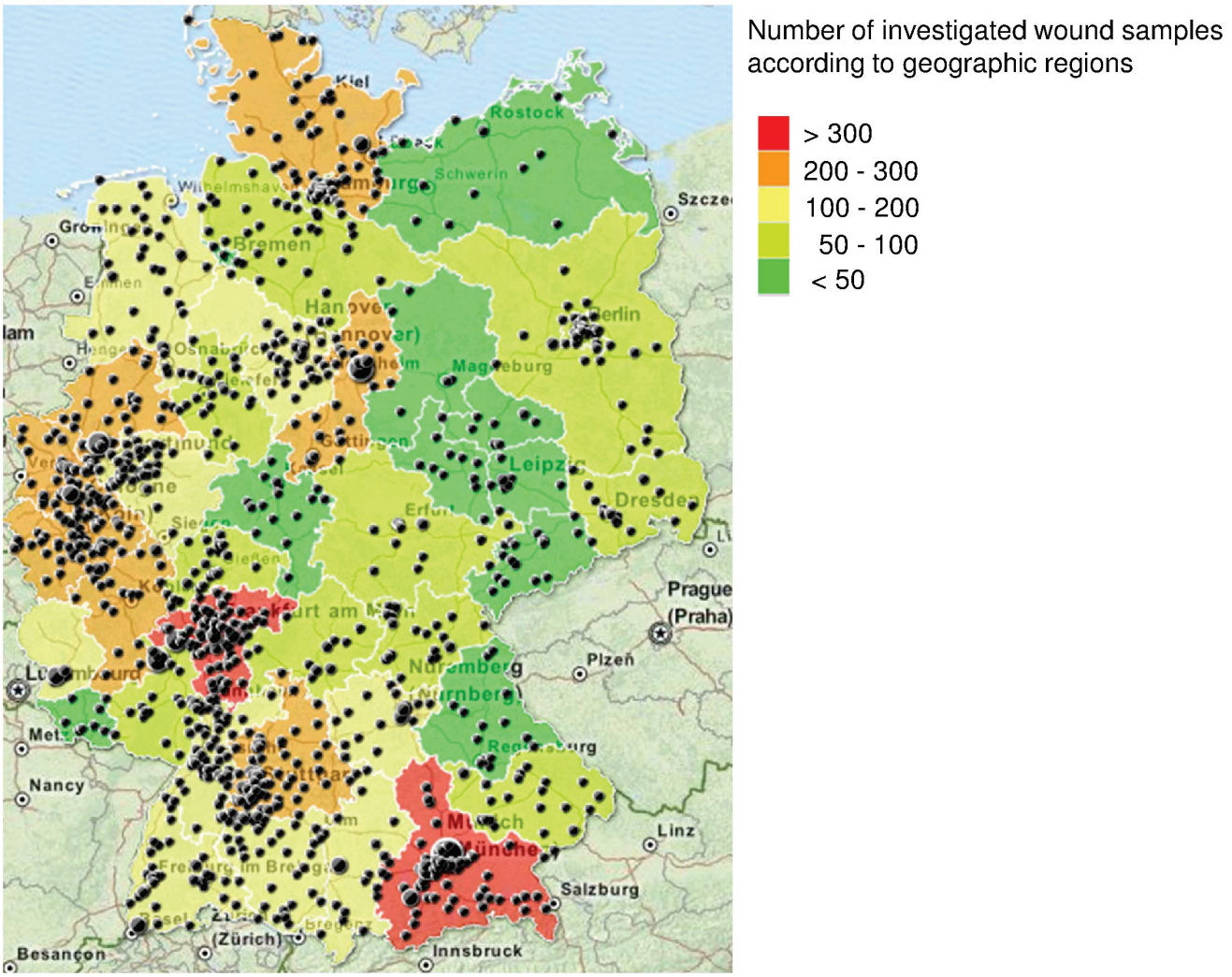


Figure 2. Sample origin. Figure 1 shows the Germany-wide origin of the 5,229 wound swabs from dogs, cats and horses. Areas are shaped in color with regard to the sample frequency. Black dots represent the sample origin with regard to the postal code. The dot size displays the submission frequency of each veterinary practice/clinic.
doi:10.1371/journal.pone.0085656.g001

Table 2. *Spa* type frequency and predicted clonal complexes based on *spa* typing and MLST for representative MRSA from wound infections of canine, feline and equine origin in Germany.

		MRSA		
CC	***spa* type**	**dog (n = 121)**	**cat (n = 62)**	**horse (57)**
1	t127	0	0	1
5	t002	1	1	0
	t003	31	17	1
	t045	4	1	0
	t1007	1	0	0
	t264	1	0	0
8	t008	2	6	0
	t009	1	0	4
	t024	1	0	0
	t12131	0	0	1
	t036	1	0	0
9	t1430	1	0	0
22	t016	0	1	0
	t020	0	4	0
	t022	11	6	0
	t032	43	17	0
	t3846	0	1	0
	t294	1	0	0
	t557	1	0	0
	t613	0	1	0
	t1292	1	0	0
	t747	2	0	0
	t7982	0	2	0
	t910	1	0	0
599	t278	0	1	0
398	t011	10	5	24
	t034	2	0	0
	t108	1	0	0
	t6867	4	0	24
	t10643	0	0	2

doi:10.1371/journal.pone.0085656.t002

for PVL revealed one positive canine CC8-MRSA (t008) as well as one positive feline CC22-MRSA (t016).

Antimicrobial susceptibility testing was performed for all MRSA. A detailed overview of all phenotypic resistance profiles is given in table 3.

Discussion

This survey describes for the first time the Germany-wide occurrence of MRSA and methicillin-susceptible *S. aureus* (MSSA) in wound samples of companion animal origin. The considerable high proportion of *S. aureus*-positive wound swabs from horses, cats and dogs clearly shows the prominent clinical importance of this pathogen in companion animal medicine. In view of MRSA being a zoonotic pathogen, proportions of 9.4% (horses), 5.7% (cats) and 3.6% (dogs) among wound swabs are alarming and provide a significant source for the spread in both veterinary clinics and the community.

The genotypic characterization of MRSA in this study provides comprehensive insights into the clonal background of MRSA in wound infection samples of companion animal origin (figure 2) and confirms the previous finding of CC22 and CC5 as predominant lineages in dogs and cats [14,20] for Germany. The predominance of these lineages in dogs and cats is in accordance with findings in human medicine within Germany, where most wound infections are caused by CC22- and CC5-MRSA as well [29]. These findings lead to the suggestion that humans might be a likely infection source for dogs and cats. In addition, dogs and cats should be considered as (re-)infection and/or colonization source for humans as well.

However, a recent study by Holden et al. demonstrated the genetic changes that accompanied the emergence of EMRSA-15, a typical hospital associated CC22-MRSA. A series of gene acquisition events and mutations seemed to promote the success of this clone in (human) hospitals. Accordingly, further comparative genomic studies on MRSA obtained from veterinary and human health care settings are warranted to evaluate factors influencing success in veterinary environments (e.g. prescription of certain antibiotics, acquisition of phages encoding virulence-enhancing factors) [30].

Reports about LA-MRSA in pet animals [15,31,32] raised the question whether these results represent incidental findings from screening studies in companion animals [33]. As shown in this study, the frequent finding of LA-MRSA CC398 among canine and feline MRSA isolates demonstrates that CC398-MRSA is an important lineage for canine and feline infections in Germany at present. Further investigation is needed to determine whether this genotype has the capacity to replace other common MRSA-lineages in dogs and cats. However, the characterization of different CC398 clades among humans in comparison to livestock already demonstrated the adaptability of this genetic lineage [34]. Based on this knowledge and the regular appearance of CC398-MRSA in pet animals, dogs and cats should be considered as a potential source for human CC398-MRSA colonization and infection, especially if contact to livestock or horses can be excluded.

The CC398 proportion among equine MRSA identified in this study is in concordance with previous reports of high CC398-MRSA infection- and colonization rates in horses [7,18] and gives evidence that MRSA of CC8 are almost completely replaced by CC398 in equine wound infections within Germany. The predominance of CC398-MRSA in wound swabs of horse origin leads to the assumption that this lineage might have developed some mechanisms for adaptation to the equine host.

Host adaptation has been described for several *S. aureus*-lineages in the past. The emergence of a poultry-specific ST5-clade for example was probably caused by a single human-to-poultry host jump. Further acquisition of mobile genetic elements (MGE) from avian specific lineages and the inactivation of several proteins with importance in human infection led to the occurrence of a poultry-specific clade [35]. Another example was given for CC97-*S. aureus*, a lineage that has been predominantly identified in samples from ruminants in the past. Only recently, CC97-MRSA were identified as a cause of human infections as well. The emergence of two new CC97 clones in the human population seems to be associated with the acquisition of MGE which encode factors that enhance the capacity to adapt to the human host [36].

In accordance with findings for CC5- and CC97-*S. aureus*, different studies revealed the capacity of CC398 to adapt to specific hosts including humans and livestock. The recent analysis

Table 3. Phenotypic resistance for 241 MRSA grouped according to their clonal complexes.

CC	CC22	CC5	CC398	CC8	CC1	CC9	ST599
total number	**n = 92**	**n = 58**	**n = 72**	**n = 16**	**n = 1**	**n = 1**	**n = 1**
GEN	3 (3.3%)	2 (3.4%)	57 (79.2%)	7 (43.8%)	0	0	0
KAN	3 (3.3%)	35 (60.3%)	58 (80.6%)	14 (87.5%)	1	0	0
ENR	90 (97.8%)	57 (98.2%)	28 (38.9%)	14 (87.5%)	0	1	0
MAR	90 (97.8%)	57 (98.2%)	30 (41.7%)	14 (87.5%)	0	1	0
ERY	32 (34.8%)	57 (98.2%)	7 (9.7%)	3 (18.8%)	1	1	0
CLI	34 (37.0%)	57 (98.2%)	8 (11.1%)	2 (12.5%)	1	1	0
TET	7 (7.6%)	1 (1.7%)	68 (94.4%)	7 (43.8%)	1	1	0
NIT	0	0	0	2 (12.5%)	0	0	0
CHL	1 (1.1%)	0	0	0	0	0	0
SXT	1 (1.1%)	1 (1.7%)	25 (34.7%)	6 (37.5%)	0	1	0

Number (n) and percentage (%) of resistant strains according to VITEK®2 system (bioMérieux, Germany).
Abbreviations: GEN: gentamicin, KAN: kanamycin, ENR: enrofloxacin, MAR: marbofloxacin, ERY: erythromycin, CLI: clindamycin, TET: tetracycline, NIT: nitrofurantoin, CHL: chloramphenicol and SXT: trimethoprim- sulfamethoxazole.
doi:10.1371/journal.pone.0085656.t003

of 89 diverse CC398-*S. aureus* from Price et al. strongly suggests an initial jump from humans to livestock, followed by adaptive processes like the loss of phage-carried human-associated virulence genes and the acquisition of tetracycline and methicillin resistance [37]. Besides, a recent study showed the emergence of an animal-independent ST398-MSSA with relevance in human infections in northern Manhattan. In addition to a different subset of MGE, these strains varied according to the set of intact adhesins, resulting in enhanced adhesion to human keratinocytes and keratin and demonstrated the adaptive capacity of ST398 strains to humans [38]. Further investigation is required to see if CC398-MRSA of equine origin harbour similar adaptive features.

Only one of the 243 MRSA yielded a positive signal for the *mec*A-homologue *mec*C, indicating a sporadic occurrence of *mec*C-MRSA in companion animals in Germany, as previously reported [28]. A rather sporadic occurrence of PVL-carriage among companion animal MRSA strains (two positives) is in accordance with previous findings [39,40].

Phenotypic resistance profiles of all MRSA revealed a high number of multi-resistant strains, in particular for CC5- and CC398-MRSA, highlighting the limited therapeutic options to treat most of these MRSA infections (table 3). Resistance profiles from isolates of human origin associated with epidemic MRSA lineages like CC22, CC5 and CC8 within German hospitals were regularly reported by the national reference laboratory Robert Koch-Institute (RKI) [29]. For ST22, the "Barnim epidemic strain" (*spa* types t005, t002 and t032), resistance against fluoroquinolones (ciprofloxacin, moxifloxacin) was frequently observed. While care should be taken before comparing study results (CC22-MRSA reported on here comprise more than these three *spa* types), the frequent resistance (97.8%) against fluoroquinolones (enrofloxacin, marbofloxacin) is also present in CC22-MRSA of companion animal origin. CC5-MRSA, human as well as companion animal strains, seem to express resistance against fluoroquinolones, clindamycin and erythromycin on a regular basis. For CC8-MRSA, a diverse phenotypic resistance pattern was observed. These results are in accordance with findings for CC8-MRSA of human origin [41].

The study was biased to some degree due to inhomogeneity of the case definition "wound infection" which was dependent on veterinarians who sent their samples to Vet Med Labor GmbH (Ludwigsburg, Germany). This survey focused on wound infections which have been sent to a diagnostic lab for microbiological examination but does not take empirically treated wound infections into account. Thus, calculated MRSA proportions might not reflect the MRSA and MSSA rates in the general companion animal population.

Another limitation is the lack of information for seven strains (five canine and two feline MRSA), which were not available for genotypic characterization.

Conclusion

This study provides evidence for the importance and impact of *S. aureus* in general, and MRSA in particular, as a cause of wound infections in dogs, cats and horses. The emergence of CC398-MRSA as predominant genotype in equine wound infections demonstrates the adaptive capacity of this lineage to other hosts than livestock and humans. Since CC398-MRSA can be also regularly identified in samples from dogs and cats, each of these animals should be considered as potential infection and/or colonization source for humans as well. Our findings clearly demonstrate the need for on-going screening studies to gain information about the replacement and emergence of certain genetic lineages among these animals. Further investigation including whole genome sequencing, phenotypic appearance and functional studies are needed to unveil factors and structures influencing the host adaptation processes like it has been successfully demonstrated for adaptation of CC398 to humans [38] and a CC5-clade to poultry [35]. Further attempts to reduce the total burden of MRSA, in each field afflicted with this pathogen, are clearly needed. Transmission of MRSA between different hosts (humans, animals) as well as between different ecological niches (hospital, community, veterinarian hospitals) should be regarded as a major "One Health" problem.

Acknowledgments

We thank E. Antao for critical reading of the manuscript.

Author Contributions

Conceived and designed the experiments: BW JH SV ALB. Performed the experiments: SV IS PAK. Analyzed the data: SV IS CA TS. Contributed reagents/materials/analysis tools: PAK IS LHW ALB. Wrote the paper: SV BW ALB.

References

1. Pinho MG, de Lencastre H, Tomasz A (2001) An acquired and a native penicillin-binding protein cooperate in building the cell wall of drug-resistant staphylococci. Proc Natl Acad Sci U S A 98: 10886–10891.
2. Otto M (2012) MRSA virulence and spread. Cell Microbiol 14: 1513–1521.
3. Manian FA (2003) Asymptomatic nasal carriage of mupirocin-resistant, methicillin-resistant *Staphylococcus aureus* (MRSA) in a pet dog associated with MRSA infection in household contacts. Clin Infect Dis 36: e26–28.
4. van Duijkeren E, Wolfhagen MJ, Box AT, Heck ME, Wannet WJ, et al. (2004) Human-to-dog transmission of methicillin-resistant *Staphylococcus aureus*. Emerg Infect Dis 10: 2235–2237.
5. Weese JS, Dick H, Willey BM, McGeer A, Kreiswirth BN, et al. (2006) Suspected transmission of methicillin-resistant *Staphylococcus aureus* between domestic pets and humans in veterinary clinics and in the household. Vet Microbiol 115: 148–155.
6. Wieler LH, Ewers C, Guenther S, Walther B, Lubke-Becker A (2011) Methicillin-resistant staphylococci (MRS) and extended-spectrum beta-lactamases (ESBL)-producing *Enterobacteriaceae* in companion animals: nosocomial infections as one reason for the rising prevalence of these potential zoonotic pathogens in clinical samples. Int J Med Microbiol 301: 635–641.
7. van Duijkeren E, Moleman M, Sloet van Oldruitenborgh-Oosterbaan MM, Multem J, Troelstra A, et al. (2010) Methicillin-resistant *Staphylococcus aureus* in horses and horse personnel: an investigation of several outbreaks. Vet Microbiol 141: 96–102.
8. Leonard FC, Markey BK (2008) Meticillin-resistant *Staphylococcus aureus* in animals: a review. Vet J 175: 27–36.
9. Walther B, Wieler LH, Friedrich AW, Hanssen AM, Kohn B, et al. (2008) Methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from small and exotic animals at a university hospital during routine microbiological examinations. Vet Microbiol 127: 171–178.
10. Axon JE, Carrick JB, Barton MD, Collins NM, Russell CM, et al. (2011) Methicillin-resistant *Staphylococcus aureus* in a population of horses in Australia. Aust Vet J 89: 221–225.
11. Malik S, Coombs GW, O'Brien FG, Peng H, Barton MD (2006) Molecular typing of methicillin-resistant staphylococci isolated from cats and dogs. J Antimicrob Chemother 58: 428–431.
12. Moodley A, Stegger M, Bagcigil AF, Baptiste KE, Loeffler A, et al. (2006) *spa* typing of methicillin-resistant *Staphylococcus aureus* isolated from domestic animals and veterinary staff in the UK and Ireland. J Antimicrob Chemother 58: 1118–1123.
13. Strommenger B, Kehrenberg C, Kettlitz C, Cuny C, Verspohl J, et al. (2006) Molecular characterization of methicillin-resistant *Staphylococcus aureus* strains from pet animals and their relationship to human isolates. J Antimicrob Chemother 57: 461–465.
14. Vincze S, Stamm I, Monecke S, Kopp PA, Semmler T, et al. (2013) Molecular Analysis of Human and Canine *Staphylococcus aureus* Strains Reveals Distinct Extended-Host-Spectrum Genotypes Independent of Their Methicillin Resistance. Appl Environ Microbiol 79: 655–662.
15. Witte W, Strommenger B, Stanek C, Cuny C (2007) Methicillin-resistant *Staphylococcus aureus* ST398 in humans and animals, Central Europe. Emerg Infect Dis 13: 255–258.
16. Weese JS, van Duijkeren E (2010) Methicillin-resistant *Staphylococcus aureus* and *Staphylococcus pseudintermedius* in veterinary medicine. Vet Microbiol 140: 418–429.
17. Walther B, Monecke S, Ruscher C, Friedrich AW, Ehricht R, et al. (2009) Comparative molecular analysis substantiates zoonotic potential of equine methicillin-resistant *Staphylococcus aureus*. J Clin Microbiol 47: 704–710.
18. Van den Eede A, Martens A, Lipinska U, Struelens M, Deplano A, et al. (2009) High occurrence of methicillin-resistant *Staphylococcus aureus* ST398 in equine nasal samples. Vet Microbiol 133: 138–144.
19. Weese JS, Caldwell F, Willey BM, Kreiswirth BN, McGeer A, et al. (2006) An outbreak of methicillin-resistant *Staphylococcus aureus* skin infections resulting from horse to human transmission in a veterinary hospital. Vet Microbiol 114: 160–164.
20. Loeffler A, Lloyd DH (2010) Companion animals: a reservoir for methicillin-resistant *Staphylococcus aureus* in the community? Epidemiol Infect 138: 595–605.
21. Wayne P (2008) Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals; Approved Standard – Third Edition. CLSI document M31-A3.Vol. 28 No. 8 ed. Pennsylvania: Clinical and Laboratory Standards Institute (CLSI).
22. Wayne P (2010) Performance Standards for Antimicrobial Susceptibility Testing; Twentieth Informational Supplement. CLSI document M100-S20. Pennsylvania: Clinical and Laboratory Standards Institute (CLSI).
23. Cuny C, Layer F, Strommenger B, Witte W (2011) Rare occurrence of methicillin-resistant *Staphylococcus aureus* CC130 with a novel mecA homologue in humans in Germany. PLoS One 6: e24360.
24. Merlino J, Watson J, Rose B, Beard-Pegler M, Gottlieb T, et al. (2002) Detection and expression of methicillin/oxacillin resistance in multidrug-resistant and non-multidrug-resistant *Staphylococcus aureus* in Central Sydney, Australia. J Antimicrob Chemother 49: 793–801.
25. Harmsen D, Claus H, Witte W, Rothganger J, Turnwald D, et al. (2003) Typing of methicillin-resistant *Staphylococcus aureus* in a university hospital setting by using novel software for *spa* repeat determination and database management. J Clin Microbiol 41: 5442–5448.
26. Enright MC, Day NP, Davies CE, Peacock SJ, Spratt BG (2000) Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. J Clin Microbiol 38: 1008–1015.
27. Lina G, Piemont Y, Godail-Gamot F, Bes M, Peter MO, et al. (1999) Involvement of Panton-Valentine leukocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia. Clin Infect Dis 29: 1128–1132.
28. Walther B, Wieler LH, Vincze S, Antao EM, Brandenburg A, et al. (2012) MRSA variant in companion animals. Emerg Infect Dis 18: 2017–2020.
29. Robert-Koch-Institut (2011) Epidemiologic Bulletin Nr. 26. 12.
30. Holden MT, Hsu LY, Kurt K, Weinert LA, Mather AE, et al. (2013) A genomic portrait of the emergence, evolution, and global spread of a methicillin-resistant *Staphylococcus aureus* pandemic. Genome Res 23: 653–664.
31. Zhang W, Hao Z, Wang Y, Cao X, Logue CM, et al. (2011) Molecular characterization of methicillin-resistant *Staphylococcus aureus* strains from pet animals and veterinary staff in China. Vet J 190: e125–129.
32. Nienhoff U, Kadlec K, Chaberny IF, Verspohl J, Gerlach GF, et al. (2009) Transmission of methicillin-resistant *Staphylococcus aureus* strains between humans and dogs: two case reports. J Antimicrob Chemother 64: 660–662.
33. McCarthy AJ, Lindsay JA, Loeffler A (2012) Are all meticillin-resistant *Staphylococcus aureus* (MRSA) equal in all hosts? Epidemiological and genetic comparison between animal and human MRSA. Vet Dermatol 23: 267-e254.
34. McCarthy AJ, van Wamel W, Vandendriessche S, Larsen J, Denis O, et al. (2012) *Staphylococcus aureus* CC398 clade associated with human-to-human transmission. Appl Environ Microbiol 78: 8845–8848.
35. Lowder BV, Guinane CM, Ben Zakour NL, Weinert LA, Conway-Morris A, et al. (2009) Recent human-to-poultry host jump, adaptation, and pandemic spread of *Staphylococcus aureus*. Proc Natl Acad Sci U S A 106: 19545–19550.
36. Spoor LE, McAdam PR, Weinert LA, Rambaut A, Hasman H, et al. (2013) Livestock Origin for a Human Pandemic Clone of Community-Associated Methicillin-Resistant *Staphylococcus aureus*. MBio 4.
37. Price LB, Stegger M, Hasman H, Aziz M, Larsen J, et al. (2012) *Staphylococcus aureus* CC398: Host Adaptation and Emergence of Methicillin Resistance in Livestock. MBio 3.
38. Uhlemann AC, Porcella SF, Trivedi S, Sullivan SB, Hafer C, et al. (2012) Identification of a Highly Transmissible Animal-Independent *Staphylococcus aureus* ST398 Clone with Distinct Genomic and Cell Adhesion Properties. MBio 3.
39. Rankin S, Roberts S, O'Shea K, Maloney D, Lorenzo M, et al. (2005) Panton valentine leukocidin (PVL) toxin positive MRSA strains isolated from companion animals. Vet Microbiol 108: 145–148.
40. Sing A, Tuschak C, Hormansdorfer S (2008) Methicillin-resistant *Staphylococcus aureus* in a family and its pet cat. N Engl J Med 358: 1200–1201.
41. Strommenger B, Bartels MD, Kurt K, Layer F, Rohde SM, et al. (2013) Evolution of methicillin-resistant *Staphylococcus aureus* towards increasing resistance. J Antimicrob Chemother.

4. Supplement of unpublished data

The two publications have a strict focus on specific aspects of the entire PhD-work and thus, display only a part of this project. Additional information and further data are given in the following chapter.

4.1 Additional information concerning MRSA isolated from wound infections of dogs, cats and horses

Like described in publication 2, MRSA of companion animal origin were genotypically characterized using different methods including *spa* typing and the detection of the Panton-Valentine leukocidin (PVL)-factor. In addition to the methods mentioned in the publication, PFGE was performed for each isolate as well as DNA microarray analysis for selected strains. Table 2 gives an overview on the frequency of all detected *spa* types for all analyzed isolates with respect to different host origins. Further it provides information regarding the association of each *spa* type to a specific clonal complex (CC). The number of representative isolates that are characterized by DNA microarray analysis and MLST are displayed for each *spa* type and host species. PVL-positive strains (n=2) and the *mec*C-positive MRSA are specifically mentioned in the table. The PFGE profile as well as additional information for each isolate is shown in supplemental tables S1 and S2.

CC	number of *spa* types	dog origin			cat origin			horse origin		
		Isolates (n)	Microarray (n)	MLST (n)	Isolates (n)	Microarray (n)	MLST (n)	Isolates (n)	Microarray (n)	MLST (n)
CC1	8	1	1	1	2	2	1	21	9	6
CC5	4	7	2	2	5	3	0	0	0	0
CC6	1	1	1	1	0	0	0	0	0	0
CC7	1	3	2	1	7	1	0	1	1	0
CC8	4	6	3	2	3	3	2	1	1	0
CC9	6	0	0	0	2	2	2	11	6	6
CC12	2	1	1	1	1	1	1	0	0	0
CC15	7	5	4	4	4	3	2	1	1	1
CC22	6	5	2	2	3	3	3	1	1	0
CC25	3	1	1	1	2	1	2	0	0	0
CC30	8	5	5	4	4	4	4	2	2	0
CC45	10	9	4	3	9	8	7	0	0	0
CC49	1	0	0	0	0	0	0	1	1	0
CC59	2	1	1	1	1	1	1	0	0	0
CC97	1	0	0	0	1	1	1	0	0	0
CC101	2	1	1	1	1	1	1	0	0	0
CC120	1	0	0	0	1	1	1	0	0	0
CC121	2	2	2	1	0	0	0	0	0	0
CC130	1	0	0	0	1	1	1	0	0	0
CC133	1	0	0	0	0	0	0	2	2	2
CC188	2	1	1	1	2	2	1	0	0	0
CC398	1	1	1	1	0	0	0	0	0	0
CC479	1	0	0	0	0	0	0	8	3	3
CC509	1	0	0	0	1	1	1	0	0	0
CC890	1	0	0	0	0	0	0	1	1	1
n (total)	**77**	**50**	**32**	**27**	**50**	**39**	**31**	**50**	**28**	**19**

Table 2: Overview: 241 isolated MRSA grouped according to their clonal complexes. The table provides information about the frequency of detected *spa* types for each host origin as well as the number of further typed isolates for each distinct *spa* type using DNA microarray analysis and MLST. Positive results for the detection of PVL and *mec*C are included.

4.1.1 PFGE analysis

Macrorestriction pattern obtained from PFGE analysis are exemplarily shown for the predominant *spa* types of CC5- (t003) and CC22-MRSA (t032) within this study. Accordingly, figure 3 shows the PFGE pattern of 61 CC22-MRSA, *spa* type t032 while figure 4 displays results for 49 CC5-MRSA, *spa* type t003. Macrorestriction analysis was performed as described in publication 1, using the band based dice coefficient analysis (optimization=0.5%, tolerance 1.2%). The percent similarity of each pattern is displayed in a dendrogram on the left site of the figure. A PFGE-based clonal group was defined as group of isolates with 85% similarity [109]. Since CC398-MRSA are non-typeable using the macrorestriction enzyme *Sma*I due to methylation of the restriction sites [110], strains

belonging to this particular lineage were PFGE-typed after digestion with the macrorestriction enzyme *Apa*I (supplemental table S2) like it has been described before [111].

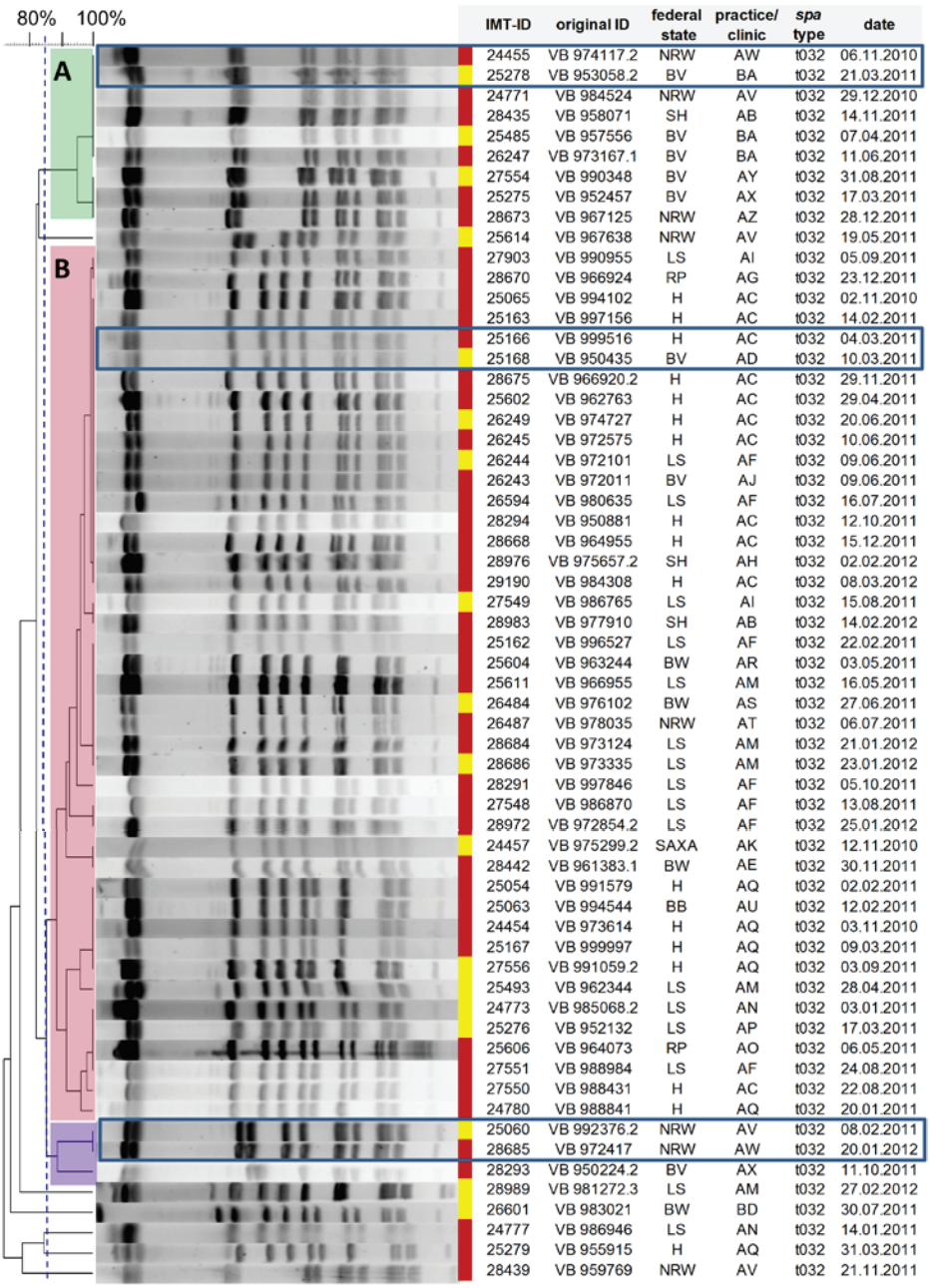
Additional strain information (original ID, geographic origin, date) is provided within the supplemental tables S1 and S2. Further, the host origin is indicated by a colored square.

PFGE: CC22-MRSA

The macrorestriction analysis of CC22-MRSA (*spa* type t032) provides information about the putative relatedness of the 61 investigated isolates. The analysis revealed three distinct PFGE clusters, named as cluster A (green shaped part of the dendrogram), B (red shaped) and C (purple shaped). In total, 43 isolates obtained from dogs (n=32) and cats (n=11) were associated to cluster B. Nine isolates belonged to cluster A (dog origin: n=6; cat origin: n=3) and three strains were assigned to cluster C (dog origin: n=2; cat origin: n=1). Undistinguishable PFGE pattern for strains of different host- and different geographic origin (blue boxes in figure 3) were observed for MRSA within all three clusters. Each cluster consists of MRSA from different clinics / practices (n) within the same and / or different federal states (m): Cluster A: n=8, m=3; cluster B: n=18, m=8; cluster C: n=3, m=2. Six strains did not belong to clusters A, B or C.

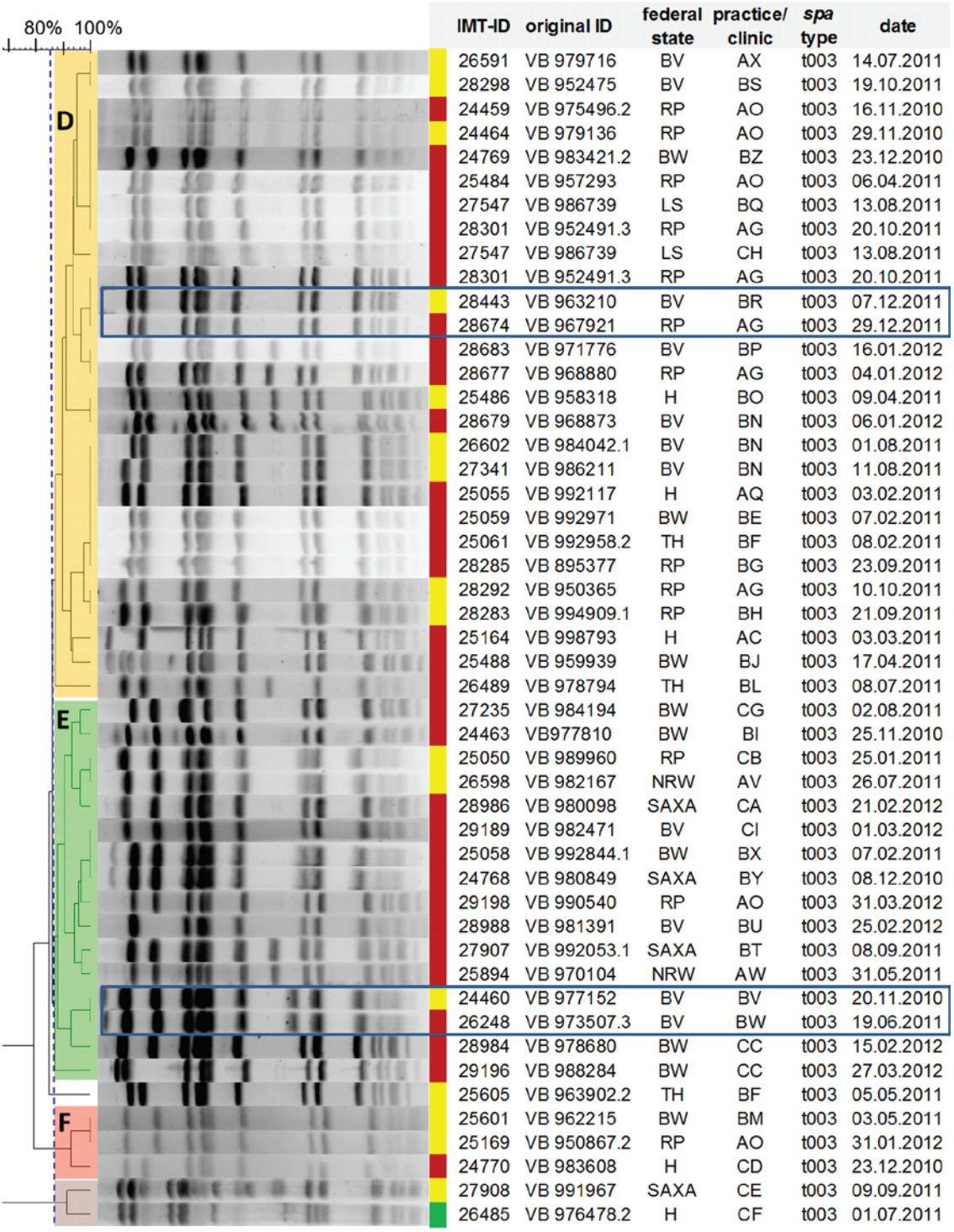
PFGE: CC5-MRSA

Macrorestriction analysis of all CC5-MRSA (*spa* type t003) revealed four distinct clusters, named as cluster D-G. Cluster D (yellow shaped part of the dendrogram) harbored 27 isolates obtained from dogs (n=18) and cats (n=9). Sixteen isolates (canine=13, feline=3) belonged to cluster E (green shaped), three (canine=1, feline=2) to cluster F (red shaped) and two MRSA (cat respectively horse origin) to cluster G (brown shaped). Similar to the findings for CC22-MRSA t032, identical PFGE-pattern were also observed for CC5-MRSA t003 from different hosts as well as from different geographic origin (see blue boxes in figure 4). Most isolates within one cluster originated from different clinics / practices (n) as well as from different federal states (m): Cluster D: n=18, m=6; cluster E: n=15, m=5, cluster F: n=3, m=3, cluster G: n=2, m=2.



IMT-ID	original ID	federal state	practice/ clinic	*spa* type	date
24455	VB 974117.2	NRW	AW	t032	06.11.2010
25278	VB 953058.2	BV	BA	t032	21.03.2011
24771	VB 984524	NRW	AV	t032	29.12.2010
28435	VB 958071	SH	AB	t032	14.11.2011
25485	VB 957556	BV	BA	t032	07.04.2011
26247	VB 973167.1	BV	BA	t032	11.06.2011
27554	VB 990348	BV	AY	t032	31.08.2011
25275	VB 952457	BV	AX	t032	17.03.2011
28673	VB 967125	NRW	AZ	t032	28.12.2011
25614	VB 967638	NRW	AV	t032	19.05.2011
27903	VB 990955	LS	AI	t032	05.09.2011
28670	VB 966924	RP	AG	t032	23.12.2011
25065	VB 994102	H	AC	t032	02.11.2010
25163	VB 997156	H	AC	t032	14.02.2011
25166	VB 999516	H	AC	t032	04.03.2011
25168	VB 950435	BV	AD	t032	10.03.2011
28675	VB 966920.2	H	AC	t032	29.11.2011
25602	VB 962763	H	AC	t032	29.04.2011
26249	VB 974727	H	AC	t032	20.06.2011
26245	VB 972575	H	AC	t032	10.06.2011
26244	VB 972101	LS	AF	t032	09.06.2011
26243	VB 972011	BV	AJ	t032	09.06.2011
26594	VB 980635	LS	AF	t032	16.07.2011
28294	VB 950881	H	AC	t032	12.10.2011
28668	VB 964955	H	AC	t032	15.12.2011
28976	VB 975657.2	SH	AH	t032	02.02.2012
29190	VB 984308	H	AC	t032	08.03.2012
27549	VB 986765	LS	AI	t032	15.08.2011
28983	VB 977910	SH	AB	t032	14.02.2012
25162	VB 996527	LS	AF	t032	22.02.2011
25604	VB 963244	BW	AR	t032	03.05.2011
25611	VB 966955	LS	AM	t032	16.05.2011
26484	VB 976102	BW	AS	t032	27.06.2011
26487	VB 978035	NRW	AT	t032	06.07.2011
28684	VB 973124	LS	AM	t032	21.01.2012
28686	VB 973335	LS	AM	t032	23.01.2012
28291	VB 997846	LS	AF	t032	05.10.2011
27548	VB 986870	LS	AF	t032	13.08.2011
28972	VB 972854.2	LS	AF	t032	25.01.2012
24457	VB 975299.2	SAXA	AK	t032	12.11.2010
28442	VB 961383.1	BW	AE	t032	30.11.2011
25054	VB 991579	H	AQ	t032	02.02.2011
25063	VB 994544	BB	AU	t032	12.02.2011
24454	VB 973614	H	AQ	t032	03.11.2010
25167	VB 999997	H	AQ	t032	09.03.2011
27556	VB 991059.2	H	AQ	t032	03.09.2011
25493	VB 962344	LS	AM	t032	28.04.2011
24773	VB 985068.2	LS	AN	t032	03.01.2011
25276	VB 952132	LS	AP	t032	17.03.2011
25606	VB 964073	RP	AO	t032	06.05.2011
27551	VB 988984	LS	AF	t032	24.08.2011
27550	VB 988431	H	AC	t032	22.08.2011
24780	VB 988841	H	AQ	t032	20.01.2011
25060	VB 992376.2	NRW	AV	t032	08.02.2011
28685	VB 972417	NRW	AW	t032	20.01.2012
28293	VB 950224.2	BV	AX	t032	11.10.2011
28989	VB 981272.3	LS	AM	t032	27.02.2012
26601	VB 983021	BW	BD	t032	30.07.2011
24777	VB 986946	LS	AN	t032	14.01.2011
25279	VB 955915	H	AQ	t032	31.03.2011
28439	VB 959769	NRW	AV	t032	21.11.2011

Figure 3: Macrorestriction analysis of all CC22-MRSA, *spa* type t032. The dendrogram provides information about the similarity of the investigated strains. A PFGE-based clonal group was defined as group of isolates with 85% similarity (blue broken line) which are considered as putatively clonally related and shaped in different colors (A-D). The host origin of each strain is indicated by color (red= dog-, yellow= cat origin). Blue boxes: examples for identical pattern of strains from different host- and geographic origin. Abbreviations: BB=Brandenburg, BV=Bavaria, BW=Baden-Wuerttemberg, H=Hesse, LS= Lower Saxony, NRW= North Rhine-Westphalia, RP= Rhineland-Palatinate, SAXA=Saxony Anhalt, SH= Schleswig-Holstein, TH= Thuringia.



IMT-ID	original ID	federal state	practice/ clinic	*spa* type	date
26591	VB 979716	BV	AX	t003	14.07.2011
28298	VB 952475	BV	BS	t003	19.10.2011
24459	VB 975496.2	RP	AO	t003	16.11.2010
24464	VB 979136	RP	AO	t003	29.11.2010
24769	VB 983421.2	BW	BZ	t003	23.12.2010
25484	VB 957293	RP	AO	t003	06.04.2011
27547	VB 986739	LS	BQ	t003	13.08.2011
28301	VB 952491.3	RP	AG	t003	20.10.2011
27547	VB 986739	LS	CH	t003	13.08.2011
28301	VB 952491.3	RP	AG	t003	20.10.2011
28443	VB 963210	BV	BR	t003	07.12.2011
28674	VB 967921	RP	AG	t003	29.12.2011
28683	VB 971776	BV	BP	t003	16.01.2012
28677	VB 968880	RP	AG	t003	04.01.2012
25486	VB 958318	H	BO	t003	09.04.2011
28679	VB 968873	BV	BN	t003	06.01.2012
26602	VB 984042.1	BV	BN	t003	01.08.2011
27341	VB 986211	BV	BN	t003	11.08.2011
25055	VB 992117	H	AQ	t003	03.02.2011
25059	VB 992971	BW	BE	t003	07.02.2011
25061	VB 992958.2	TH	BF	t003	08.02.2011
28285	VB 895377	RP	BG	t003	23.09.2011
28292	VB 950365	RP	AG	t003	10.10.2011
28283	VB 994909.1	RP	BH	t003	21.09.2011
25164	VB 998793	H	AC	t003	03.03.2011
25488	VB 959939	BW	BJ	t003	17.04.2011
26489	VB 978794	TH	BL	t003	08.07.2011
27235	VB 984194	BW	CG	t003	02.08.2011
24463	VB977810	BW	BI	t003	25.11.2010
25050	VB 989960	RP	CB	t003	25.01.2011
26598	VB 982167	NRW	AV	t003	26.07.2011
28986	VB 980098	SAXA	CA	t003	21.02.2012
29189	VB 982471	BV	CI	t003	01.03.2012
25058	VB 992844.1	BW	BX	t003	07.02.2011
24768	VB 980849	SAXA	BY	t003	08.12.2010
29198	VB 990540	RP	AO	t003	31.03.2012
28988	VB 981391	BV	BU	t003	25.02.2012
27907	VB 992053.1	SAXA	BT	t003	08.09.2011
25894	VB 970104	NRW	AW	t003	31.05.2011
24460	VB 977152	BV	BV	t003	20.11.2010
26248	VB 973507.3	BV	BW	t003	19.06.2011
28984	VB 978680	BW	CC	t003	15.02.2012
29196	VB 988284	BW	CC	t003	27.03.2012
25605	VB 963902.2	TH	BF	t003	05.05.2011
25601	VB 962215	BW	BM	t003	03.05.2011
25169	VB 950867.2	RP	AO	t003	31.01.2012
24770	VB 983608	H	CD	t003	23.12.2010
27908	VB 991967	SAXA	CE	t003	09.09.2011
26485	VB 976478.2	H	CF	t003	01.07.2011

Figure 4: Macrorestriction analysis of all CC5-MRSA, *spa* type t003. The dendrogram provides information about the similarity of the investigated strains. Isolates with more than 85% similarity (blue broken line) are considered as putatively clonally related and shaped in different colors (E-H). The host origin of each strain is indicated by color (red= dog-, yellow= cat-, green= horse origin). Abbreviations: BV=Bavaria, BW=Baden-Wuerttemberg, H=Hesse, LS= Lowver Saxony, NRW= North Rhine-Westpahlia, RP= Rhineland-Palatinate, SAXA=Saxony Anhalt, TH= Thuringia.

4.1.2 Putative nosocomial relationship of MRSA strains

In order to gain information about the putative nosocomial spread of MRSA from each individual veterinary settings which contributed more than one isolate to this study, undistinguishable MRSA isolates (with respect to PFGE and *spa* typing results) were investigated regarding a putative spatiotemporal relationship by consideration of the isolation timeframe. Thus, genotypically identical samples isolated within 14 days were considered as putatively related [112].

As already shown in figures 3 and 4, some clinics / practices provided more than one isolate during the sampling period (17 months). Consequently, this might have been a result of MRSA transmission (direct / indirect) between different patients, indicating nosocomial spread within a distinct veterinary setting. In total, the 241 MRSA originated from 113 different clinics / practices. 75 veterinarians contributed a single MRSA-positive wound swab within the 17-months sampling period, while the remaining 166 isolates originated from 38 different clinics / practices. Figure 5 gives an overview on the frequency of single and multiple MRSA-positive wound swabs from these settings.

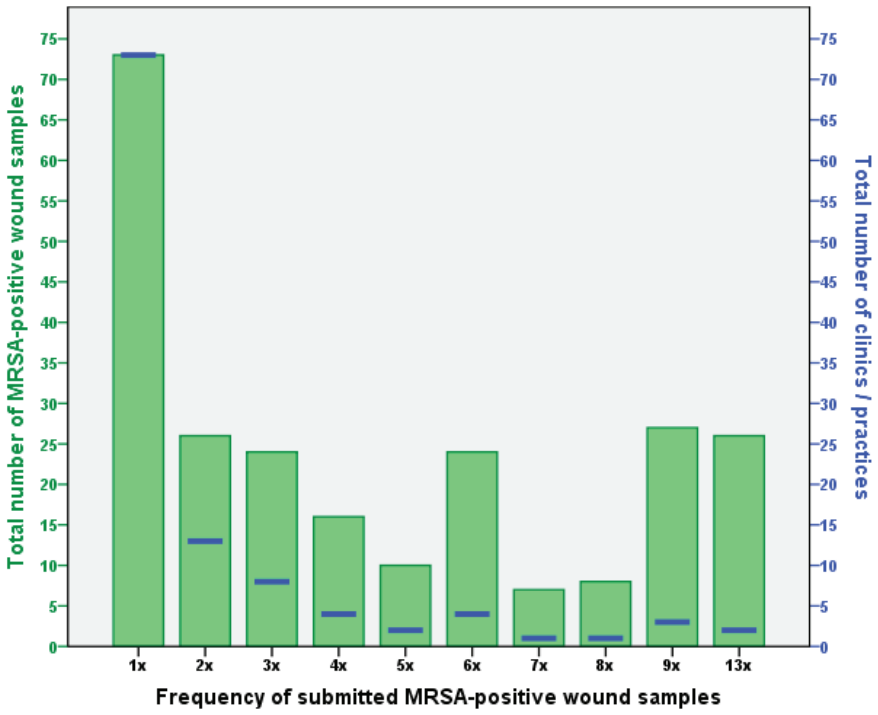
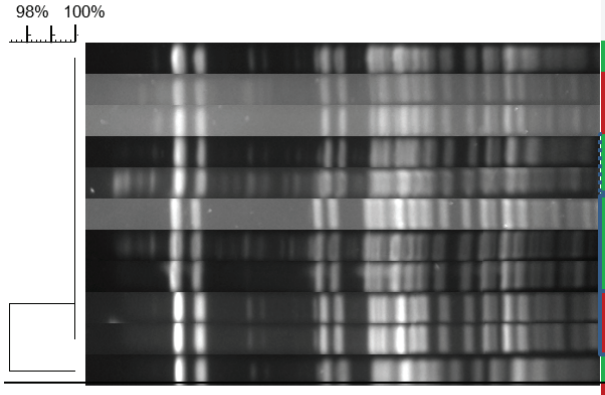


Figure 5: Overview: Submission rates of MRSA-positive wound samples per veterinary institution. The x-axis shows the submission rate (1 to 13) of MRSA-positive wound samples per individual clinic / practice. The green bars indicate the total number of positive samples for each of these rates (green y-axis on the left side). The blue lines provide information about the total number of clinics / practices that reached each of the listed frequencies (blue y-axis on the right side).

Most of the veterinary facilities sent one to three MRSA-positive samples (n=96 / 85%). However, 17 clinics / practices provided more than three MRSA-positive wound samples (15%). The highest submission rate (13 MRSA-positive samples / institution) was reached by two different settings. Since MRSA is known as an important nosocomial pathogen, genotyping results were analyzed with respect to the putative nosocomial appearance of certain strains within these two clinics / practices.

Putative nosocomial spread of MRSA: Results obtained for veterinary setting “A”

13 MRSA-positive wound swabs from veterinary setting “A” originated from horses (n=7) and dogs (n=6). Nine isolates were CC398-MRSA belonging to *spa* type t6867. To gain more knowledge about the genotypic variation of those isolates PFGE analysis was performed for MRSA strains obtained from the practice of interest. Since CC398-MRSA cannot be digested using the macrorestriction enzyme *Sma*I, PFGE analysis was performed using the endonuclease *Apa*I (figure 6).



IMT-ID	original ID	*spa* type	date	PFGE profile (*Apa* I)
25064	VB 994572.1	t6867	12.02.2011	H1
25171	VB 951312	t6867	15.03.2011	H1
25616	VB 968353	t6867	21.05.2011	H1
26488	VB 978192	t6867	06.07.2011	H1
26600	VB 982982.2	t6867	27.07.2011	H1
28297	VB 951375.2	t6867	19.10.2011	H1
28302	VB 952633.2	t6867	20.10.2011	H1
28304	VB 954489	t6867	27.10.2011	H1
28669	VB 966726.2	t6867	22.12.2011	H1
28671	VB 967034.2	t6867	23.12.2011	H1
28985	VB 979685	t6867	20.02.2012	H2
28435	VB 958071	t032	14.11.2011	n.t.
28983	VB 977910	t032	14.02.2012	n.t.

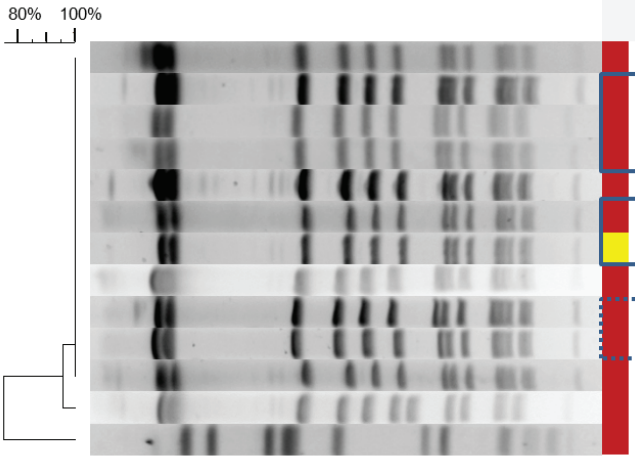
Figure 6: PFGE analysis of 11 isolates from veterinary setting A (Schleswig Holstein) using the macrorestriction enzyme *Apa*I. The analysis was performed like described in publication 1. The blue boxes show isolates with a close spatiotemporal relationship (≤14 days). Blue dotted box: two indistinguishable isolates obtained within 21 days.

Eight out of the nine isolates of interest from clinic A revealed an undistinguishable PFGE pattern (H1) and strain IMT28985 (pattern H2) showed more than 97% similarity with pattern H1.

Within facility “A”, isolates associated with PFGE pattern H1 were twice obtained within an acceptable timeframe (≤14 days) (figure 6). Further two MRSA (both: H1) were received within 21 days (dotted blue line in figure 6).

Putative nosocomial spread of MRSA: Results obtained for veterinary setting “B”

The PFGE analysis of 13 MRSA strains received from veterinary setting “B” is shown in figure 7. Twelve MRSA originated from dogs (red squares) and one from a cat (yellow square). The majority of isolates belonged to CC22, *spa* type t032 (n=11). Further, one CC22-MRSA, *spa* type t022 and one CC5-MRSA, *spa* type t003 had been isolated within this clinic. PFGE analysis revealed undistinguishable macrorestriction pattern for 11 CC22-MRSA including the single t022-isolate. Considering the individual isolation dates of these 11 isolates assigned to pattern B1, a putative relationship of two respectively three isolates (marked in blue boxes) might be reasonable for two different timeframes. Additionally, two MRSA have been isolated within 16 days (blue dotted box within figure 7).



IMT-ID	original ID	*spa* type	date	PFGE profile (*Sma* I)
24453	VB 973026.2	t022	02.11.2010	B1
25065	VB 994102	t032	14.02.2011	B1
25163	VB 997156	t032	24.02.2011	B1
25166	VB 999516	t032	04.03.2011	B1
25602	VB 962763	t032	29.04.2011	B1
26245	VB 972575	t032	10.06.2011	B1
26249	VB 974727	t032	20.06.2011	B1
28294	VB 950881	t032	12.10.2011	B1
28675	VB 966920.2	t032	29.11.2011	B1
28668	VB 964955	t032	15.12.2011	B1
29190	VB 984308	t032	08.03.2012	B1
27550	VB 988431	t032	22.08.2011	B2
25164	VB 998793	t003	03.03.2011	E1

Figure 7: PFGE analysis of 13 MRSA isolates from clinic / practice “B” (Hesse) using the macrorestriction enzyme *Sma*I. The analysis was performed like described in publication 1. The two blue boxes indicate isolates with a close spatiotemporal relationship (≤14 days). Blue dotted box: two indistinguishable isolates obtained within 16 days.

While a nosocomial spread of the pulsotype H1 within veterinary setting “A” and B1 for setting “B” can be assumed, further epidemiological data regarding putative transmission routes would be necessary to proof this assumption.

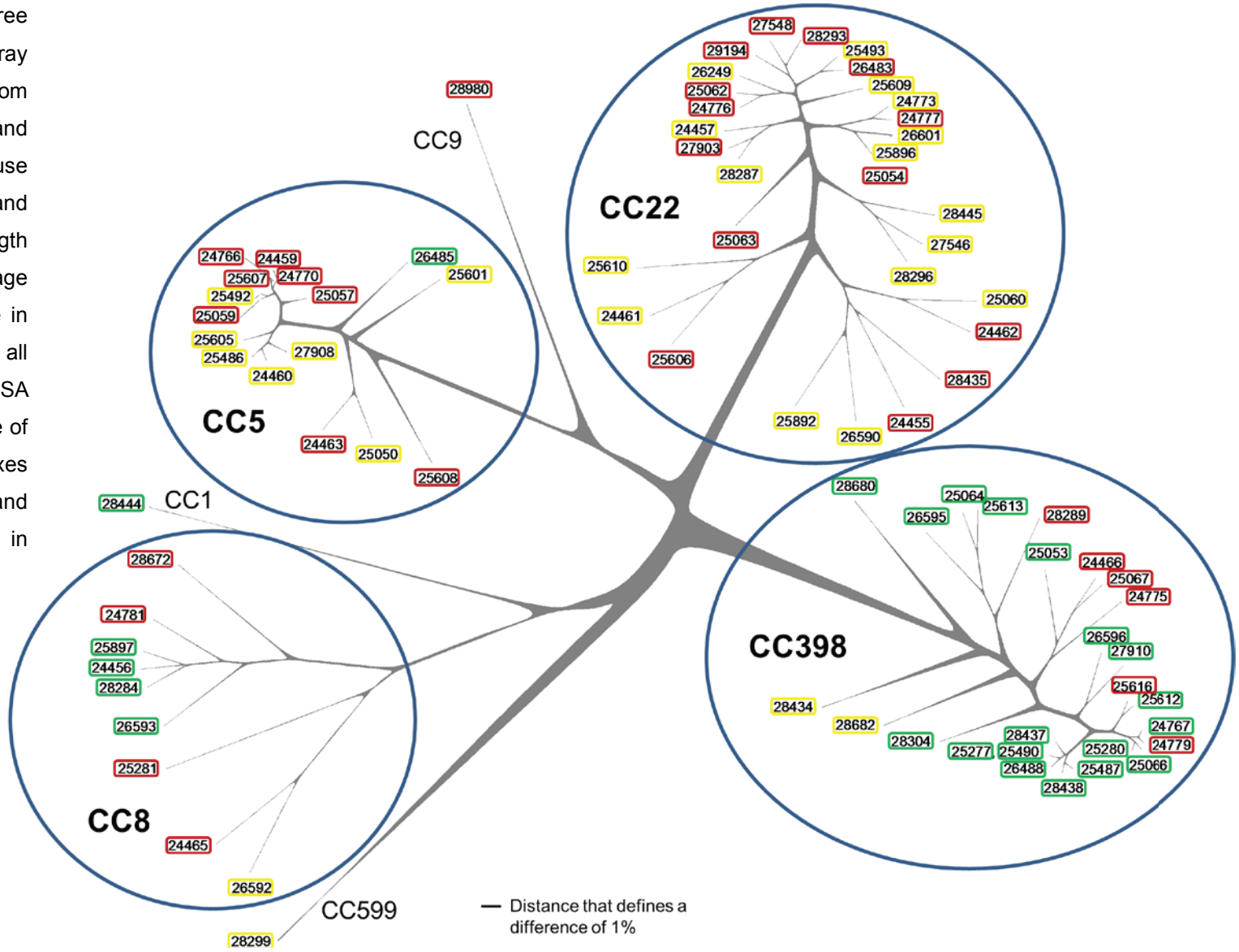
4.1.3 DNA microarray analysis

In addition to PFGE analysis that was performed for all MRSA strains from publication 2, DNA microarray analysis was carried out for a subset of 83 MRSA of dog (n=33), cat (n=26) and horse origin (n=24) to gain knowledge about the genetic diversity of these isolates. The selection was based on all previously gained genotyping results including *spa* typing, detection of the *mec*A / *mec*C gene, PVL-detection and PFGE, with the aim to include isolates with a high variability with regard to genetic diversity (see table 2). DNA microarray analysis provides information about the presence or absence of 334 defined gene loci for each investigated isolate (see also 2.7.4).

Based on these data it is possible to create an unrooted tree as described in publication 1. The unrooted tree provided in figure 8 (83 MRSA) allows a discrimination of seven distinct main branches. Each of these branches consists of isolates from a certain clonal complex. The host origin of each MRSA is indicated by the color of the square around the IMT-ID (green: horse-, yellow: cat-, red: dog origin). This method was used to visualize results of the DNA microarray analysis and revealed unique pattern for 81 of the 83 investigated MRSA.

Only two CC398-MRSA showed identical results (IMT25490, IMT25277). However, all MRSA clustered according to their clonal complexes (displayed in blue circles for more than one isolate per CC) showing that DNA microarray analysis is another tool to identify the clonal background of *S. aureus* isolates. In accordance with results from earlier mentioned genotyping methods, DNA microarray analysis using the Identibac *S. aureus* genotyping chip from Alere ® is not capable to show host specific genetic differences for isolates of dog, cat and horse origin for each investigated genetic lineage.

Figure 8: Unrooted tree based on DNA microarray results for 83 MRSA from dog (red), cat (yellow) and horse (green) origin by use of Pearson correlation and UPGMA. The branch length displays the percentage difference of each isolate in comparison to results for all investigated strains. MRSA with an association to one of the main clonal complexes (CC5, CC22, CC8 and CC398) are encircled in blue.



4.2 Investigation of methicillin-susceptible *S. aureus* isolated from infections of dogs, cats and horses

Investigation of *S. aureus*-mediated infections in animals is mainly focused on MRSA since they are frequently associated with multi-drug resistance and therefore difficult to treat. Examples from the recent past however show the necessity to include methicillin-susceptible *S. aureus* (MSSA) isolates into epidemiological studies to obtain a complete picture about the spread and adaptation processes of investigated lineages [80,113].

Therefore, another aim of this PhD-thesis was to gain more knowledge about the diversity of MSSA isolated from dogs, cats and horses. Initially, a convenience sample of 50 canine MSSA from different infection sites and different geographic origin, which have been isolated between 2008 and 2010 within Europe, were genotypically investigated using PFGE, *spa* typing as well as DNA microarray analysis and MLST (for selected MSSA) as shown in publication 1. This survey revealed high lineage diversity within these strains including MSSA from 16 different clonal complexes (figure 9).

In order to determine the diversity of MSSA from cats and horses, further 50 isolates of each host origin were characterized using *spa* typing (all strains) as well as DNA microarray analysis and MLST (for representative isolates). Table three shows the genotyping results for all 150 MSSA (= 50 per animals species: dog, cat, horse) grouped according to their host origin and clonal complexes. Because of the high variability of *spa* types, this table provides information about the number of different *spa* types that has been identified within a certain clonal complex. Within all CC1-MSSA, for example, eight different *spa* types were found among 24 isolates of dog (n=1), cat (n=2) and horse origin (n=21). A detailed overview on each identified *spa* type can be found in supplemental table S3. In Table 3, the number of further characterized isolates per animal species using DNA microarray analysis and MLST is given. Inclusion criteria for further characterization were based on the *spa* types with the aim to select at least one representative MSSA for each identified *spa* type.

CC	number of *spa* types	dog origin			cat origin			horse origin		
		Isolates (n)	Microarray (n)	MLST (n)	Isolates (n)	Microarray (n)	MLST (n)	Isolates (n)	Microarray (n)	MLST (n)
CC1	8	1	1	1	2	2	1	21	9	6
CC5	4	7	2	2	5	3	0	0	0	0
CC6	1	1	1	1	0	0	0	0	0	0
CC7	1	3	2	1	7	1	0	1	1	0
CC8	4	6	3	2	3	3	2	1	1	0
CC9	6	0	0	0	2	2	2	11	6	6
CC12	2	1	1	1	1	1	1	0	0	0
CC15	7	5	4	4	4	3	2	1	1	1
CC22	6	5	2	2	3	3	3	1	1	0
CC25	3	1	1	1	2	1	2	0	0	0
CC30	8	5	5	4	4	4	4	2	2	0
CC45	10	9	4	3	9	8	7	0	0	0
CC49	1	0	0	0	0	0	0	1	1	0
CC59	2	1	1	1	1	1	1	0	0	0
CC97	1	0	0	0	1	1	1	0	0	0
CC101	2	1	1	1	1	1	1	0	0	0
CC120	1	0	0	0	1	1	1	0	0	0
CC121	2	2	2	1	0	0	0	0	0	0
CC130	1	0	0	0	1	1	1	0	0	0
CC133	1	0	0	0	0	0	0	2	2	2
CC188	2	1	1	1	2	2	1	0	0	0
CC398	1	1	1	1	0	0	0	0	0	0
CC479	1	0	0	0	0	0	0	8	3	3
CC509	1	0	0	0	1	1	1	0	0	0
CC890	1	0	0	0	0	0	0	1	1	1
n (total)	**77**	**50**	**32**	**27**	**50**	**39**	**31**	**50**	**28**	**19**

Table 3: Overview: MSSA strains from dog, cat and horse origin grouped according to their clonal complexes. The table provides information about the number of different *spa* types and the number of isolates per CC for each host origin. In addition, it displays the number of further typed isolates for each CC using DNA microarray analysis and MLST.

Results for MSSA characterization are visualized within figures 8 to 10. In total, the 50 canine MSSA showed a high lineage-diversity (figure 9) with strains belonging to 16 different clonal complexes. Clonal complexes that occurred 1-3 times are either displayed in stripes (n=1) or checkerboard pattern (n= 2-3). More frequently occurring lineages (n>3), which are entirely colored, included the clonal complexes CC45, CC5, CC8, CC30, CC22 and CC15.

In accordance with findings for canine isolates, MSSA of cat origin displayed a high lineage-diversity including 18 distinct clonal complexes. Frequently detected lineages within this group were CC45, CC7, CC5, CC30 and CC15.

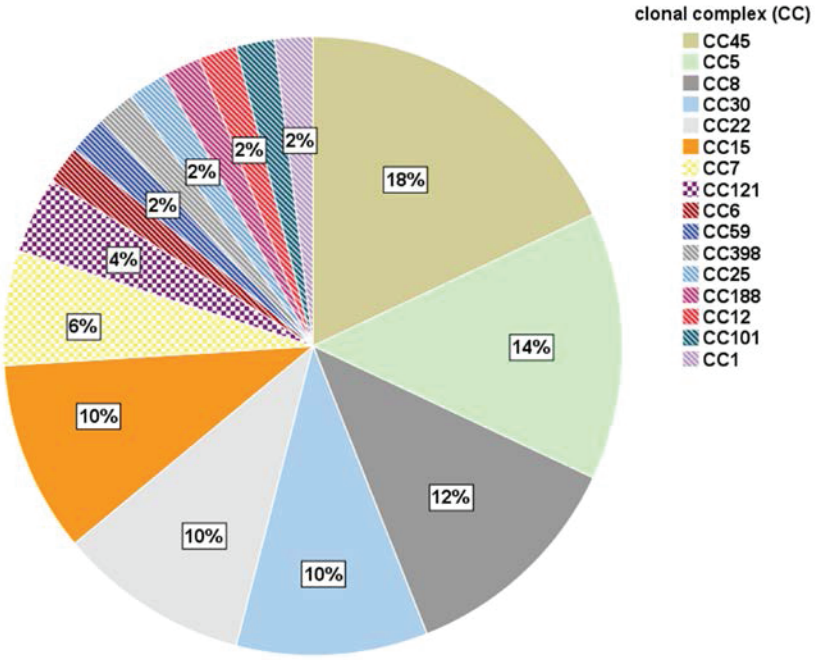


Figure 9: Lineage-diversity of 50 canine MSSA (based on *spa* typing results)

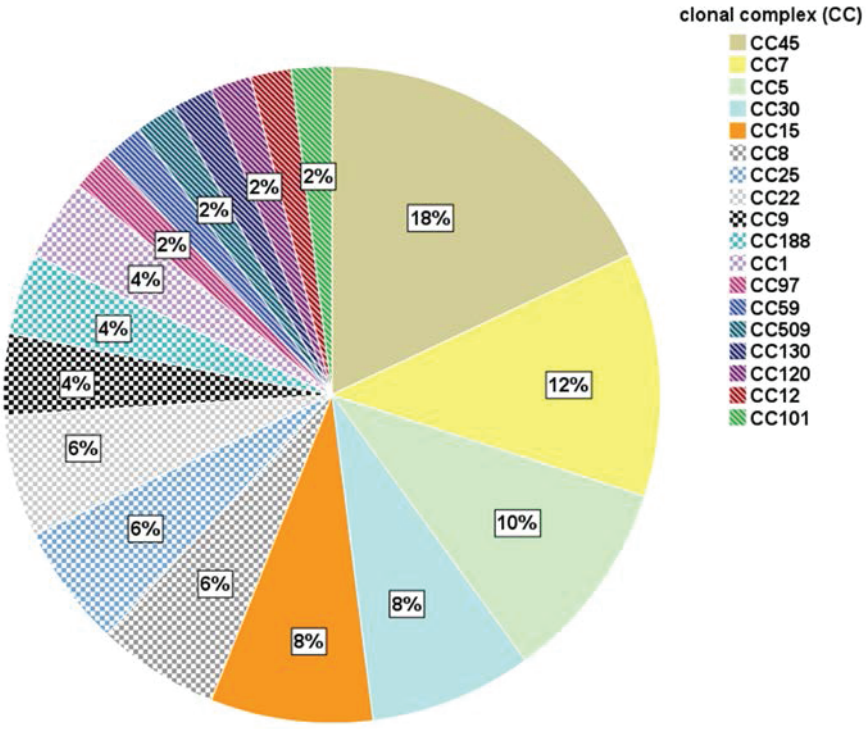


Figure 10: Lineage-diversity of 50 feline MSSA (based on *spa* typing results)

In horses, 12 different clonal complexes were identified. However, in contrast to the findings for MSSA of dog and cat origin, 80% of these isolates belonged to one of three clonal complexes: CC1, CC9 or CC479 (see figure 11).

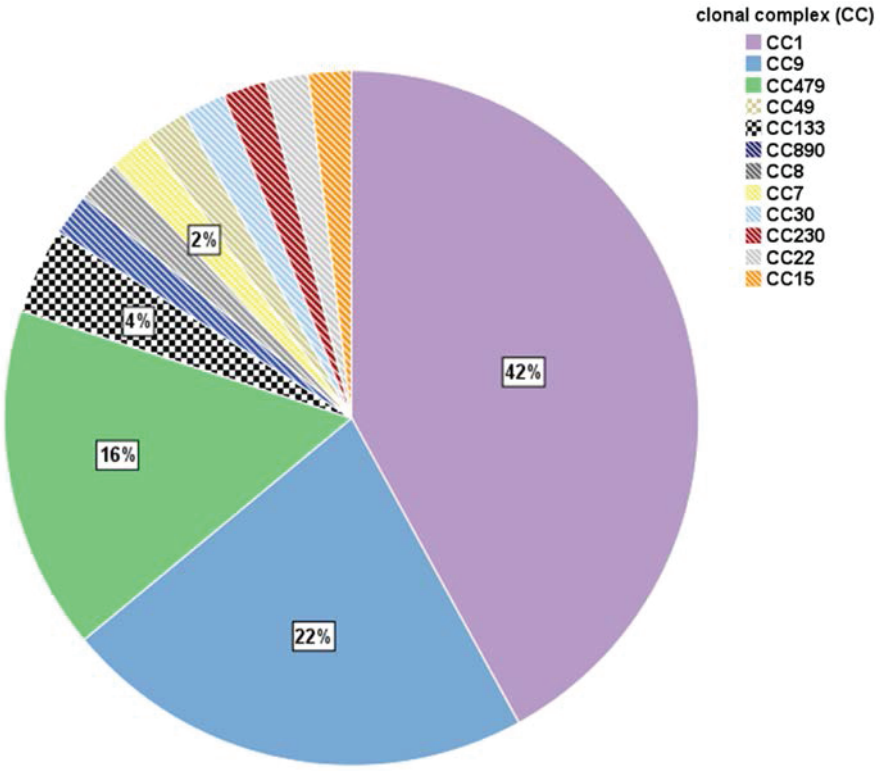


Figure 11: Lineage-diversity of 50 equine MSSA (based on *spa* typing results)

5. Discussion

Within this thesis, data on the frequency, geographic origin as well as information on numerous molecular characteristics of *S. aureus* isolated from infected dogs, cats and horses are presented. Thus, this work provides for the first time both reliable and representative data, obtained by investigation of a large sample size within Germany. Comparative analysis of the genotypic diversity of *S. aureus* from infectious diseases of companion animals with epidemic human strains allow a deeper understanding of the current epidemiology and potential pathways of successful methicillin-susceptible and -resistant *S. aureus* lineages. Thus, data presented here are an example for the necessity of interdisciplinary collaborative efforts to combat multi-drug resistant pathogens widely distributed in human and veterinary medicine with respect to the “One Health”-idea.

5.1 Host specific aspects of *Staphylococcus aureus*

Data shown in publication 1 demonstrate the lack of host restricted lineages for *S. aureus* isolated from infected dogs. While different studies examined the genetic diversity of MRSA from dog infections in the recent past [1,63,83,114-118], information about the genetic diversity of methicillin-susceptible *S. aureus* in dogs and other companion animals is still scarce [119]. In order to close this gap, the data summarized in publication 1 included MSSA as well. Based on the genotypic characterization of *S. aureus* in publication 1 it was possible to identify both MRSA and MSSA from infected dogs as extended host spectrum genotypes (EHSG). Further, this study demonstrated that frequently occurring lineages from *S. aureus* mediating infections in dogs belong to the group of so called “human associated” lineages. These findings are particularly interesting as they indicate the possibility of all EHSG-*S. aureus* to spread (at least) between dogs and humans and vice versa. Further, data obtained during this work (see publication 2 and section 4.2) show that this is also true for *S. aureus* from feline infections, which means that a differentiation between strains isolated from humans, dogs and cats is not possible with the methods applied during this work. Combined with described transmission events between dogs and cats and their owners [3-6], these data imply the lack of host specific factors for these isolates.

The most discussed human specific structure in these “human associated” lineages is the Immune Evasion Cluster (IEC), which is encoded on a phage. Several studies published data concerning the distribution of the IEC in isolates from humans and animals, providing epidemiologic evidence that this cluster can be found in important lineages from humans

[77,78] but is missing in isolates of animal origin [36,77,79]. Further, in 2013 Spoor et al. were able to describe an epidemiologic association between the integration of the phage-encoded IEC and the host spectrum of CC97-*S. aureus*. Formerly, CC97 was known as ruminant specific lineage that lacked the IEC, but nowadays CC97-*S. aureus* are also regularly identified in human samples. Investigations based on whole genome analysis identified independent host jumps from cattle to humans that were accompanied by the integration of the IEC-encoding phage [80]. Although these epidemiological data seem to support the general accepted hypothesis of the IEC being a human specific virulence cluster, the nature of mechanisms that could contribute to host specific colonization and / or infection are still poorly understood.

The main shortcoming of all studies with a focus on the occurrence of the IEC in strains of animal origin however is the selection of a unilateral study population. Representative isolates of animal origin were only chosen from livestock but lacked the investigation of *S. aureus* from companion animals. Since especially dogs and cats are predominantly infected with *S. aureus* of so-called common human lineages, it is of special interest to identify the frequency of IEC-carrying isolates from dogs and cats. Therefore, DNA microarray data from 48 representative canine *S. aureus* were evaluated (publication 1). The detected IEC-carriage rate was 79% in isolates of dog origin demonstrating the regular occurrence of the IEC among canine isolates that belong to typical human lineages (CC5, CC15, CC22, CC30, and CC45) within Germany.

Even though the effects of genes encoded on the IEC are described as specific for humans, knowledge is scarce about the expression and functional effects of factors carried on the IEC in dogs. Lijnen et al. compared fibrinolytic and fibrinogenolytic activities of staphylokinase (Sak) in different species and identified high activity in humans and dogs, indicating a potential role of Sak in the canine host as well [120]. However, whether the IEC may possess a beneficial effect in the canine host still needs to be investigated in further experimental studies. Possible explanations for the high IEC detection rate in isolates of dog origin might be:

i) that the probability or frequency of the loss of the IEC phages is low
ii) that there might be a potential benefit of IEC genes in at least some nonhuman hosts
iii) that the timeframe for the deletion of this phage was too short since companion animals including dogs are only recently recognized as species with frequently occurring *S. aureus*-mediated infections.

In general, adaptation is a response to changes of the local environment and strong selection pressure can lead to rapid genetic adaptation of bacterial pathogens driven by spontaneous mutations as well as horizontal gene transfer. Beside this genetic flexibility bacteria developed several non-genetic strategies to respond to hostile environments or to adapt to a specific niche. One of these strategies is the phenotypic variability of bacteria as a result of changes in the gene expression [121,122]. This phenotypic diversity within one bacterial population that is defined as (non-genetic) individuality allows the existence in heterogeneous environments and points out the flexibility of bacteria to respond to temporal environmental changes independent of genetic changes of the organisms. The best studied example of bacterial individuality is persistence [121]. Persistence was first described by Bigger in 1944, who investigated the behavior of *S. aureus* during treatment with penicillin. In this experiment he observed that the majority of bacterial cells were immediately killed while treated with penicillin. A small fraction however showed the ability of prolonged survival. The small fraction that showed prolonged survival was cultivated without antibiotic pressure. This subculture was used for repetition of the experiment revealing identical results in comparison to the first experiment where again only a small number of cells were able to survive longer during treatment with penicillin. This finding implies that this bacterial behavior is not based on the acquisition of a resistance mechanism but that only a small subpopulation exhibits resistance [123]. Even though persistence was first described in 1944 and afterwards identified for many other bacteria, it was not before 2004 that Balaban et al. where able to proof the hypothesis that persistence is linked to preexisting heterogeneity in bacterial populations [124]. Since *S. aureus* encodes a variety of virulence factors and adhesins which are expressed under different conditions this facultative pathogen is highly flexible with respect to non-genetic phenotypic heterogeneity due to changes in the gene expression like it has been shown for the growth in human and calf serum in comparison to bacterial culture medium in the past [125,126]. It might be this broad spectrum of virulence-associated factors that enables EHSG-*S. aureus* to infect various different hosts.

In contrast to EHSG-*S. aureus* “host restricted lineages” seem to lack certain factors, for example due to the formation of pseudogenes, like it has been exemplarily shown for ST5-*S. aureus* adapted to poultry. Pseudogenes can be the result of so called null mutations leading to a specific loss of function for the bacterial population. While mutations with a gain of function are quite rare, null mutations are much more common and should be considered as a powerful tool of bacteria with regard to various adaptation processes like it has been demonstrated for *Escherichia coli* by Hottes and colleagues [127]. In addition to the formation of pseudogenes due to null mutations, the acquisition of factors enhancing virulence in a

particular host via uptake of mobile genetic elements (MGEs) is another powerful tool for adaptation of bacteria [128]. Thus, the variety of mobile genetic elements that can integrate into the staphylococcal genome might serve as a source of adaptive factors by providing more host-restricted virulence encoding genes [129]. The modification of the staphylococcal genome is highly dynamic and even successful adaptation to a specific niche of one lineage does not necessarily prevent a further niche-independent spread of this lineage. The best studied example is given by CC398-*S. aureus*. In 2004, CC398-MRSA was described as a new emerging lineage that is highly adapted to livestock. Swine were identified as the main reservoir for CC398-MRSA possessing a risk for humans in close contact with livestock like farmers and veterinarians [130]. Nowadays CC398-MRSA cause regularly infections in various mammalians including companion animals (publication 2) and humans [131]. The rapid dissemination of this lineage resulted in detailed population analysis based on WGS where Price and colleagues were able to unravel the epidemiological pathways of CC398-*S. aureus* with respect to host changes. The investigation of 89 CC398-*S. aureus* from various host species and countries by use of whole genome sequencing revealed an initial host switch of CC398-MSSA from humans to livestock. This change was accompanied by the acquisition of methicillin resistance as well as tetracycline resistance. The existence of CC398-MRSA harboring different SCC*mec* elements as well as different tetracycline resistance encoding genes provides evidence that distinct resistances were introduced to this lineages at several independent time points, driven by the selective pressure in a new environment. Beside the successful adaptation to the niche “livestock”, this lineage demonstrates the enormous capacity of *S. aureus* to spread from one successful niche to various other hosts. Nowadays, CC398-MRSA is not restricted to livestock but gains more and more importance in companion animal medicine like it has been clearly demonstrated in publication 2. Beside the regular occurrence of CC398-MRSA in wound infections from dogs and cats (13.5%, 7.7%) this lineage further replaced CC8-MRSA as predominant lineage in horse infections (and colonization). Further, increasing reports show the rising relevance of CC398-MRSA in human medicine [131,132]. The phenotypic investigation of CC398-MRSA including host-cell interaction performed by Ballhausen and colleagues demonstrated a human-pathogenic potential for isolates from this lineage *in vitro* [82]. Furthermore, the survey identified a substantial phenotypic heterogeneity for CC398 sub-populations independent of the host origin which supports the hypothesis that *S. aureus* shows rather lineage specific features in contrast to host specific aspects (see chapter 5.2). The example of CC398-MRSA clearly highlights the flexibility of *S. aureus* to change within a short time frame from a host-adapted pathogen to a broad host spectrum and thus underlines the

advantage of *S. aureus* to undergo rapid epidemiological changes. This behavior is of special concern with regard to the acquisition and spread of new resistance encoding genes.

The capacity of bacteria to disable the functionality of antibiotics is probably one of the most impressive examples for adaptation from the recent past. Driven by a high selection pressure, bacteria use several mechanisms to develop resistance to antibiotics like point mutations or the acquisition of MGEs via horizontal gene transfer [133]. In the past, it was assumed that the acquisition of resistance against a specific antimicrobial agent results in fitness cost for bacteria without exposure to the antibiotic which is mostly expressed in reduced growth rates [128]. In 2014, Baker and colleagues were able to point out the selective advantage of fluoroquinolone-resistant *Salmonella enterica* serovar Typhi isolates compared to sensitive strains even in absence of antimicrobial selection pressure when the resistance was caused by point mutations of the *gyr*A or *par*C gene [134]. Increasing resistance against first choice antibiotics in *Enterobacteriaceae* led to an increased use of fluoroquinolones in the recent past, thus selecting for resistance against this antibiotic class. These findings are particularly worrisome as they indicate an advantage of resistant bacteria even without antimicrobial use and thus might result in the dissemination of resistant bacteria in future.

To treat infections with MRSA that exhibit resistance against several antimicrobial classes the glycopeptide vancomycin has become the “last resort antibiotic”. Like expected, the number of vancomycin-resistant *S. aureus* increased with the use of this particular antimicrobial agent. Dependent on the resistance mechanism, it is possible to distinguish vancomycin-resistant *S. aureus* (VRSA) and vancomycin-intermediate *S. aureus* (VISA). While the vancomycin resistance of VISA is caused by point mutations, VRSA acquire vancomycin resistance due to the uptake of the *vanA* operon [135]. Only recently, the first report on a vancomycin resistant MRSA containing a plasmid encoding the *van*A cluster was published. The vancomycin resistance encoding plasmid was acquired by a CC8-MRSA during treatment with vancomycin [136]. While acquisition of the *van*A operon does not result in a higher fitness like shown for fluoroquinolone resistance in *Salmonella enterica* serovar Typhi it is also not responsible for fitness cost in MRSA. Thus, once integrated into the staphylococcal genome there is (at least in vitro) no selective advantage with respect to the level of fitness to lose the *van*A operon in an environment without selective pressure [137]. Along with the identification of the *vanA* cluster on a highly mobile genetic element (plasmid) these findings are particularly worrisome with regard to a potential spread in the community

and environment and therefore the occurrence of VRSA containing the *van*A cluster needs to be closely monitored.

5.2 *S. aureus*: lineage specificity

During this work it was shown that the attempt from various researchers in the past to identify *S. aureus* genotypes with a narrow host restriction succeeds only for a few lineages. However, a broad molecular analysis of a large number of *S. aureus* isolated from different hosts and different geographic origin over a long time period provided information about the structure and organization of the *S. aureus* genome, identifying a lineage-specific organization of *S. aureus* genomes in general. While the core genome is present in all *S. aureus* and provides most baseline and essential molecular mechanisms the core variable genome consists mostly of virulence-associated genes (including adhesins) and enables a differentiation of genetic lineages based on core variable gene profiles. Mobile genetic elements promote diversification within lineages and thus define sub-lineages within a distinct clonal complex [85].

The variability of MRSA lineages within each clonal complex was subject of intensive research in the past and a detailed overview of predominant clonal complexes and their sub-lineages was given by Monecke and colleagues in 2011 [138]. The best studied successful lineage is the epidemic MRSA (EMRSA)-15, a typical hospital associated CC22-MRSA. Only recently, Holden et al. investigated the adaptive capacity of this lineage by whole genome analysis of 193 EMRSA-15 from human origin. All MRSA originated from 15 different countries and were collected between 1990 and 2009. Based on results of whole genome data, genetic changes that accompanied the emergence of EMRSA-15 were identified. A series of gene acquisition events and mutations seemed to promote the broad success of this lineage in (human) hospitals [102]. In 2013, Loeffler et al. addressed the question, whether EMRSA-15 show host specific differences by comparison of isolates from human, dog and cat origin. For genotypic characterization the so called “whole genome microarray SAM62” was used. In comparison to the Alere Identibac *S. aureus* Genotyping chip that was used for the work of this PhD-thesis, the SAM62 includes more putative host specific genes that have been identified so far. In accordance with the findings of our study (publication 1), the survey was not able to identify host specific pattern for the investigated isolates from dogs and cats to those from humans, indicating again the initial spill-over from humans to pets [73]. These findings were verified by Harrison and colleagues who conducted a comparative analysis of EMRSA-15 isolated from companion animals and humans based on whole genome sequencing. In addition to the study outcome from Loeffler et al. the

comparison of whole genomes with regard to SNPs as well as the distribution of mobile genetic elements did not reveal species specific differences for isolates from companion animals and humans within this specific lineage [105].

While detailed comparisons of human and companion animal isolates based on the whole genome are only available for EMRSA-15 until now, data obtained during this work show that a genotypic distinction between MRSA originating from infected dogs and cats is also not possible for other genetic lineages like for example CC5. Based on the results of genotyping methods used in this study (PFGE, *spa* typing, DNA microarray analysis, see publication 2 and 4.1) it was not possible to identify a host species specific marker to distinguish between canine and feline MRSA as well as canine and human *S. aureus* (publication 1). In contrast, macrorestriction analysis revealed identical or very similar PFGE patterns for isolates from dog and cat respectively dog and human origin. The unrooted tree that was build based on the results of DNA microarray analysis (figure 7 and publication 1) further shows that a distinction between feline and canine MRSA as well as human and canine *S. aureus* is not possible based on information about the presence or absence of 334 different gene loci. Taken together, none of the applied genotyping methods allowed a host specific distinction for the examined *S. aureus*, supporting the hypothesis that the investigated genotypes show lineage specific characteristics rather than host specific elements and thus, should be addressed as EHSG-*S. aureus*. However, since this investigation was not based on the whole genome, it cannot be excluded, that unknown host specific structures might exist. Accordingly, further comparative genomic studies based on whole genome analysis of *S. aureus* obtained from veterinary and human health care settings are warranted to evaluate factors influencing success in veterinary environments (e.g. prescription of certain antibiotics, acquisition of phages encoding virulence-enhancing factors).

In order to unravel the microevolution of a bacterial species it is crucial to understand the mechanisms of diversification of bacteria. In principle, there are two different mechanisms (i) asexual and (ii) sexual diversification contributing to genetic diversity in bacteria. (i) Asexual diversification is caused by *de novo* mutations. Thus, the genetic change will be transferred exclusively via cell division. An accumulation of mutations with a benefit over bacterial populations within the same species without these mutations results in the emergence of new lineages. Asexual diversification leads to a clonal population structure. Hence, it provides information about the evolutionary history of the investigated bacterial species and further can be used to define genetic lineages within bacterial species. Multilocus sequence typing is one method for identification of lineages based on asexual diversification.

The term (ii) sexual diversification includes all mechanisms involved in horizontal genetic exchange of chromosomal DNA enabling closely related as well as distinct related bacterial populations and subpopulations to exchange genetic material. In consequence, these events enable mutations to escape the lineage they originated from and to spread independently to other lineages. Therefore, recombination events can – depending on the frequency – strongly contribute to genetic diversity in bacterial populations. Furthermore, they complicate to unravel the microevolution of bacteria. The impact of asexual and sexual diversification differs for each bacterial population ranging from highly clonal populations with occasional recombination events (for example *Mycobacterium* species [139]) to non-clonal (panmictic) bacteria with frequent recombination events that prevent the emergence of a stable sub-lineage like it has been shown for *Helicobacter pylori* [140]. Most bacterial species however diversify due to asexual and sexual changes. While recombination is an important mechanism in those bacterial populations it is not sufficiently frequent to prevent the emergence of distinct lineages [141] resulting in the widespread occurrence of distinct clusters of genotypes. For many bacterial populations (including *S. aureus*) these clusters of genotypes are mostly stable in space and time [142]. In theory, bacterial populations consist of various different genotypes displaying the “neutral diversity” of each species that is exclusively depending on mutation- and recombination rates [143]. The effective population size that is observed in surveys however is much smaller and several different reasons should be taken into consideration including among others the following points:

(i) The neutral diversity of bacterial populations can be estimated by different models that have been developed in the recent past [144]. These models are based on mutation- and recombination rates but ignore the process of natural selection. Despite this fact, these models show the dynamics of diversification in bacterial species resulting in the development of distinct lineages for species with relatively low recombination rates even in absence of selective pressure [143].

(ii) Selective pressure plays a key role in adaptation like it has been already discussed for use of antibiotics (chapter 5.1). Extreme environmental conditions can result in the survival of well-adapted populations in combination with the extinction of un-adapted lineages within the specific ecological niche. This process has been termed as selective sweep [144].

(iii) The sample collection is often heavily biased with an enormous overrepresentation of particularly virulent or resistant isolates. Less virulent or resistant strains are frequently neglected even though they mostly comprise the majority of the population. Thus, epidemiological investigations mostly

underestimate the diversity within one bacterial species and accordingly overestimate the extend of clonality [141]. This is particularly important for facultative pathogens like *S. aureus* as most sample collections are based on clinical isolates lacking a representative number of commensal isolates.

5.3 Molecular epidemiology of methicillin-susceptible *S. aureus* (MSSA)

Due to the enormous medical and financial burden associated with the spread of multi-drug resistant transferrable bacteria in humans and animals, research on *S. aureus* focusses particularly on MRSA resulting in a heavily biased study population like it has been discussed in the previous chapter. Analysis of MSSA however is crucial for a better understanding of the evolution, spread and adaptation of this opportunistic pathogen like has been shown for CC398 by Uhleman et al. [113] where CC398-MSSA were frequently collected from human infections. In contrast to well-characterized CC398-MRSA, isolates of the methicillin-susceptible lineage were easily transmissible between humans and further exhibited an increased ability to adhere to human keratinocytes. Genomic analysis revealed differences for CC398-MRSA and CC398-MSSA, indicating the (re-)adaptation of CC398-*S. aureus* to the human host.

A review from McCarthy et al. summarized the current knowledge on the genetic diversity of *S. aureus* in animals and revealed a wide knowledge gap concerning MSSA in companion animals [119]. To obtain data on the genetic diversity of MSSA from infectious diseases in dogs, cats and horses, a convenience sample of 150 isolates (50 per host origin) was genotypically characterized. A high variability was detected for MSSA of dog and cat origin (figures 8 and 9). MSSA belonging to clonal complexes CC45, CC5, CC15 CC30, CC8 and CC7 were identified for several canine and feline isolates. In accordance with findings for MRSA from dog and cat origin, these MSSA lineages mirror frequently occurring genotypes from human MSSA infections as well. A possible reason for the detection of similar lineages in humans as well as dogs and cats could be a spill-over from humans to dogs and cats. In line with these findings, comparative analysis of 50 canine MSSA with representative human isolates revealed no species specific differences for MSSA from clonal complexes CC5, CC8, CC15, CC22, CC30, and CC45 (publication 1). Furthermore, macrorestriction pattern from isolates of human and canine origin showed similar results, indicating the same clonal background for MSSA as well (figure 1 in publication 1). Results of microarray analysis for selected canine MSSA and human reference strains confirmed that no specific differences were detectable with regard to the composition of virulence-associated and resistance genes. While CC8-, CC5- and CC22-*S. aureus* were detected both as MSSA and MRSA,

other frequently identified lineages like CC15, CC30 and CC45 were only detected as MSSA of feline and canine origin. This is particularly interesting as these three lineages are also relevant in MRSA infections from humans [145]. The absence of CC30- and CC45-MRSA in canine and feline isolates however might be a result of the small sample size. Other possible reasons could be the limited occurrence of these lineages in Germany or the loss of the *mec*A gene. However, canine CC30-MRSA strains had been reported in the past as well showing the general possibility of dogs to get infected with MRSA from this lineage [62]. The lack of data concerning the relevance of MSSA as colonizer of small animals like dogs and cats in conjunction with missing data for MSSA-infections in these animals prohibits drawing conclusions with respect to possible infection and / or colonization routes between humans and the investigated animals. Nevertheless, the identification of similar lineages in dogs, cats and humans as well as the absence of host specific pattern indicate a general transferability of MSSA between these species as well. The characterization of canine and feline MSSA showed more diversity with regard to the identified genetic lineages in comparison to MRSA genotypes underlining the importance to include a broad study population like discussed in chapter 5.2 to get insight into the variability of *S. aureus* sub-populations for each host. Even though the sample collection included MSSA as well as MRSA from dogs and cats it needs to be taken into account that all samples originated from infected animals. Since it is known that *S. aureus* can colonize dogs and cats as well it would be necessary to include such samples to obtain a representative sample collection.

The characterization of 50 equine MSSA revealed a different picture in comparison to findings for feline and canine isolates comprising CC1, CC9 and CC479 as mainly identified genetic lineages. While CC1-MSSA have been already reported from horses in the past [77], reports about infections with CC9- and CC479-MSSA in horses are absent in the literature. Instead, CC9-MSSA have been isolated from various livestock, including cattle, goat and sheep [119] but also from rats until now. CC479 has been described as restricted to cattle so far [75]. Due to limited information about relevant genetic lineages for equine MSSA infections it is not possible to compare the findings obtained during this work with previous results. Therefore, it is impossible to evaluate whether the occurrence of CC9- and CC479-MSSA in equine infections within Germany displays a new development or if infections with MSSA of these genotypes have been present in the past as well.

Despite the fact that MSSA show more diversity with regard to genetic lineages in comparison to MRSA they usually include predominant MRSA genotypes within the same host and region like for example CC5 and CC22 for humans [145] as well as dogs and cats

(publication 1, 2 and chapter 4.2) indicating an advantage of these lineages independent of multi-drug resistance. Comparison of predominant genotypes in equine MRSA and MSSA infections shows that this is not the case for samples from horses investigated during this work. While CC398- and CC8-MRSA were the main genotypes in equine MRSA infections (publication 2) only one CC8-and no CC398-MSSA was identified within 50 MSSA of equine origin. The predominant MSSA genotype CC1 that accounted for 42% of 50 isolates was only present in one equine MRSA (publication 2). Infections with CC1-MRSA have been reported sporadically in horses in the past [69,146] while occurrence in human infections is quite more common [138,145]. Reports about CC1-MRSA occurred also in bulk milk from cattle [147] and in pigs (nasal colonization) [148]. Together with CC398, CC9-MRSA is described as another prominent so called LA-MRSA that can be regularly detected as colonizer in pigs [148-152].

In contrast to the study results obtained for dogs and cats, frequently occurring equine MSSA lineages are no common genotypes in MRSA infections of horses. This is particularly interesting since it is known that CC1- and CC9-*S. aureus* are able to integrate the SCC*mec* element with the *mec*A gene into the genome and thus are able to develop resistance against all beta-lactam antibiotics. While examples of successful CC1-MRSA in humans [145] and CC9-MRSA in swine [148-152] have been reported only recently, these lineages are absent in equine MRSA infections within Germany until now. If CC1- and CC9-MSSA from horses differ essentially from methicillin-resistant isolates of the same CCs and therefore might not be able to integrate the resistance mechanism encoding mobile genetic element into their genomes or if the lack of CC1- and CC9-MRSA in horses is only transient needs to be investigated in further studies. However, this example shows that continuous screening of both MRSA and MSSA is important for early detection of changes with regard to frequency and putative host adaptation.

It should be noted that the collection of MSSA was a convenience sample. Thus, the identified frequencies do not necessarily reflect the genotypic distribution of MSSA in canine, feline and equine infections. Nevertheless, it provides insight into the variability of genetic MSSA-lineages of dogs, cats and horses.

5.4 Relevance of *S. aureus* as pathogen in companion animals

Data concerning the proportion and impact of *S. aureus* and MRSA infections in clinical samples of companion animals are variable but give evidence of an increased infection rate in horses [26] as well as dogs and cats [25] during the last years. In particular wound infections were described as frequently caused by *S. aureus* [1,27]. The broad Germany-

wide screening study from publication 2, that was based on the investigation of 5,229 wound swabs from dogs, cats and horses, indeed proved the importance of *S. aureus* as pathogen especially for horses and cats. To our knowledge, this is currently the broadest study published on the relevance of *S. aureus* as pathogen in wound infections from dogs, cats and horses. Thus, data of this survey provide for the first time reliable information about the frequency and genetic background of *S. aureus* as a pathogen in wound infections from dogs, cats and horses in Germany. Overall, *S. aureus* was detected in 479 of 5,229 investigated wound swabs (9.2%) and 51.8% of these *S. aureus* were methicillin-resistant. The isolation frequency varied with respect to the host origin. *S. aureus*-positive wound swabs were most often detected for samples from horses (22.8%). In feline and canine wound swabs *S. aureus* was detected in 12.2% respectively 5.8% of all investigated samples. These considerable high proportions clearly show the enormous clinical importance of this pathogen for horses, cats and dogs. It is particularly noteworthy that high MRSA rates were detected in all three groups (62.7%, 46.4% and 41.3% of the *S. aureus* from canine, feline and equine wound swabs, respectively). In view of MRSA being a zoonotic pathogen, total MRSA proportions of 9.4% (horses), 5.7% (cats) and 3.6 % (dogs) among wound swabs are alarming and provide a source for the spread in both veterinary clinics and the community.

The observed comparatively small *S. aureus* proportion in wound swabs from dogs can be explained by the fact that infections in dogs mediated by coagulase-positive *Staphylococcus sp*. are mainly caused by *S. pseudintermedius* [95]. But still, with regard to the role of dogs as possible infection and / or colonization source for humans the MRSA-percentage remains high.

The study was biased to some degree due to inhomogeneity of the case definition “wound infection” that was dependent on veterinarians who sent their samples to Vet Med Labor GmbH (Ludwigsburg, Germany). Further, this survey included only wound infections sent to a diagnostic lab for microbiological examination but did not take empirically treated wound infections into account. Thus, calculated MRSA proportions might not reflect the MRSA and MSSA rates in the general companion animal population. Despite these limitations, the current survey gives clearly evidence for the importance of *S. aureus* in general and particularly MRSA as pathogens in wound infections of companion animals.

Phenotypic resistance profiles of all MRSA revealed a high number of multi-drug resistant strains, in particular for CC5- and CC398-MRSA, highlighting the limited therapeutic options to treat most of these MRSA infections (table 3, publication 2). Resistance profiles from

isolates of human origin associated with epidemic MRSA lineages like CC22, CC5 and CC8 within German hospitals were regularly reported by the national reference laboratory Robert Koch-Institute (RKI) [145]. For ST22, the “Barnim epidemic strain” (*spa* types t005, t002 and t032), resistance against fluoroquinolones (ciprofloxacin, moxifloxacin) was frequently observed. While care should be taken before comparing study results (CC22-MRSA reported on here comprise more than these three *spa* types), the frequent resistance (97.8%) against fluoroquinolones (enrofloxacin, marbofloxacin) is also present in CC22-MRSA of companion animal origin. CC5-MRSA, human as well as companion animal strains, seem to express resistance against fluoroquinolones, clindamycin and erythromycin on a regular basis. For CC8-MRSA, a diverse phenotypic resistance pattern was observed. These results are in accordance with findings for CC8-MRSA of human origin [153].

5.5 The role of *S. aureus* as a nosocomial pathogen in companion animal medicine

It is well known that MRSA are important nosocomial pathogens in both human and veterinary medicine. To evaluate the possible appearance of nosocomial infections within this study the number of MRSA-positive wound swabs was determined for each veterinarian institution (figure 5). As all samples were collected in collaboration with Vet Med Labor Ludwigsburg information about the type of the institution (clinic or practice) are not available for this study. While 75 institutions contributed a single MRSA-positive isolate to the screening study, the other 38 clinics / practices sent in multiple MRSA-positive swabs ranging from 2 to 13 positive samples within the 17 months sampling period. Most clinics / practices however sent in between one and three positive swabs (n=93). The two veterinarian institutions that provided 13 positive swabs were investigated towards possible nosocomial infections by connecting genotyping results with time of sampling. With the help of such an analysis (4.1.2) three respectively two chronologically closely connected MRSA with identical macrorestriction pattern were detected for both institutions (isolated within 14 days). Further, two MRSA with identical pattern were isolated between 14 and 30 days, indicating a possible time connection. Since MRSA is known as an important nosocomial pathogen in veterinary medicine [26,107,154] it seems very likely that nosocomial infections took place in both veterinary institutions. Nevertheless, this conclusion cannot be drawn based on the available data. In order to make a statement concerning nosocomial spread in these clinics / practices a sound epidemiologic investigation would be necessary, including sampling of veterinarians and surfaces within each clinic as well. However, the investigation of risk factors for MRSA infections in companion animals, which has been conducted recently by our working group,

identified the clinic size as an important risk factor, indicating once more the role of nosocomial transmission as an important route for MRSA infections in companion animal clinics [155]. To identify and eliminate nosocomial infection routes it will be necessary to implement sound surveillance systems in companion animal clinics. Surveillance is based on continual collection, analysis and interpretation of relevant data for important nosocomial pathogens like for example MRSA. Therefore, it is not only necessary to build but also to maintain a database which includes important patient-specific parameters as well as antibiotic treatment. If the analysis of the data shows unusually high detection rates of nosocomial pathogens possible causes need to be determined. Based on the identified cause targeted intervention strategies need to be implemented to prevent further nosocomial spread. Finally, applied strategies need to be evaluated. During the whole process, the staff (including veterinarians as well as assistants) needs to get feedback according to the evaluated data on a regular basis as well as instructions for intervention strategies to minimize the risk for nosocomial infections. In order to obtain ideal results, it would be necessary to compare data to other companion animal clinics. Only by comparing infection rates of nosocomial pathogens with other companion animal clinics it would be possible to evaluate the frequency and therefore the risk of nosocomial spread within each clinic. Hence, surveillance systems within different clinics should be comparable with regard to collection, analysis and interpretation of data.

In contrast to veterinary medicine, surveillance systems in human medicine are widely used. One of the best-known surveillance systems is the KISS (Krankenhaus-Interventions-Surveillance-System) initiative that was conducted by the national reference center from the Robert-Koch-Institut (RKI) in 1996 (http://www.nrz-hygiene.de/surveillance/kiss/). In general, each hospital is welcome to participate after qualification through successful completion of a curse introducing KISS data collection methods and training diagnosis. Although surveillance systems in human medicine can be still improved [156] the knowledge obtained during the last decade for human healthcare systems provides a solid basis for veterinary medicine and therefore should be introduced into companion animal clinics as a cornerstone to prevent infections.

6. Conclusion

Obtained data from this study provide evidence for the importance and impact of *S. aureus* in general, and MRSA in particular, as a cause of wound infections in companion animals in Germany. The high MRSA-rate is especially worrisome since multi-drug resistance of these strains leads to decreased therapeutic options resulting in a higher burden of disease.

All genetic lineages detected for both MRSA and MSSA from companion animals have been often isolated from different hosts as well, showing a lack of strict host restriction. Extended host spectrum genotypes (EHSG) from dogs and cats displayed common human lineages and were further undistinguishable from representative isolates of human origin. Furthermore, several studies described the transmission between humans and their pets in the literature so that humans should be considered as an important infection source for dogs and cats. On the other hand it is also very likely that dogs and cats are a (re) infection and / or colonization source for humans. In future, longitudinal studies should be conducted to gain more knowledge about the transmission frequency between pets and their owners as well as the risk factors involved.

The emergence of CC398-MRSA as predominant genotype in equine wound infections demonstrates the adaptive capacity of this lineage to other hosts and niches besides livestock and humans and further demonstrates an epidemiological change over time in horses. Since CC398-MRSA can be also regularly identified in samples from dogs and cats, each of these animals should be considered as potential infection and / or colonization source for humans. Our findings clearly demonstrate the need for on-going surveillance to display dynamics and spread of *S. aureus* among these animals and furthermore to implement targeted intervention strategies to prevent nosocomial spread within clinics. Further investigation including whole genome sequencing, phenotypic appearance and functional studies are needed to unveil factors and structures influencing the host adaptation processes like it has been successfully demonstrated for adaptation of a CC5-S. *aureus* sub-lineage to poultry [30]. Therefore, further attempts to reduce the total burden of MRSA, in each field afflicted with this pathogen, are clearly needed. Transmission of MRSA between different hosts (humans, animals) as well as between different ecological niches (hospital, community, veterinarian hospitals) should be regarded as a major “One Health” challenge.

7. Summary

Staphylococcus (S.) aureus colonizes the skin and mucosa of various mammalian hosts, and up to 30% of the human population is permanently colonized. As a zoonotic pathogen, *S. aureus* plays an important role in both human and veterinary medicine, affecting a broad range of animals. When the general condition of the (animal or human) patient allows the bacteria to cause an infection, the diseases range from superficial to life-threatening. Especially infections with methicillin-resistant *S. aureus* (MRSA) are a major burden in medicine due to limited treatment options. The increasing detection of MRSA as causative agent of purulent infections in companion animal patients like dogs, cats and horses in the recent past resulted in a discussion about the general relevance and impact of this observation for these animals as well as for Public Health. In consequence, several localized studies investigated and reported MRSA infection frequencies. Further, genotypic characterization of MRSA from companion animal origin indicated the occurrence of genotypes that are also commonly reported in human medicine. The aim of this study was to obtain data on the frequency, geographic origin as well as information on numerous molecular characteristics of *S. aureus* isolated from infected dogs, cats and horses. Thus, this work provides for the first time both reliable and representative data, obtained by investigation of a large sample size within Germany.

Results achieved by the analysis of 5,229 wound swabs from dogs, cats and horses demonstrated the importance and impact of *S. aureus* in general, and MRSA in particular, as a cause of wound infections in companion animals in Germany. The detected high MRSA-rates (62.7% for dogs, 46.4% for cats and 41.3% for horses) within this study are especially worrisome since multi-drug resistance in many of these strains leads to decreased therapeutic options. Genotypic characterization of MRSA, that was performed by use of various typing methods, identified in samples from dogs and cats mainly genetic lineages that are also relevant for infections in humans within Germany (for example clonal complexes CC5 and CC22). These results proof once more the existence of *S. aureus* belonging to extended host spectrum genotypes (EHSG) with the ability to infect several hosts. For MRSA of equine origin, most of the isolates (87.7%) belonged to CC398, a genetic lineage that can be isolated from livestock, dogs, cats and humans. The emergence of CC398-MRSA as predominant genotype in equine wound infections demonstrates the adaptive capacity of this lineage to other hosts respective niches than livestock and humans, showing an epidemiological change over time of dominating MRSA genotypes in horses.

Further comparative analysis of EHSG-*S. aureus* originating from companion animals and humans based on various genetic characteristics by use of *spa* typing, multilocus sequence typing (MLST), pulsed field gel electrophoresis (PFGE) and DNA microarray analysis revealed a lack of host adaptation for both, methicillin-resistant as well as methicillin-susceptible (MSSA) isolates. Thus, MSSA from companion animals have to be considered as EHSG as well. The minor variations of all investigated *S. aureus* was independent of each respective host origin but rather displayed the natural variation within each investigated lineage.

In conclusion, wound infections caused by *S. aureus* are a burden in companion animal medicine and the identified high infection rates with MRSA are of special concern due to limited treatment options. Genotypic investigation of isolates from companion animal origin revealed common human lineages that were further undistinguishable from representative isolates of human origin. Therefore, these animals should be considered as an infection source for humans and vice versa. To reduce the total burden of MRSA in both, human and veterinary medicine, it is necessary to take various epidemiological pathways into account and thus, to strengthen the collaboration between human and veterinary medicine according to the concept of “One Health”.

8. Zusammenfassung

Staphylococcus aureus von Begleittieren: Eine Infektionsquelle für die Gesellschaft?

Staphylococcus (S.) aureus kolonisiert die Haut und Schleimhäute vieler Mammalia und bis zu 30% der Menschen sind permanent besiedelt. Als zoonotischer Erreger ist *S. aureus* sowohl in der Human- als auch in der Tiermedizin bedeutsam, da Infektionen durch *S. aureus* bei vielen verschiedenen Tierarten relevant sind. Wenn die Gesamtkondition des Patienten (Mensch oder Tier) eine Infektion ermöglicht, können die Krankheiten entweder oberflächlich oder aber lebensbedrohlich sein. Insbesondere Infektionen mit Methicillin-resisenten *S. aureus* (MRSA) sind, bedingt durch die limitierten Behandlungsmöglichkeiten, eine schwere Belastung für die Medizin. Der vermehrte Nachweis von MRSA als Erreger purulenter Infektionen bei Begleittieren wie beispielsweise Hunden, Katzen und Pferden in jüngerer Zeit führte zu einer Diskussion über die Relevanz und Bedeutung von MRSA-Infektionen bei diesen Tieren sowie in Bezug auf die öffentliche Gesundheit. Daraufhin wurden verschiedene Studien durchgeführt, die überwiegend in kleinen Gebieten die Häufigkeit von MRSA-Infektionen untersuchten. Zusätzlich wurden isolierte MRSA genotypisch charakterisiert, wobei gezeigt werden konnte, dass MRSA von Begleittieren häufig Genotypen aufweisen, die auch humanmedizinisch relevant sind. Ziel dieser Arbeit war es, Informationen über die Häufigkeit und geographische Verteilung von *S. aureus*-bedingten Infektionen zu erhalten und des Weiteren Daten zu molekularen Charakteristiken zu Isolaten von infizierten Hunden, Katzen und Pferden bereitzustellen. Die Arbeit stellt zum ersten Mal repräsentative und belastbare Zahlen zur Verfügung, die im Rahmen einer Deutschland-weiten Untersuchung erarbeitet wurden.

Die Ergebnisse der Untersuchung, basierend auf der Analyse von 5.229 Wundtupfern von Hunden, Katzen und Pferden, zeigt die Bedeutung von *S. aureus* allgemein sowie insbesondere von MRSA als Wund-Infektionserreger bei Begleittieren innerhalb Deutschlands. Die im Rahmen dieser Arbeit detektieren hohen MRSA-Raten (62,7% für Hunde, 46,4% für Katzen und 41,3% für Pferde) sind besonders besorgniserregend, da Multiresistenzen in vielen dieser Isolate verringerte Therapiemöglichkeiten zur Folge haben. Die genotypische Charakterisierung der isolierten MRSA wurde mit Hilfe verschiedener Typisierungsmethoden durchgeführt, wobei identifizierte Genotypen der Begleittier-Proben überwiegend relevante genetische Linien aus der Humanmedizin wiederspiegelten (zum Beispiel klonale Komplexe (CC)5 und CC22). Dieses Ergebnis bestätigt einmal mehr die

Existenz von *S. aureus*, die Genotypen mit erweiterten Wirtsspektren aufweisen (aus dem englischen: extended host spectrum genotypes: EHSG) und somit die Möglichkeit besitzen, verschiedene Wirte zu infizieren. Bei Pferden hingegen wurden überwiegend CC398-MRSA isoliert (87,7%). Hierbei handelt es sich um einen Genotypen der auch bei landwirtschaftlichen Nutztieren, Hunden, Katzen und Menschen isoliert werden kann. Das Auftreten von CC398-MRSA als prädominanter Genotyp in equinen Wundinfektionen zeigt die adaptive Kapazität dieser genetischen Linie zu anderen Wirten beziehungsweise Nischen als landwirtschaftliche Nutztiere und Menschen. Außerdem zeigt die Prädominanz von CC398-MRSA den epidemiologischen Wandel dominanter MRSA-Genotypen bei Pferden im Laufe der Jahre auf.

Basierend auf verschiedenen genetischen Eigenschaften wurden unter der Nutzung von *Spa*-Typisierung, Multilokus-Sequenztypisierung (MLST), Pulsfeldgelelektrophorese (PFGE) und DNA-Microarray-Analyse weitere vergleichende Untersuchungen von EHSG-*S. aureus* von Begleittieren und Menschen durchgeführt. Dabei konnte gezeigt werden, dass sowohl untersuchte Methicillin-resistente als auch Methicillin-sensible (MSSA) *S. aureus* keine Wirts-spezifischen Eigenschaften aufwiesen. Deshalb müssen auch MSSA von Begleittieren als EHSG in Betracht gezogen werden. Vorhandene genetische Variationen in den untersuchten Isolaten waren unabhängig von der jeweiligen Wirtsspezies. Vielmehr stellte sie die natürliche Variation innerhalb jeder untersuchten genetischen Linie dar.

Mit Hilfe dieser Arbeit konnte gezeigt werden, dass *S. aureus* ein wichtiger Wundinfektionserreger bei den untersuchten Begleittieren ist. Insbesondere die hohe MRSA-Rate ist wegen der limitierten Behandlungsmöglichkeiten sehr besorgniserregend. Die genetische Untersuchung der Isolate von Begleittieren zeigte eine Übereinstimmung zu humanmedizinisch bedeutsamen Genotypen und mit Hilfe der durchgeführten Methoden war eine Unterscheidung der Isolate mit *S. aureus* von humanen Infektionen nicht möglich. Daher sollten die untersuchten Tierarten als Infektionsquelle für Menschen sowie Menschen als Infektionsquelle für Begleittiere in Betracht gezogen werden. Um das Infektionsrisiko mit MRSA sowohl im Bereich der Human- als auch der Tiermedizin zu verringern ist es nötig, verschiedene epidemiologische Routen in Betracht zu ziehen. Dafür muss im Sinne des Konzeptes „One Health“ die Zusammenarbeit zwischen Human- und Tiermedizin ausgeweitet und verstärkt werden.

9. Literature

1. Walther B, Wieler LH, Friedrich AW, Hanssen AM, Kohn B, et al. (2008) Methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from small and exotic animals at a university hospital during routine microbiological examinations. Vet Microbiol 127: 171-178.

2. Walther B, Hermes J, Cuny C, Wieler LH, Vincze S, et al. (2012) Sharing More than Friendship - Nasal Colonization with Coagulase-Positive Staphylococci (CPS) and Co-Habitation Aspects of Dogs and Their Owners. PLoS One 7: e35197.

3. Faires MC, Tater KC, Weese JS (2009) An investigation of methicillin-resistant *Staphylococcus aureus* colonization in people and pets in the same household with an infected person or infected pet. J Am Vet Med Assoc 235: 540-543.

4. Ferreira JP, Anderson KL, Correa MT, Lyman R, Ruffin F, et al. (2011) Transmission of MRSA between companion animals and infected human patients presenting to outpatient medical care facilities. PLoS One 6: e26978.

5. Manian FA (2003) Asymptomatic nasal carriage of mupirocin-resistant, methicillin-resistant *Staphylococcus aureus* (MRSA) in a pet dog associated with MRSA infection in household contacts. Clin Infect Dis 36: e26-28.

6. Weese JS, Archambault M, Willey BM, Hearn P, Kreiswirth BN, et al. (2005) Methicillin-resistant *Staphylococcus aureus* in horses and horse personnel, 2000-2002. Emerg Infect Dis 11: 430-435.

7. Kloos WE (1980) Natural populations of the genus *Staphylococcus*. Annu Rev Microbiol 34: 559-592.

8. Schleifer K-H, Bell J. A. (2009) Bergey´s Manual of Systematic Bacteriology. In: De Vos P, Garrity G. M., Jones, D., Krieg, N. R., Ludwig, W.,, Rainey FA, Schleifer, K.-H. & Whitman, W. B., editors. The Firmicutes. second edition ed. New York: Springer. pp. 392.

9. Euzéby J (2010) List of new names and new combinations previously effectively, but not validly, published. International Journal of Systematic and Evolutionary Microbiology 60: 469-472.

10. Bannermann TPS (2007) *Staphylococcus*, *Micrococcus*, and other catalase-positive Cocci. Manual of Clinical Microbiology. Washington DC: ASM Press. pp. 390-412.

11. Hermans K, Devriese, L.A.; Haesebrouck, F. (2004) *Staphylococcus*. In: Gyles CL PJ, Songer JG, Thoen CO, editor. Pathogenesis of Bacterial Infections in Animals. Third edition ed: Blackwell Publishing. pp. 43-56.

12. Cowan ST, Shaw C, Williams RE (1954) Type strain for *Staphylococcus aureus* Rosenbach. J Gen Microbiol 10: 174-176.

13. Kluytmans J, van Belkum A, Verbrugh H (1997) Nasal carriage of *Staphylococcus aureus*: epidemiology, underlying mechanisms, and associated risks. Clin Microbiol Rev 10: 505-520.

14. Morgan M (2008) Methicillin-resistant *Staphylococcus aureus* and animals: zoonosis or humanosis? J Antimicrob Chemother 62: 1181-1187.

15. Foster Tj (2002) *Staphylococcus aureus*. In: Sussmann M, editor. Molecular Medical Microbiology. Newcastle upon Tyne: Academic Press. pp. 839-888.

16. Calfee DP, Salgado CD, Classen D, Arias KM, Podgorny K, et al. (2008) Strategies to prevent transmission of methicillin-resistant *Staphylococcus aureus* in acute care hospitals. Infect Control Hosp Epidemiol 29 Suppl 1: S62-80.

17. Jevons MP (1961) Celbenin-resistant staphylococci. British Medical Journal: 124-125.

18. Parker MT, Jevons MP (1964) A Survey of Methicillin Resistance in *Staphylococcus Aureus*. Postgrad Med J 40: SUPPL:170-178.

19. Lyon BR, Skurray R (1987) Antimicrobial resistance of *Staphylococcus aureus*: genetic basis. Microbiol Rev 51: 88-134.

20. Otto M (2012) MRSA virulence and spread. Cell Microbiol 14: 1513-1521.

21. Devriese LA, Van Damme LR, Fameree L (1972) Methicillin (cloxacillin)-resistant *Staphylococcus aureus* strains isolated from bovine mastitis cases. Zentralbl Veterinarmed B 19: 598-605.

22. Ola Ojo M (1972) Bacteriophage types and antibiotic sensitivity of *Staphylococcus aureus* isolated from swabs of the noses and skins of dogs. Vet Rec 91: 152-153.

23. Catry B, Van Duijkeren E, Pomba MC, Greko C, Moreno MA, et al. (2010) Reflection paper on MRSA in food-producing and companion animals: epidemiology and control options for human and animal health. Epidemiol Infect 138: 626-644.

24. Weese JS (2005) Methicillin-resistant *Staphylococcus aureus*: an emerging pathogen in small animals. J Am Anim Hosp Assoc 41: 150-157.

25. Leonard FC, Markey BK (2008) Meticillin-resistant *Staphylococcus aureus* in animals: a review. Vet J 175: 27-36.

26. van Duijkeren E, Moleman M, Sloet van Oldruitenborgh-Oosterbaan MM, Multem J, Troelstra A, et al. (2010) Methicillin-resistant *Staphylococcus aureus* in horses and horse personnel: an investigation of several outbreaks. Vet Microbiol 141: 96-102.

27. Axon JE, Carrick JB, Barton MD, Collins NM, Russell CM, et al. (2011) Methicillin-resistant *Staphylococcus aureus* in a population of horses in Australia. Aust Vet J 89: 221-225.

28. O'Mahony R, Abbott Y, Leonard FC, Markey BK, Quinn PJ, et al. (2005) Methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from animals and veterinary personnel in Ireland. Vet Microbiol 109: 285-296.

29. Hasman H, Moodley A, Guardabassi L, Stegger M, Skov RL, et al. (2010) Spa type distribution in *Staphylococcus aureus* originating from pigs, cattle and poultry. Vet Microbiol 141: 326-331.

30. Lowder BV, Guinane CM, Ben Zakour NL, Weinert LA, Conway-Morris A, et al. (2009) Recent human-to-poultry host jump, adaptation, and pandemic spread of *Staphylococcus aureus*. Proc Natl Acad Sci U S A 106: 19545-19550.

31. Smith JA, McNamee PT (2001) Staphylococci, streptococci and enterococci, Poultry Diseases. In: Jordan F, Pattison M, Alexander D, Faragher T, editors. 6th edition ed: Elsevier. pp. 191-199.

32. Vanderhaeghen W, Hermans K, Haesebrouck F, Butaye P (2010) Methicillin-resistant *Staphylococcus aureus* (MRSA) in food production animals. Epidemiol Infect 138: 606-625.

33. Weese JS (2010) Methicillin-resistant *Staphylococcus aureus* in animals. ILAR J 51: 233-244.

34. Fessler A, Scott C, Kadlec K, Ehricht R, Monecke S, et al. (2010) Characterization of methicillin-resistant *Staphylococcus aureus* ST398 from cases of bovine mastitis. J Antimicrob Chemother 65: 619-625.

35. Hauschild T, Fessler AT, Kadlec K, Billerbeck C, Schwarz S (2012) Detection of the novel vga(E) gene in methicillin-resistant *Staphylococcus aureus* CC398 isolates from cattle and poultry. J Antimicrob Chemother 67: 503-504.

36. Monecke S, Kuhnert P, Hotzel H, Slickers P, Ehricht R (2007) Microarray based study on virulence-associated genes and resistance determinants of *Staphylococcus aureus* isolates from cattle. Vet Microbiol 125: 128-140.

37. Lambert PA, Allison D.G., Gilbert P. (2002) Antibiotics that act on the Cell Wall and Membrane. In: Sussmann M, editor. Molecular Medical Microbiology. Newcastle upon Tyne: Academic Press. pp. 591-593.

38. Bondi A, Jr., Dietz CC (1945) Penicillin resistant staphylococci. Proc Soc Exp Biol Med 60: 55-58.

39. Barber M (1947) Staphylococcal infection due to penicillin-resistant strains. Br Med J 2: 863-865.

40. Barber M, Rozwadowska-Dowzenko M (1948) Infection by penicillin-resistant staphylococci. Lancet 2: 641-644.

41. Jovetic S, Zhu Y, Marcone GL, Marinelli F, Tramper J (2010) beta-Lactam and glycopeptide antibiotics: first and last line of defense? Trends Biotechnol 28: 596-604.

42. Brown DF, Edwards DI, Hawkey PM, Morrison D, Ridgway GL, et al. (2005) Guidelines for the laboratory diagnosis and susceptibility testing of methicillin-resistant *Staphylococcus aureus* (MRSA). J Antimicrob Chemother 56: 1000-1018.

43. Hartman B, Tomasz A (1981) Altered penicillin-binding proteins in methicillin-resistant strains of *Staphylococcus aureus*. Antimicrob Agents Chemother 19: 726-735.

44. Garcia-Alvarez L, Holden MT, Lindsay H, Webb CR, Brown DF, et al. (2011) Meticillin-resistant *Staphylococcus aureus* with a novel *mec*A homologue in human and bovine populations in the UK and Denmark: a descriptive study. Lancet Infect Dis 11: 595-603.

45. Murchan S, Kaufmann ME, Deplano A, De Ryck R, Struelens M, et al. (2003) Harmonization of pulsed-field gel electrophoresis protocols for epidemiological typing of strains of methicillin-resistant *Staphylococcus aureus*: A single approach developed by consensus in 10 European laboratories and its application for tracing the spread of related strains. Journal of Clinical Microbiology 41: 1574-1585.

46. Schlievert PM, Case LC (2007) Molecular analysis of staphylococcal superantigens. Methods Mol Biol 391: 113-126.

47. Schneewind O, Fowler A, Faull KF (1995) Structure of the cell wall anchor of surface proteins in *Staphylococcus aureus*. Science 268: 103-106.

48. Foster TJ, Hook M (1998) Surface protein adhesins of *Staphylococcus aureus*. Trends Microbiol 6: 484-488.

49. Verdon J, Girardin N, Lacombe C, Berjeaud JM, Hechard Y (2009) delta-hemolysin, an update on a membrane-interacting peptide. Peptides 30: 817-823.

50. Grumann D, Nubel U, Broker BM (2013) *Staphylococcus aureus* toxins - Their functions and genetics. Infect Genet Evol.

51. Feil EJ, Cooper JE, Grundmann H, Robinson DA, Enright MC, et al. (2003) How clonal is *Staphylococcus aureus*? J Bacteriol 185: 3307-3316.

52. Chambers HF, Deleo FR (2009) Waves of resistance: *Staphylococcus aureus* in the antibiotic era. Nat Rev Microbiol 7: 629-641.

53. Enright MC, Robinson DA, Randle G, Feil EJ, Grundmann H, et al. (2002) The evolutionary history of methicillin-resistant *Staphylococcus aureus* (MRSA). Proc Natl Acad Sci U S A 99: 7687-7692.

54. Herold BC, Immergluck LC, Maranan MC, Lauderdale DS, Gaskin RE, et al. (1998) Community-acquired methicillin-resistant *Staphylococcus aureus* in children with no identified predisposing risk. JAMA 279: 593-598.

55. Morrison MA, Hageman JC, Klevens RM (2006) Case definition for community-associated methicillin-resistant *Staphylococcus aureus*. J Hosp Infect 62: 241.

56. Aires-de-Sousa M, Parente CE, Vieira-da-Motta O, Bonna IC, Silva DA, et al. (2007) Characterization of *Staphylococcus aureus* isolates from buffalo, bovine, ovine, and caprine milk samples collected in Rio de Janeiro State, Brazil. Appl Environ Microbiol 73: 3845-3849.

57. Hata E, Katsuda K, Kobayashi H, Uchida I, Tanaka K, et al. (2010) Genetic variation among *Staphylococcus aureus* strains from bovine milk and their relevance to methicillin-resistant isolates from humans. J Clin Microbiol 48: 2130-2139.

58. Ikawaty R, Brouwer EC, Jansen MD, van Duijkeren E, Mevius D, et al. (2009) Characterization of Dutch *Staphylococcus aureus* from bovine mastitis using a Multiple Locus Variable Number Tandem Repeat Analysis. Vet Microbiol 136: 277-284.

59. Rabello RF, Moreira BM, Lopes RM, Teixeira LM, Riley LW, et al. (2007) Multilocus sequence typing of *Staphylococcus aureus* isolates recovered from cows with mastitis in Brazilian dairy herds. J Med Microbiol 56: 1505-1511.

60. Smith EM, Green LE, Medley GF, Bird HE, Fox LK, et al. (2005) Multilocus sequence typing of intercontinental bovine *Staphylococcus aureus* isolates. J Clin Microbiol 43: 4737-4743.

61. Guinane CM, Ben Zakour NL, Tormo-Mas MA, Weinert LA, Lowder BV, et al. (2010) Evolutionary genomics of *Staphylococcus aureus* reveals insights into the origin and molecular basis of ruminant host adaptation. Genome Biol Evol 2: 454-466.

62. Loeffler A, Pfeiffer DU, Lloyd DH, Smith H, Soares-Magalhaes R, et al. (2010) Meticillin-resistant *Staphylococcus aureus* carriage in UK veterinary staff and owners of infected pets: new risk groups. J Hosp Infect 74: 282-288.

63. Strommenger B, Kehrenberg C, Kettlitz C, Cuny C, Verspohl J, et al. (2006) Molecular characterization of methicillin-resistant *Staphylococcus aureus* strains from pet animals and their relationship to human isolates. J Antimicrob Chemother 57: 461-465.

64. Armand-Lefevre L, Ruimy R, Andremont A (2005) Clonal comparison of *Staphylococcus aureus* isolates from healthy pig farmers, human controls, and pigs. Emerg Infect Dis 11: 711-714.

65. Lewis HC, Molbak K, Reese C, Aarestrup FM, Selchau M, et al. (2008) Pigs as source of methicillin-resistant *Staphylococcus aureus* CC398 infections in humans, Denmark. Emerg Infect Dis 14: 1383-1389.

66. Nemati M, Hermans K, Lipinska U, Denis O, Deplano A, et al. (2008) Antimicrobial resistance of old and recent *Staphylococcus aureus* isolates from poultry: first detection of livestock-associated methicillin-resistant strain ST398. Antimicrob Agents Chemother 52: 3817-3819.

67. Vanderhaeghen W, Cerpentier T, Adriaensen C, Vicca J, Hermans K, et al. (2010) Methicillin-resistant *Staphylococcus aureus* (MRSA) ST398 associated with clinical and subclinical mastitis in Belgian cows. Vet Microbiol 144: 166-171.

68. Van den Eede A, Martens A, Lipinska U, Struelens M, Deplano A, et al. (2009) High occurrence of methicillin-resistant *Staphylococcus aureus* ST398 in equine nasal samples. Vet Microbiol 133: 138-144.

69. Walther B, Monecke S, Ruscher C, Friedrich AW, Ehricht R, et al. (2009) Comparative molecular analysis substantiates zoonotic potential of equine methicillin-resistant *Staphylococcus aureus*. J Clin Microbiol 47: 704-710.

70. Witte W, Strommenger B, Stanek C, Cuny C (2007) Methicillin-resistant *Staphylococcus aureus* ST398 in humans and animals, Central Europe. Emerg Infect Dis 13: 255-258.

71. Devriese LA (1984) A simplified system for biotyping *Staphylococcus aureus* strains isolated from animal species. J Appl Bacteriol 56: 215-220.

72. Devriese LA, Nzuambe D, Godard C (1984) Identification and characterization of staphylococci isolated from cats. Vet Microbiol 9: 279-285.

73. Zadoks R, van Leeuwen W, Barkema H, Sampimon O, Verbrugh H, et al. (2000) Application of pulsed-field gel electrophoresis and binary typing as tools in veterinary clinical microbiology and molecular epidemiologic analysis of bovine and human *Staphylococcus aureus* isolates. J Clin Microbiol 38: 1931-1939.

74. Reinoso E, Bettera S, Frigerio C, DiRenzo M, Calzolari A, et al. (2004) RAPD-PCR analysis of *Staphylococcus aureus* strains isolated from bovine and human hosts. Microbiol Res 159: 245-255.

75. Reinoso EB, El-Sayed A, Lammler C, Bogni C, Zschock M (2008) Genotyping of *Staphylococcus aureus* isolated from humans, bovine subclinical mastitis and food samples in Argentina. Microbiol Res 163: 314-322.

76. Herron-Olson L, Fitzgerald JR, Musser JM, Kapur V (2007) Molecular correlates of host specialization in *Staphylococcus aureus*. PLoS One 2: e1120.

77. Sung JM, Lloyd DH, Lindsay JA (2008) *Staphylococcus aureus* host specificity: comparative genomics of human versus animal isolates by multi-strain microarray. Microbiology 154: 1949-1959.

78. van Wamel WJ, Rooijakkers SH, Ruyken M, van Kessel KP, van Strijp JA (2006) The innate immune modulators staphylococcal complement inhibitor and chemotaxis inhibitory protein of *Staphylococcus aureus* are located on beta-hemolysin-converting bacteriophages. J Bacteriol 188: 1310-1315.

79. Verkaik NJ, Benard M, Boelens HA, de Vogel CP, Nouwen JL, et al. (2011) Immune evasion cluster-positive bacteriophages are highly prevalent among human *Staphylococcus aureus* strains, but they are not essential in the first stages of nasal colonization. Clin Microbiol Infect 17: 343-348.

80. Spoor LE, McAdam PR, Weinert LA, Rambaut A, Hasman H, et al. (2013) Livestock Origin for a Human Pandemic Clone of Community-Associated Methicillin-Resistant *Staphylococcus aureus*. MBio 4.

81. Price LB, Stegger M, Hasman H, Aziz M, Larsen J, et al. (2012) *Staphylococcus aureus* CC398: Host Adaptation and Emergence of Methicillin Resistance in Livestock. MBio 3.

82. Ballhausen B, Jung P, Kriegeskorte A, Makgotlho PE, Ruffing U, et al. (2014) LA-MRSA CC398 differ from classical community acquired-MRSA and hospital acquired-MRSA lineages: Functional analysis of infection and colonization processes. Int J Med Microbiol.

83. Haenni M, Saras E, Chatre P, Medaille C, Bes M, et al. (2012) A USA300 variant and other human-related methicillin-resistant *Staphylococcus aureus* strains infecting cats and dogs in France. J Antimicrob Chemother 67: 326-329.

84. Loeffler A, Pfeiffer DU, Lindsay JA, Magalhaes RJ, Lloyd DH (2010) Prevalence of and risk factors for MRSA carriage in companion animals: a survey of dogs, cats and horses. Epidemiol Infect: 1-10.

85. Stefani S, Chung DR, Lindsay JA, Friedrich AW, Kearns AM, et al. (2012) Meticillin-resistant *Staphylococcus aureus* (MRSA): global epidemiology and harmonisation of typing methods. Int J Antimicrob Agents 39: 273-282.

86. Enright MC, Spratt BG (1999) Multilocus sequence typing. Trends Microbiol 7: 482-487.

87. Enright MC, Day NP, Davies CE, Peacock SJ, Spratt BG (2000) Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. J Clin Microbiol 38: 1008-1015.

88. Spratt BG (2004) Exploring the concept of clonality in bacteria. Methods Mol Biol 266: 323-352.

89. Gomes AR, Vinga S, Zavolan M, de Lencastre H (2005) Analysis of the genetic variability of virulence-related loci in epidemic clones of methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother 49: 366-379.

90. Frenay HM, Theelen JP, Schouls LM, Vandenbroucke-Grauls CM, Verhoef J, et al. (1994) Discrimination of epidemic and nonepidemic methicillin-resistant *Staphylococcus aureus* strains on the basis of protein A gene polymorphism. J Clin Microbiol 32: 846-847.

91. Shopsin B, Gomez M, Montgomery SO, Smith DH, Waddington M, et al. (1999) Evaluation of protein A gene polymorphic region DNA sequencing for typing of *Staphylococcus aureus* strains. J Clin Microbiol 37: 3556-3563.

92. Harmsen D, Claus H, Witte W, Rothganger J, Turnwald D, et al. (2003) Typing of methicillin-resistant *Staphylococcus aureus* in a university hospital setting by using novel software for *spa* repeat determination and database management. J Clin Microbiol 41: 5442-5448.

93. David MZ, Taylor A, Lynfield R, Boxrud DJ, Short G, et al. (2013) Comparing pulsed-field gel electrophoresis with multilocus sequence typing, *spa* typing, staphylococcal cassette chromosome *mec* (SCC*mec*) typing, and PCR for panton-valentine leukocidin, arcA, and opp3 in methicillin-resistant *Staphylococcus aureus* isolates at a U.S. Medical Center. J Clin Microbiol 51: 814-819.

94. Pomba C, Baptista FM, Couto N, Loucao F, Hasman H (2010) Methicillin-resistant *Staphylococcus aureus* CC398 isolates with indistinguishable *Apa*I restriction patterns in colonized and infected pigs and humans. J Antimicrob Chemother 65: 2479-2481.

95. Aires de Sousa M, Sanches IS, Ferro ML, Vaz MJ, Saraiva Z, et al. (1998) Intercontinental spread of a multidrug-resistant methicillin-resistant *Staphylococcus aureus* clone. J Clin Microbiol 36: 2590-2596.

96. Roman RS, Smith J, Walker M, Byrne S, Ramotar K, et al. (1997) Rapid geographic spread of a methicillin-resistant *Staphylococcus aureus* strain. Clin Infect Dis 25: 698-705.

97. Monecke S, Berger-Bachi B, Coombs G, Holmes A, Kay I, et al. (2007) Comparative genomics and DNA array-based genotyping of pandemic *Staphylococcus aureus* strains encoding Panton-Valentine leukocidin. Clin Microbiol Infect 13: 236-249.

98. Lindsay JA (2010) Genomic variation and evolution of *Staphylococcus aureus*. Int J Med Microbiol 300: 98-103.

99. Wilson DJ (2012) Insights from genomics into bacterial pathogen populations. PLoS Pathog 8: e1002874.

100. Castillo-Ramirez S, Harris SR, Holden MT, He M, Parkhill J, et al. (2011) The impact of recombination on dN/dS within recently emerged bacterial clones. PLoS Pathog 7: e1002129.

101. Strommenger B, Braulke C, Heuck D, Schmidt C, Pasemann B, et al. (2008) spa Typing of *Staphylococcus aureus* as a frontline tool in epidemiological typing. J Clin Microbiol 46: 574-581.

102. Holden MT, Hsu LY, Kurt K, Weinert LA, Mather AE, et al. (2013) A genomic portrait of the emergence, evolution, and global spread of a methicillin-resistant *Staphylococcus aureus* pandemic. Genome Res 23: 653-664.

103. Holmes A, McAllister G, McAdam PR, Hsien Choi S, Girvan K, et al. (2014) Genome-wide single nucleotide polymorphism-based assay for high-resolution epidemiological analysis of the methicillin-resistant *Staphylococcus aureus* hospital clone EMRSA-15. Clin Microbiol Infect 20: O124-131.

104. Harrison EM, Paterson GK, Holden MT, Larsen J, Stegger M, et al. (2013) Whole genome sequencing identifies zoonotic transmission of MRSA isolates with the novel *mec*A homologue *mec*C. EMBO Mol Med.

105. Harrison EM, Weinert LA, Holden MT, Welch JJ, Wilson K, et al. (2014) A shared population of epidemic methicillin-resistant *Staphylococcus aureus* 15 circulates in humans and companion animals. MBio 5: e00985-00913.

106. van Duijkeren E, Kamphuis M, van der Mije IC, Laarhoven LM, Duim B, et al. (2011) Transmission of methicillin-resistant *Staphylococcus pseudintermedius* between infected dogs and cats and contact pets, humans and the environment in households and veterinary clinics. Vet Microbiol 150: 338-343.

107. Walther B, Wieler LH, Friedrich AW, Kohn B, Brunnberg L, et al. (2009) *Staphylococcus aureus* and MRSA colonization rates among personnel and dogs in a small animal hospital: association with nosocomial infections. Berl Munch Tierarztl Wochenschr 122: 178-185.

108. Price JR, Golubchik T, Cole K, Wilson DJ, Crook DW, et al. (2014) Whole-genome sequencing shows that patient-to-patient transmission rarely accounts for acquisition of *Staphylococcus aureus* in an intensive care unit. Clin Infect Dis 58: 609-618.

109. Vincze S, Stamm I, Monecke S, Kopp PA, Semmler T, et al. (2013) Molecular Analysis of Human and Canine *Staphylococcus aureus* Strains Reveals Distinct Extended-Host-Spectrum Genotypes Independent of Their Methicillin Resistance. Appl Environ Microbiol 79: 655-662.

110. Bens CC, Voss A, Klaassen CH (2006) Presence of a novel DNA methylation enzyme in methicillin-resistant *Staphylococcus aureus* isolates associated with pig farming leads to uninterpretable results in standard pulsed-field gel electrophoresis analysis. J Clin Microbiol 44: 1875-1876.

111. Gomez-Sanz E, Torres C, Lozano C, Fernandez-Perez R, Aspiroz C, et al. (2010) Detection, molecular characterization, and clonal diversity of methicillin-resistant *Staphylococcus aureus* CC398 and CC97 in Spanish slaughter pigs of different age groups. Foodborne Pathog Dis 7: 1269-1277.

112. Ruscher C (2010) Molekulare Epidemiologie Methicillin-Resistenter Staphylokokken der Intermedius-Gruppe. Berlin: Mensch und Buch Verlag 2010.

113. Uhlemann AC, Porcella SF, Trivedi S, Sullivan SB, Hafer C, et al. (2012) Identification of a Highly Transmissible Animal-Independent *Staphylococcus aureus* ST398 Clone with Distinct Genomic and Cell Adhesion Properties. MBio 3.

114. Lin Y, Barker E, Kislow J, Kaldhone P, Stemper ME, et al. (2011) Evidence of multiple virulence subtypes in nosocomial and community-associated MRSA genotypes in companion animals from the upper midwestern and northeastern United States. Clin Med Res 9: 7-16.

115. Malik S, Coombs GW, O'Brien FG, Peng H, Barton MD (2006) Molecular typing of methicillin-resistant staphylococci isolated from cats and dogs. J Antimicrob Chemother 58: 428-431.

116. Moodley A, Stegger M, Bagcigil AF, Baptiste KE, Loeffler A, et al. (2006) *spa* typing of methicillin-resistant *Staphylococcus aureus* isolated from domestic animals and veterinary staff in the UK and Ireland. J Antimicrob Chemother 58: 1118-1123.

117. Walther B, Wieler LH, Vincze S, Antao EM, Brandenburg A, et al. (2012) MRSA variant in companion animals. Emerg Infect Dis 18: 2017-2020.

118. Zhang W, Hao Z, Wang Y, Cao X, Logue CM, et al. (2011) Molecular characterization of methicillin-resistant *Staphylococcus aureus* strains from pet animals and veterinary staff in China. Vet J 190: e125-129.

119. McCarthy AJ, Lindsay JA, Loeffler A (2012) Are all meticillin-resistant *Staphylococcus aureus* (MRSA) equal in all hosts? Epidemiological and genetic comparison between animal and human MRSA. Vet Dermatol 23: 267-e254.

120. Lijnen HR, Stassen JM, Vanlinthout I, Fukao H, Okada K, et al. (1991) Comparative fibrinolytic properties of staphylokinase and streptokinase in animal models of venous thrombosis. Thromb Haemost 66: 468-473.

121. Davidson CJ, Surette MG (2008) Individuality in bacteria. Annu Rev Genet 42: 253-268.

122. Casadesus J, Low DA (2013) Programmed heterogeneity: epigenetic mechanisms in bacteria. J Biol Chem 288: 13929-13935.

123. Bigger WB (1944) Treatment of staphylococcal infections with penicillin by intermittent sterilization. Lancet 2: 497-500.

124. Balaban NQ, Merrin J, Chait R, Kowalik L, Leibler S (2004) Bacterial persistence as a phenotypic switch. Science 305: 1622-1625.

125. Malachowa N, Whitney AR, Kobayashi SD, Sturdevant DE, Kennedy AD, et al. (2011) Global changes in *Staphylococcus aureus* gene expression in human blood. PLoS One 6: e18617.

126. Oogai Y, Matsuo M, Hashimoto M, Kato F, Sugai M, et al. (2011) Expression of virulence factors by *Staphylococcus aureus* grown in serum. Appl Environ Microbiol 77: 8097-8105.

127. Hottes AK, Freddolino PL, Khare A, Donnell ZN, Liu JC, et al. (2013) Bacterial adaptation through loss of function. PLoS Genet 9: e1003617.

128. Andersson DI, Hughes D (2010) Antibiotic resistance and its cost: is it possible to reverse resistance? Nat Rev Microbiol 8: 260-271.

129. Lindsay JA (2013) Hospital-associated MRSA and antibiotic resistance-what have we learned from genomics? Int J Med Microbiol 303: 318-323.

130. Graveland H, Duim B, van Duijkeren E, Heederik D, Wagenaar JA (2011) Livestock-associated methicillin-resistant *Staphylococcus aureus* in animals and humans. Int J Med Microbiol 301: 630-634.

131. Kock R, Schaumburg F, Mellmann A, Koksal M, Jurke A, et al. (2013) Livestock-associated methicillin-resistant *Staphylococcus aureus* (MRSA) as causes of human infection and colonization in Germany. PLoS One 8: e55040.

132. Cuny C, Kock R, Witte W (2013) Livestock associated MRSA (LA-MRSA) and its relevance for humans in Germany. Int J Med Microbiol 303: 331-337.

133. Martinez JL (2012) Natural antibiotic resistance and contamination by antibiotic resistance determinants: the two ages in the evolution of resistance to antimicrobials. Front Microbiol 3: 1.

134. Baker S, Duy PT, Nga TV, Dung TT, Phat VV, et al. (2013) Fitness benefits in fluoroquinolone-resistant *Salmonella* Typhi in the absence of antimicrobial pressure. Elife 2: e01229.

135. Qureshi NK, Yin S, Boyle-Vavra S (2014) The role of the Staphylococcal VraTSR regulatory system on vancomycin resistance and vanA operon expression in vancomycin-resistant *Staphylococcus aureus*. PLoS One 9: e85873.

136. Rossi F, Diaz L, Wollam A, Panesso D, Zhou Y, et al. (2014) Transferable vancomycin resistance in a community-associated MRSA lineage. N Engl J Med 370: 1524-1531.

137. Foucault ML, Courvalin P, Grillot-Courvalin C (2009) Fitness cost of *Van*A-type vancomycin resistance in methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother 53: 2354-2359.

138. Monecke S, Coombs G, Shore AC, Coleman DC, Akpaka P, et al. (2011) A field guide to pandemic, epidemic and sporadic clones of methicillin-resistant *Staphylococcus aureus*. PLoS One 6: e17936.

139. Smith NH, Dale J, Inwald J, Palmer S, Gordon SV, et al. (2003) The population structure of *Mycobacterium bovis* in Great Britain: clonal expansion. Proc Natl Acad Sci U S A 100: 15271-15275.

140. Falush D, Kraft C, Taylor NS, Correa P, Fox JG, et al. (2001) Recombination and mutation during long-term gastric colonization by *Helicobacter pylori*: estimates of clock rates, recombination size, and minimal age. Proc Natl Acad Sci U S A 98: 15056-15061.

141. Spratt BG, Maiden MC (1999) Bacterial population genetics, evolution and epidemiology. Philos Trans R Soc Lond B Biol Sci 354: 701-710.

142. Tibayrenc M, Ayala FJ (2012) Reproductive clonality of pathogens: a perspective on pathogenic viruses, bacteria, fungi, and parasitic protozoa. Proc Natl Acad Sci U S A 109: E3305-3313.

143. Fraser C, Hanage WP, Spratt BG (2007) Recombination and the nature of bacterial speciation. Science 315: 476-480.

144. Fraser C, Alm EJ, Polz MF, Spratt BG, Hanage WP (2009) The bacterial species challenge: making sense of genetic and ecological diversity. Science 323: 741-746.

145. Robert-Koch-Institut (2011) Epidemiologic Bulletin Nr. 26. 12.

146. Sieber S, Gerber V, Jandova V, Rossano A, Evison JM, et al. (2011) Evolution of multidrug-resistant *Staphylococcus aureus* infections in horses and colonized personnel in an equine clinic between 2005 and 2010. Microb Drug Resist 17: 471-478.

147. Jorgensen HJ, Mork T, Caugant DA, Kearns A, Rorvik LM (2005) Genetic variation among *Staphylococcus aureus* strains from Norwegian bulk milk. Appl Environ Microbiol 71: 8352-8361.

148. Stegmann R, Perreten V (2010) Antibiotic resistance profile of *Staphylococcus rostri*, a new species isolated from healthy pigs. Vet Microbiol 145: 165-171.

149. Arriola CS, Guere ME, Larsen J, Skov RL, Gilman RH, et al. (2011) Presence of methicillin-resistant *Staphylococcus aureus* in pigs in Peru. PLoS One 6: e28529.

150. Wagenaar JA, Yue H, Pritchard J, Broekhuizen-Stins M, Huijsdens X, et al. (2009) Unexpected sequence types in livestock associated methicillin-resistant *Staphylococcus aureus* (MRSA): MRSA ST9 and a single locus variant of ST9 in pig farming in China. Vet Microbiol 139: 405-409.

151. Battisti A, Franco A, Merialdi G, Hasman H, Iurescia M, et al. (2010) Heterogeneity among methicillin-resistant *Staphylococcus aureus* from Italian pig finishing holdings. Vet Microbiol 142: 361-366.

152. Neela V, Mohd Zafrul A, Mariana NS, van Belkum A, Liew YK, et al. (2009) Prevalence of ST9 methicillin-resistant *Staphylococcus aureus* among pigs and pig handlers in Malaysia. J Clin Microbiol 47: 4138-4140.

153. Strommenger B, Bartels MD, Kurt K, Layer F, Rohde SM, et al. (2013) Evolution of methicillin-resistant *Staphylococcus aureus* towards increasing resistance. J Antimicrob Chemother.

154. Vasudevan P, Nair MK, Annamalai T, Venkitanarayanan KS (2003) Phenotypic and genotypic characterization of bovine mastitis isolates of *Staphylococcus aureus* for biofilm formation. Vet Microbiol 92: 179-185.

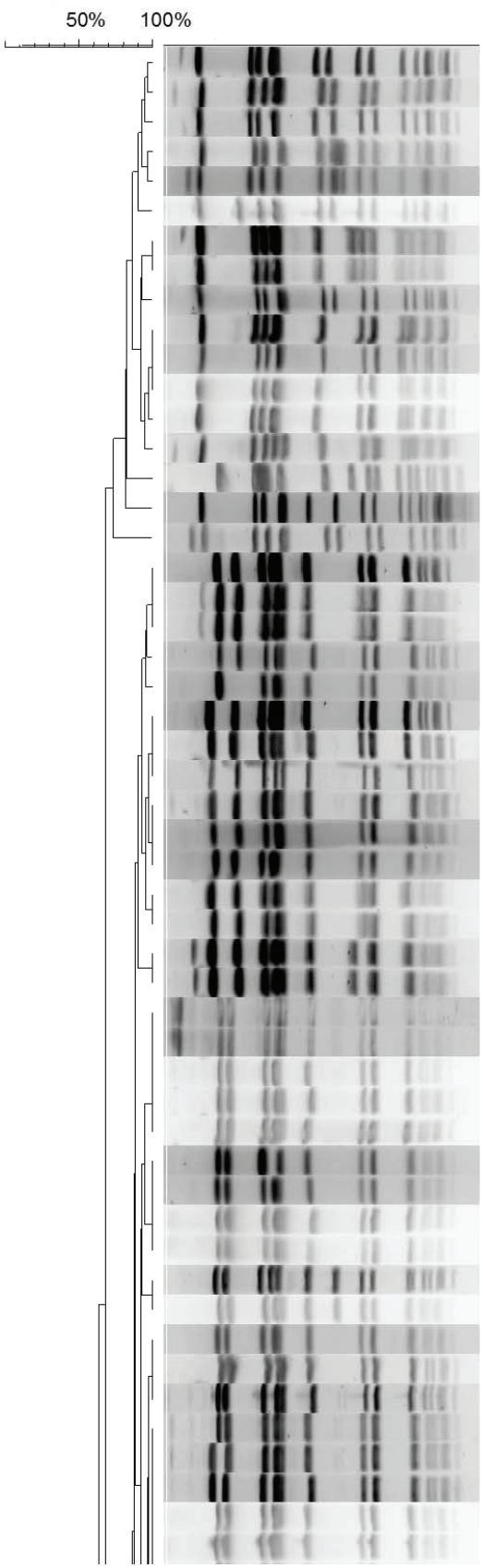
155. Vincze S, Brandenburg AG, Espelage W, Stamm I, Wieler LH, et al. (2014) Risk factors for MRSA infection in companion animals: Results from a case-control study within Germany. Int J Med Microbiol.

156. van Mourik MS, Troelstra A, van Solinge WW, Moons KG, Bonten MJ (2013) Automated surveillance for healthcare-associated infections: opportunities for improvement. Clin Infect Dis 57: 85-93.

10. Appendix

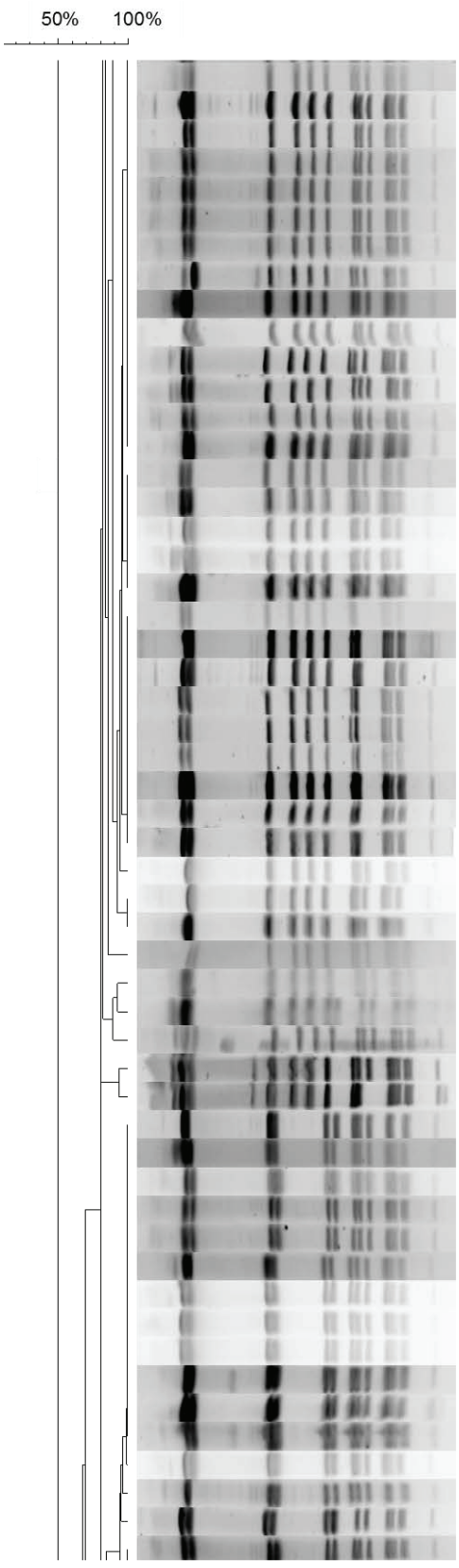
10.1 Supplementary table S1: PFGE-pattern of MRSA using the macrorestriction enzyme *Sma*I

Results are shown for all MRSA investigated within this PhD project except for CC398-MRSA. Additional information are shown for each isolate including host origin, *spa* type, date of isolation, the geographic origin, results of DNA microarray analysis and multilocus sequence typing (MLST). Furthermore, the specific clonal complex is shown for each isolate. PFGE analysis was performed using BioNumerics (cluster analysis; Dice coefficient [1.2% tolerance and 0.5% optimization]) The dendrogram provides information about the similarity of the investigated isolates.

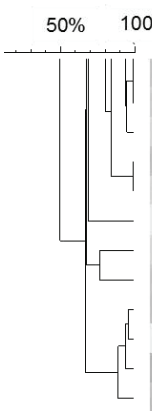


Host origin	IMT-ID	*spa* type	date	origin	DNA-micorarray	ST (MLST)	clonal complex
horse	24782	t12131	22.01.2011	NRW	CC8-atyp	ST254	CC8
horse	26593	t009	16.07.2011	BV	CC8-atyp	ST254	CC8
dog	28672	t009	24.12.2012	H	CC8-atyp		CC8
dog	24781	t036	22.01.2011	LS	CC8-atyp	ST254	CC8
horse	24456	t009	08.11.2010	NRW	CC8-atyp	ST254	CC8
horse	28284	t009	21.09.2011	NRW	CC8-atyp		CC8
cat	28977	t008	03.02.2012	BW			CC8
cat	28981	t008	13.02.2012	BW			CC8
cat	26592	t008	14.07.2011	H	CC8-IV	ST8	CC8
dog	24465	t024	30.11.2010	BW	CC8-IV		CC8
cat	27904	t008	06.09.2011	NRW			CC8
cat	28295	t008	15.10.2011	NRW			CC8
dog	28282	t008	21.09.2011	NRW			CC8
dog	25281	t008	05.04.2011	BV	CC8-IV	ST8	CC8
horse	28444	t127	02.12.2012	RP	CC1-IV	ST1	CC1
dog	25608	t264	06.05.2011	BV	CC5-II	ST225	CC5
horse	25897	t009	06.06.2011	BV	CC8-atyp		CC8
dog	25607	t1007	06.05.2011	BW	CC5-II	ST225	CC5
dog	29198	t003	31.03.2012	RP			CC5
dog	28988	t003	25.02.2012	BV			CC5
dog	27907	t003	08.09.2011	SAX			CC5
dog	25894	t003	31.05.2011	NRW			CC5
cat	25605	t003	06.05.2011	TH	CC5-II		CC5
dog	27555	t045	02.09.2011	NRW			CC5
dog	26489	t003	08.07.2011	TH			CC5
dog	25058	t003	07.02.2011	BW			CC5
dog	24768	t003	08.12.2010	SAX			CC5
dog	24769	t003	23.12.2010	BW			CC5
dog	28986	t003	21.02.2012	SAX			CC5
dog	29189	t003	01.03.2012	BV			CC5
dog	28984	t003	15.02.2012	BW			CC5
dog	29196	t003	27.03.2012	BW			CC5
dog	24459	t003	16.11.2010	RP	CC5-II		CC5
cat	24464	t003	29.11.2010	RP	CC5-II		CC5
dog	27547	t003	13.08.2011	LS			CC5
dog	28301	t003	20.10.2011	RP			CC5
dog	25484	t003	06.04.2011	RP			CC5
dog	24766	t045	08.12.2010	BW	CC5-II	ST225	CC5
cat	26591	t003	14.07.2011	BV			CC5
cat	28298	t003	19.10.2011	BV			CC5
dog	28300	t045	20.10.2011	BV			CC5
dog	28679	t003	06.01.2012	BV			CC5
cat	25486	t003	09.04.2011	H	CC5-II		CC5
cat	25165	t045	03.03.2011	LS			CC5
dog	28441	t045	24.11.2011	RP			CC5
cat	25601	t003	03.05.2011	BW	CC5-II		CC5
dog	25055	t003	03.02.2011	H			CC5
dog	25059	t003	07.02.2011	BW	CC5-II		CC5
dog	25061	t003	08.02.2011	TH			CC5
dog	28285	t003	23.09.2011	RP			CC5
cat	28292	t003	10.10.2011	RP			CC5

Host origin	IMT-ID	*spa* type	date	origin	DNA-micorarray	ST (MLST)	clonal complex
cat	28283	t003	21.09.2011	RP			CC5
dog	28677	t003	04.01.2012	RP			CC5
dog	25164	t003	03.03.2011	H			CC5
dog	28683	t003	16.01.2012	BV			CC5
dog	28674	t003	28.12.2012	RP			CC5
dog	25057	t002	05.02.2011	BW	CC5-II	ST5	CC5
dog	25488	t003	17.04.2011	BW			CC5
cat	25492	t002	21.04.2011	BV	CC5-II		CC5
cat	28443	t003	07.12.2011	BV			CC5
cat	24460	t003	20.11.2010	BV	CC5-II	ST225	CC5
dog	26248	t003	19.06.2011	BV			CC5
cat	26602	t003	01.08.2011	BV			CC5
cat	27341	t003	11.08.2011	BV			CC5
dog	24463	t003	25.11.2010	BW	CC5-II	ST225	CC5
cat	25169	t003	12.03.2011	RP			CC5
cat	25050	t003	25.01.2011	RP	CC5-II		CC5
cat	26598	t003	26.07.2011	NRW			CC5
dog	27235	t003	02.08.2011	BW			CC5
dog	28980	t1430	13.02.2012	NRW	CC9	ST9	CC9
dog	24770	t003	23.12.2010	H	CC5-II		CC5
cat	27908	t003	09.09.2011	SAX	CC5-II		CC5
horse	26485	t003	01.07.2011	H	CC5-II		CC5
dog	26589	t003	09.07.2011	BV			CC5
cat	27545	t003	12.08.2011	BV			CC5
cat	28299	t278	20.10.2011	BV	CC133-MSSA	ST599	CC599
dog	27553	t022	27.08.2011	SAXA			CC22
cat	25060	t032	08.02.2011	NRW	CC22-IV		CC22
dog	28685	t032	20.01.2012	NRW			CC22
cat	25614	t032	19.05.2011	NRW			CC22
dog	28293	t032	11.10.2011	BV	CC22-IV		CC22
dog	25054	t032	02.02.2011	H	CC22-IV		CC22
dog	25063	t032	12.02.2011	BB	CC22-IV		CC22
dog	24454	t032	03.11.2010	H			CC22
dog	25167	t032	08.03.2011	H			CC22
cat	27556	t032	03.09.2011	H			CC22
cat	25493	t032	28.04.2011	LS	CC22-IV		CC22
cat	24773	t032	31.12.2010	LS	CC22-IV	ST2743	CC22
dog	24774	t022	07.01.2011	BW			CC22
dog	25606	t032	06.05.2011	RP	CC22-IV	ST22	CC22
cat	27546	t7982	13.08.2011	H	CC22-IV	ST22	CC22
dog	27550	t032	22.08.2011	H			CC22
dog	27551	t032	24.08.2011	LS			CC22
cat	25276	t032	17.03.2011	LS			CC22
dog	24780	t032	20.01.2011	H			CC22
dog	28294	t032	12.10.2011	H			CC22
dog	28675	t032	29.12.2012	H			CC22
dog	25065	t032	14.02.2011	H			CC22
dog	24453	t022	02.11.2010	H			CC22
dog	24462	t022	24.11.2010	BW	CC22-IV	ST1117	CC22
dog	25166	t032	04.03.2011	H			CC22



Host origin	IMT-ID	*spa* type	date	origin	DNA-micorarray	ST (MLST)	clonal complex
cat	25168	t032	10.03.2011	BV			CC22
dog	25602	t032	29.04.2011	H			CC22
cat	26249	t032	20.06.2011	H	CC22-IV	ST22	CC22
dog	26245	t032	10.06.2011	H			CC22
dog	26243	t032	09.06.2011	BV			CC22
cat	26244	t032	09.06.2011	LS			CC22
dog	27903	t032	05.09.2011	LS	CC22-IV	ST22	CC22
dog	26594	t032	16.07.2011	LS			CC22
cat	28436	t022	17.11.2011	BV			CC22
dog	28442	t032	30.11.2011	BW			CC22
dog	28668	t032	15.12.2011	H			CC22
dog	28670	t032	23.12.2012	RP			CC22
dog	29190	t032	08.03.2012	H			CC22
dog	29193	t022	16.03.2012	BV			CC22
dog	25163	t032	24.02.2011	H			CC22
dog	28983	t032	14.02.2012	SH			CC22
cat	27549	t032	15.08.2011	LS			CC22
dog	27552	t022	27.08.2011	H			CC22
dog	28976	t032	02.02.2012	SH			CC22
dog	25162	t032	22.02.2011	LS			CC22
cat	25609	t613	10.05.2011	B	CC22-IV	ST22	CC22
dog	25604	t032	03.05.2011	BW			CC22
dog	26483	t557	22.06.2011	LS	CC22-IV	ST22	CC22
cat	26484	t032	27.06.2011	BW			CC22
dog	26487	t032	06.07.2011	NRW			CC22
dog	25611	t032	16.05.2011	LS			CC22
dog	28684	t032	21.01.2012	LS			CC22
cat	28686	t032	23.01.2012	LS			CC22
dog	28291	t032	05.10.2011	LS			CC22
dog	27548	t032	13.08.2011	LS	CC22-IV		CC22
dog	28972	t032	25.01.2012	LS			CC22
cat	24457	t032	11.11.2010	SAXA	CC22-IV	ST22	CC22
dog	24776	t910	14.01.2011	BW	CC22-IV	ST22	CC22
dog	24777	t032	14.01.2011	LS	CC22-IV		CC22
cat	26590	t7982	11.07.2011	H	CC22-IV		CC22
cat	28445	t020	10.12.2011	NRW	CC22-IV		CC22
cat	28989	t032	27.02.2012	LS		ST22	CC22
dog	25062	t747	10.02.2011	LS	CC22-IV	ST2745	CC22
dog	24455	t032	06.11.2010	NRW	CC22-IV	ST22	CC22
dog	24771	t032	29.12.2010	NRW			CC22
dog	26247	t032	11.06.2011	BV			CC22
cat	27911	t022	17.09.2011	BV	CC22-IV	ST1117	CC22
dog	26246	t022	10.06.2011	BV			CC22
cat	28287	t022	29.09.2011	BV	CC22-IV		CC22
dog	28288	t022	07.10.2011	BV			CC22
dog	28290	t022	02.10.2011	BV			CC22
dog	28435	t032	14.11.2012	SH	CC22-IV		CC22
dog	28979	t022	08.02.2012	BV			CC22
cat	25278	t032	21.03.2011	BV			CC22
cat	25485	t032	07.04.2011	BV			CC22
dog	25494	t747	28.04.2011	LS			CC22
cat	28681	t022	10.01.2012	BV			CC22
dog	25275	t032	17.03.2011	BV			CC22

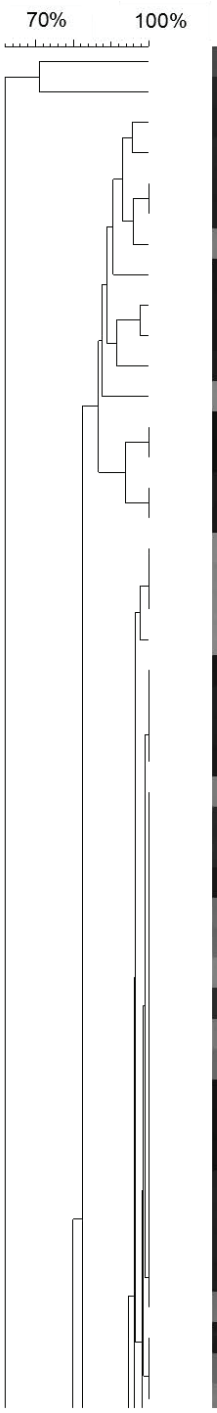


Host origin	IMT-ID	*spa* type	date	origin	DNA-micorarray	ST (MLST)	clonal complex
cat	25892	t3846	28.05.2011	NRW	CC22-IV	ST22	CC22
dog	28673	t032	28.12.2012	NRW			CC22
cat	27554	t022	31.08.2011	BV	CC22-IV		CC22
cat	24461	t022	24.11.2010	BV	CC22-IV	ST1117	CC22
dog	28974	t022	30.01.2012	BV			CC22
cat	28296	t016	17.10.2011	HB	CC22-IV	ST22	CC22
dog	28439	t032	21.11.2011	NRW			CC22
dog	25279	t032	31.03.2011	H			CC22
cat	25896	t020	03.06.2011	BW	CC22-IV		CC22
cat	28305	t020	01.11.2011	BW			CC22
cat	25610	t020	12.05.2011	BW	CC22-IV	ST22	CC22
cat	26601	t032	28.07.2011	BW	CC22-IV		CC22

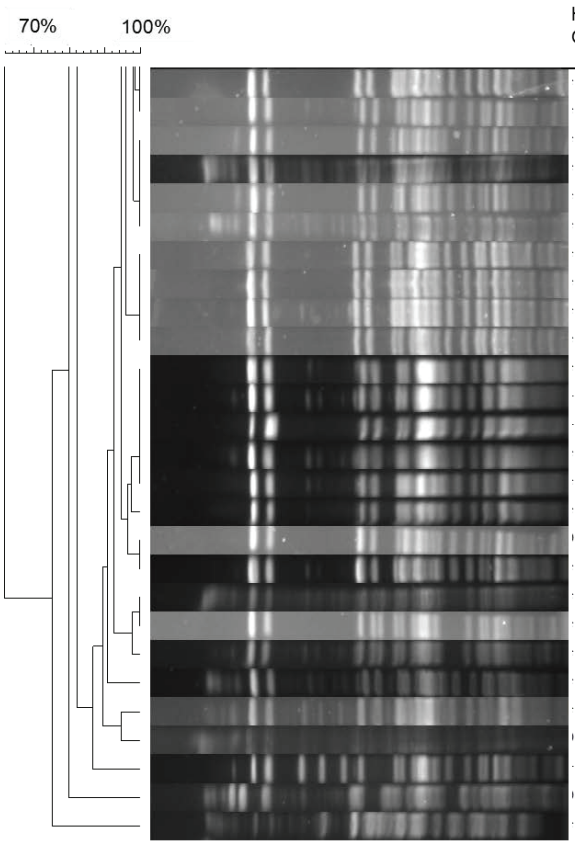
Abbreviations: ST= sequence type, CC=clonal complex, BB=Brandenburg, BV=Bavaria, BW=Baden-Wuerttemberg, H=Hesse, LS= Lower Saxony, NRW= North Rhine-Westphalia, RP= Rhineland-Palatinate, SAXA=Saxony Anhalt, SH= Schleswig-Holstein, TH= Thuringia.

10.2 Supplementary table S2: PFGE-pattern of CC398-MRSA after digestion with *Apa*I

Results are shown for all CC398-MRSA investigated within this PhD project. Additional information are shown for each isolate including host origin, *spa* type, date of isolation, the geographic origin, results of DNA microarray analysis and multilocus sequence typing (MLST). Furthermore, the specific clonal complex is shown for each isolate. PFGE analysis was performed using BioNumerics (cluster analysis; Dice coefficient [1.2% tolerance and 0.5% optimization]) The dendrogram provides information about the similarity of the investigated isolates.



Host origin	IMT-ID	*spa* type	date	origin	DNA-micorarray	ST (MLST)	clonal complex
horse	26486	t011	.02.07.2011	BV			CC398
dog	28667	t011	.14.12.2011	NRW			CC398
dog	24466	t011	01.12.2010	RP	CC398-V	ST398	CC398
dog	24775	t034	11.01.2011	BV	CC398-V	ST398	CC398
dog	24458	t011	12.11.2010	BV			CC398
cat	24784	t011	25.01.2011	BW			CC398
dog	25067	t011	16.02.2011	BV	CC398-V	ST398	CC398
dog	28289	t108	.30.09.2011	H	CC398-V	ST398	CC398
cat	28434	t011	.12.11.2011	SH	CC398-V		CC398
horse	28438	t011	.19.11.2011	H	CC398-IV		CC398
horse	25053	t011	02.02.2011	NRW	CC398-V	ST398	CC398
dog	25489	t011	.19.04.2011	NRW			CC398
horse	28306	t011	.02.11.2011	NRW			CC398
horse	28433	t011	.07.11.2011	NRW			CC398
horse	27906	t011	.08.09.2011	NRW			CC398
horse	27909	t011	.09.09.2011	NRW			CC398
horse	27340	t011	.09.08.2011	SH			CC398
horse	25612	t011	.20.05.2011	NRW	CC398-IV		CC398
horse	25490	t011	.19.04.2011	NRW	CC398-IV		CC398
horse	27251	t011	.06.08.2011	BV			CC398
horse	27544	t6867	.12.08.2011	LSAX			CC398
horse	28678	t011	.04.01.2012	LSAX			CC398
horse	27905	t6867	.08.09.2011	LSAX			CC398
horse	27910	t6867	.05.09.2011	LSAX	CC398-IV		CC398
dog	25171	t6867	15.03.2011	SH			CC398
dog	24779	t011	20.01.2011	LSAX	CC398-IV	ST398	CC398
horse	24783	t6867	24.01.2011	RP			CC398
horse	25064	t6867	12.02.2011	SH	CC398-IV		CC398
dog	25068	t011	17.02.2011	BV			CC398
dog	25616	t6867	.21.05.2011	SH	CC398-IV		CC398
dog	25603	t011	.02.05.2011	BW			CC398
horse	26488	t6867	.06.07.2011	SH	CC398-IV		CC398
horse	25487	t6867	.08.04.2011	RP	CC398-IV		CC398
horse	26600	t6867	.27.07.2011	SH			CC398
horse	28297	t6867	.19.10.2011	SH			CC398
horse	28302	t6867	.20.10.2011	SH			CC398
horse	28304	t6867	.27.10.2011	SH	CC398-IV		CC398
dog	28669	t6867	.22.12.2011	SH			CC398
dog	28671	t6867	.23.12.2011	SH			CC398
horse	28676	t011	.04.01.2012	RP			CC398
horse	28680	t6867	.07.01.2012	SH	CC398-IV		CC398
horse	25615	t6867	.20.05.2011	RP			CC398
horse	25051	t6867	27.01.2011	RP			CC398
horse	25893	t6867	28.05.2011	RP			CC398
horse	25277	t011	.19.03.2011	NRW	CC398-IV	ST398	CC398



Host Origin	IMT-ID	*spa* type	date	origin	DNA-micorarray	ST (MLST)	clonal complex
horse	25893	t6867	28.05.2011	RP			CC398
horse	25277	t011	19.03.2011	NRW	CC398-IV	ST398	CC398
horse	25066	t6867	14.02.2011	LSAX	CC398-IV		CC398
horse	26599	t6867	27.07.2011	LSAX			CC398
horse	25170	t6867	13.03.2011	LSAX		ST398	CC398
horse	25280	t011	04.04.2011	BV	CC398-IV		CC398
horse	26595	t10.	18.07.2011	SH	CC398-IV	ST398	CC398
horse	25613	t6867	19.05.2011	RP	CC398-IV		CC398
horse	26596	t6867	21.07.2011	RP	CC398-IV		CC398
horse	26597	t10.	22.07.2011	SH			CC398
horse	28973	t011	30.01.2012	BV			CC398
cat	28987	t011	25.02.2012	HH			CC398
cat	28978	t011	04.02.2012	BV			CC398
horse	28982	t011	13.02.2012	SH			CC398
horse	28985	t6867	20.02.2012	SH			CC398
horse	28975	t6867	01.02.2012	RP			CC398
dog	25161	t011	19.02.2011	BV			CC398
horse	28286	t011	25.09.2011	BV			CC398
horse	24772	t011	30.12.2010	NRW		ST398	CC398
horse	25491	t011	20.04.2011	NRW			CC398
horse	24778	t011	14.01.2011	NRW			CC398
horse	28440	t011	21.11.2011	NRW			CC398
horse	25052	t011	01.02.2011	LSAX		ST398	CC398
dog	29195	t011	22.03.2012	SH			CC398
cat	28682	t011	13.01.2012	NRW	CC398-IV		CC398
dog	25056	t034	03.02.2011	LSAX			CC398
horse	24767	t6867	09.12.2010	LSAX	CC398-IV	ST398	CC398

Abbreviations: ST= sequence type, CC=clonal complex, BB=Brandenburg, BV=Bavaria, BW=Baden-Wuerttemberg, H=Hesse, LS= Lower Saxony, NRW= North Rhine-Westphalia, RP= Rhineland-Palatinate, SAXA=Saxony Anhalt, SH= Schleswig-Holstein, TH= Thuringia.

10.3 Supplementary table S3: Methicillin-susceptible *S. aureus* (MSSA) isolated from cats and horses within this study

This table provides additional information about all methicillin-susceptible *S. aureus* that were investigated during this study. Additional information are shown for each isolate including host origin, infection site, *spa* type, date of isolation, the geographic origin, results of DNA microarray analysis and multilocus sequence typing (MLST). Furthermore, the clonal complex or sequence type (ST) is shown for each isolate.

IMT	isolation date	original ID	host	infection site	region	*spa*	CC (according to *spa* type)	ST	Microarray
18271	29.03.2010		cat	wound	unknown	t003	CC5	**not typed**	**not typed**
19487	20.05.2010		cat	wound	unknown	t331	unknown	**ST508**	**CC45-MSSA**
19488	20.05.2010		cat	ear	unknown	t015	CC45	**not typed**	**CC45-MSSA**
19494	20.05.2010		cat	nose	unknown	t3358	unknown	**ST101**	**CC101-MSSA**
19496	20.05.2010		cat	skin	unknown	t223	unknown	**ST22**	**none**
19680	20.05.2010		cat	wound	unknown	t488	unknown	**ST509**	**none**
19681	20.05.2010		cat	eye	unknown	t383	unknown	**ST1**	**CC1-MSSA**
19682	02.08.2010		cat	eye	unknown	t065	CC45	**ST45**	**none**
19684	20.05.2010		cat	eye	unknown	t091	CC7	**not typed**	**CC7-MSSA**
19689	20.05.2010		cat	nose	unknown	t091	CC7	**not typed**	**not typed**
19693	20.05.2010		cat	wound	unknown	t050	CC45	**ST45**	**CC45-MSSA**
19699	29.03.2010		cat	trachea	unknown	t803	unknown	**ST15**	**CC15-MSSA**
19701	29.03.2010		cat	eye	unknown	t012	CC30	**ST30**	**CC30-MSSA**
19702	29.03.2010		cat	skin	unknown	t230	CC45	**ST1096**	**CC45-MSSA**
21230	05.03.2010	VB971700	cat	hair	LS	t002	CC5	**not typed**	**CC5-MSSA**
21232	11.03.2010	VB973004	cat	ear	NRW	t091	CC7	**not typed**	**not typed**
21236	12.03.2010	VB973371	cat	wound	Bavaria	t091	CC7	**not typed**	**not typed**
21469	12.03.2010	VB928332	cat	unknown	NRW	neu	unknown	**ST2077**	**not typed**
22127	07.07.2010	VB999257	cat	skin	NRW	t084	CC15	**not typed**	**CC15-MSSA**
23961	20.03.2010	VB975191	cat	nose	BW	t780	unknown	**ST45**	**CC45-MSSA**
24069	07.09.2010	VB961917	cat	wound	NRW	neu	unknown	**ST737**	**CC22-MSSA**
25039	23.09.2010	VB965477	cat	wound	LS	t078	unknown	**ST25**	**CC25-MSSA**
25041	12.10.2010	VB969125	cat	nose	BW	neu	unknown	**ST25**	**none**
25049	21.10.2010	VB971299-2	cat	wound	Hesse	t3032	unknown	**ST15**	**CC15-MSSA**
25148	13.10.2010	VB969446	cat	palate	Hesse	t3085	unknown	**NEW**	**none**
25154	19.10.2010	VB970872	cat	skin	Bavaria	t211	unknown	**ST8**	**CC8-MSSA**
25156	21.10.2010	VB971294	cat	nose	Bavaria	t005	CC5	**not typed**	**not typed**
25158	22.10.2010	VB971550	cat	nose	BW	t091	CC7	**not typed**	**not typed**
25283	02.10.2010	VB967251	cat	eye	Bavaria	t3694	unknown	**ST30**	**CC30-MSSA**
25285	27.10.2010	VB972465	cat	skin	NRW	new	unknown	**ST9**	**CC9-MSSA**
25291	01.10.2010	VB967035	cat	ear	Bremen	t5529	unknown	**ST45**	**CC45-MSSA**
25297	09.10.2010	VB968839	cat	wound	Hesse	t091	CC7	**not typed**	**not typed**
25477	20.11.2010	VB977576	cat	wound	Hesse	t1151	CC59	**ST59**	**CC59-MSSA**
25618	26.10.2010	VB972039	cat	urin	Bavaria	t003	CC5	**not typed**	**CC5-MSSA**
25652	09.11.2010	VB974876	cat	nose	NRW	t002	unknown	**not typed**	**CC5-MSSA**
25670	04.11.2010	VB973964-2	cat	nose	BW	t2883	unknown	**ST188**	**ST188-MSSA**
25728	18.03.2010	VB974582	cat	nose	BW	t084	CC15	**not typed**	**not typed**
25743	19.11.2010	VB977221	cat	ear	Hesse	t304	unknown	**ST8**	**CC8-MSSA**
25904	08.11.2010	VB974777	cat	ear	BW	t382	unknown	**ST39**	**CC30-MSSA**
25913	19.10.2010	VB970794	cat	wound	BW	t9280	unknown	**NEW**	**none**
25920	09.11.2010	VB975046	cat	nose	Thuringia	t073	unknown	**ST1877**	**CC45-MSSA**
25931	10.11.2010	VB975306	cat	wound	NRW	t189	unknown	**not typed**	**ST188MSSA**
25932	10.11.2010	VB975374-2	cat	nose	SH	t5841	unknown	**ST97**	**CC97-MSSA**
26272	30.11.2010	VB979442	cat	nose	RP	t166	unknown	**ST34**	**CC30-MSSA**
26629	20.07.2011	VB981683-2	cat	skin	LS	new	unknown	**not typed**	**not typed**
27241	30.07.2011	VB983917	cat	wound	NRW	t160	unknown	**ST12**	**CC12-MSSA**
27249	29.07.2011	VB983847	cat	nose	Brandenburg	t015	CC45	**not typed**	**not typed**
27461	26.07.2011	VB982828	cat	ear	Bavaria	t209	unknown	**ST109**	**CC9-MSSA**
27918	07.09.2011	VB992079	cat	nose	Hesse	t127	CC1	**not typed**	CC1-MSSA
23957	20.03.2010	VB975312	horse	cervix	NRW	t127	CC1	**not typed**	**not typed**
23959	22.03.2010	VB975476	horse		BW	t127	CC1	**not typed**	**not typed**
25144	04.09.2010	VB961579-2	horse	wound	Bavaria	t127	CC1	**not typed**	**not typed**
25475	05.11.2010	VB974305	horse	wound	NRW	t127	CC1	**not typed**	**CC1-MSSA**
25479	14.03.2010	VB972780	horse	skin	NRW	t127	CC1	**not typed**	**not typed**
25660	17.06.2010	VB995123	horse	joint	unknown	t127	CC1	**not typed**	**CC1-MSSA**
25735	28.09.2010	VB966225-2	horse	eye	unknown	t127	CC1	**not typed**	**not typed**
25747	06.10.2010	VB967960	horse	skin	RP	new	unknown	**ST1**	**CC1-MSSA**
25900	13.10.2010	VB969642	horse	skin	unknown	t127	CC1	**not typed**	**not typed**
25908	02.11.2010	VB973520	horse	skin	unknown	t127	CC1	**not typed**	**not typed**
25930	10.11.2010	VB975372	horse	skin	unknown	t127	CC1	**not typed**	**not typed**
25944	09.07.2010	VB999880	horse	hair	unknown	t127	CC1	**not typed**	**not typed**

IMT	isolation date	original ID	host	infection site	region	*spa*	CC (according to *spa* type)	ST	Microarray
25948	12.11.2010	VB975811	horse	eye	unknown	t1383	unknown	**ST1**	**CC1-MSSA**
26250	24.11.2010	VB978328	horse	wound	unknown	t9632	unknown	**ST1738**	**CC1-MSSA**
26258	02.11.2010	VB973539	horse	mouth	unknown	t127	CC1	**not typed**	**CC1-MSSA**
26260	24.09.2010	VB965726	horse	skin	unknown	t127	CC1	**not typed**	**not typed**
26263	13.03.2010	VB973641-2	horse	mouth	unknown	t2279	unknown	**ST1**	**CC1-MSSA**
26267	25.09.2010	VB966011	horse	nose	unknown	new	unknown	**ST1**	**CC1-MSSA**
26273	26.11.2010	VB978839	horse	wound	unknown	t1383	unknown	**not typed**	**not typed**
26630	23.07.2011	VB982320	horse	wound	Hesse	t1383	unknown	**not typed**	**not typed**
27937	02.09.2011	VB991358	horse	wound	RP	t1508	unknown	**NEW**	**CC1-MSSA**
25738	03.11.2010	VB973692	horse	skin	BW	t091	unknown	**not typed**	**CC7-MSSA**
26616	20.07.2011	VM896410	horse	synovia	unknown	t008	unknown	**not typed**	**CC8-MSSA**
24067	02.09.2010	VB961134	horse	skin	RP	t3043	unknown	**not typed**	**not typed**
25653	11.03.2010	VB973044	horse	skin	unknown	t3043	unknown	**not typed**	**not typed**
25667	22.09.2010	VB965198	horse	wound	RP	new	unknown	**ST1660**	**CC9-MSSA**
25671	12.11.2010	VB975800	horse	wound	unknown	t3043	unknown	**ST1660**	**CC9-MSSA**
25673	23.03.2010	VB975691	horse	skin	unknown	t2484	unknown	**ST1660**	**none**
25746	02.10.2010	VB967275	horse	nose	unknown	t8571	unknown	**NEW**	**CC9-MSSA**
25907	14.09.2010	VB963334	horse	skin	Hesse	t2484	unknown	**not typed**	**not typed**
25934	16.09.2010	VB964061	horse	wound	unknown	t549	unknown	**ST1660**	**none**
26627	27.07.2011	VB983157	horse	wound	unknown	new	unknown	**ST1660**	**CC9-MSSA**
26633	19.07.2011	VM896378	horse	synovia	BW	t549	unknown	**not typed**	**not typed**
27245	30.07.2011	VB983925	horse	skin	NRW	t3043	unknown	**not typed**	**not typed**
25668	22.09.2010	VB965089	horse	skin	unknown	new	unknown	**ST15**	**CC15-MSSA**
25922	31.08.2010	VB960691	horse	skin	unknown	t005	unknown	**not typed**	**CC22-MSSA**
25940	10.07.2010	VB950159	horse	wound	unknown	t136	unknown	**not typed**	**CC30-MSSA**
26626	27.07.2011	VB983130	horse	skin	NRW	t012	unknown	**not typed**	**CC30-MSSA**
25726	13.03.2010	VB973695	horse	skin	unknown	t2420	unknown	**ST133**	**CC133-MSSA**
25927	18.09.2010	VB964427	horse	Trachea	unknown	t208	unknown	**not typed**	**ST49-MSSA**
26624	23.07.2011	VB982481	horse	cervix	unknown	t1403	unknown	**ST133**	**CC133-MSSA**
25624	29.09.2010	VB966462	horse	wound	NRW	t1294	unknown	**not typed**	**not typed**
25733	23.11.2010	VB977882	horse	wound	Bavaria	new	unknown	**ST816**	**CC479-MSSA**
25915	12.10.2010	VB969119	horse	nose	unknown	t1294	unknown	**not typed**	**not typed**
25939	14.09.2010	VB963447	horse	wound	unknown	t1294	unknown	**not typed**	**not typed**
26264	03.03.2010	VB971293	horse	skin	unknown	t1294	unknown	**NEW**	**CC479-MSSA**
27250	26.07.2011	VB982744	horse	skin	unknown	t1294	unknown	**not typed**	**not typed**
27913	09.09.2011	VB992838	horse	wound	unknown	t1294	unknown	**ST816**	**CC479-MSSA**
27914	10.09.2011	VB993053	horse	skin	unknown	t1294	unknown	**not typed**	**not typed**
25928	18.09.2010	VB964426	horse	Trachea	unknown	t1773	unknown	**ST890**	**none**

Abbreviations: ST= sequence type, CC=clonal complex, BB=Brandenburg, BV=Bavaria, BW=Baden-Wuerttemberg, H=Hesse, LS= Lower Saxony, NRW= North Rhine-Westphalia, RP= Rhineland-Palatinate, SAXA=Saxony Anhalt, SH= Schleswig-Holstein, TH= Thuringia.

11. List of Publications

Research publications in scientific journals

Risk factors for MRSA infection in companion animals: Results from a case-control study within Germany

Vincze S, Brandenburg AG, Espelage W, Stamm I, Wieler LH, Kopp PA, Lübke-Becker A, Walther B.; Int J Med Microbiol. 2014 Oct;304(7):787-93. doi: 10.1016/j.ijmm.2014.07.007. Epub 2014 Jul 25.

Alarming Proportions of Methicillin-Resistant *Staphylococcus aureus* (MRSA) in Wound Samples from Companion Animals, Germany 2010-2012.

Vincze S, Stamm I, Kopp PA, Hermes J, Adlhoch C, Semmler T, Wieler LH, Lübke-Becker A, Walther B.; PLoS One. 2014 Jan 20;9(1):e85656. doi: 10.1371/journal.pone.0085656.

Molecular analysis of human and canine *Staphylococcus aureus* strains reveals distinct extended-host-spectrum genotypes independent of their methicillin resistance.

Vincze S, Stamm I, Monecke S, Kopp PA, Semmler T, Wieler LH, Lübke-Becker A, Walther B.; Appl Environ Microbiol. 2013 Jan;79(2):655-62. doi: 10.1128/AEM.02704-12.

Multidrug- and methicillin resistant *Staphylococcus pseudintermedius* as a cause of canine pyoderma: a case report.

Vincze S, Paasch A, Walther B, Ruscher C, Lübke-Becker A, Wieler LH, Barbara K.; Berl Munch Tierarztl Wochenschr. 2010 Sep-Oct;123(9-10):353-8.

Species differentiation within the *Staphylococcus intermedius* group (SIG) using a refined MALDI-TOF MS database

Murugaiyan J, Walther B, Stamm I, Abou-Elnaga Y, Brüggemann-Schwarze S, Wieler LH, Lübke-Becker A, Semmler T, Roesler U.; Clin Microbiol Infect. 2014 May 8. doi: 10.1111/1469-0691.12662.

MRSA variant in companion animals.

Walther B, Wieler LH, Vincze S, Antão EM, Brandenburg A, Stamm I, Kopp PA, Kohn B, Semmler T, Lübke-Becker A.; Emerg Infect Dis. 2012 Dec;18(12):2017-20. doi: 10.3201/eid1812.120238.

Sharing more than friendship - nasal colonization with coagulase-positive staphylococci (CPS) and co-habitation aspects of dogs and their owners.
Walther B, Hermes J, Cuny C, Wieler LH, Vincze S, Abou Elnaga Y, Stamm I, Kopp PA, Kohn B, Witte W, Jansen A, Conraths FJ, Semmler T, Eckmanns T, Lübke-Becker A.; PLoS One. 2012; 7(4):e35197. doi: 10.1371/journal.pone.0035197.

Infektionsprävention und Hygienemanagement in Pferdekliniken
Walther B, Janßen T, Gehlen H, Vincze S, Borchers K, Wieler L, Barton A, Lübke-Becker A.; Berl Münch Tierärztl Wochenschr 127, 486–497 (2014). doi: 10.2376/0005-9366-127-48

Oral presentations

MRSA infections in companion animals: characterization of risk factors
Vincze S, Brandenburg AG, Espelage W, Stamm I, Wieler LH, Kopp PA, Lübke-Becker A, Walther B.: National Symposium on Zoonosis Research 2014, Berlin Germany – 17.10.2014

Occurrence and distribution of virulence associated factors in *Staphylococcus aureus* obtained from companion animals.
Vincze S, Lübke- Becker A, Monecke S, Brandenburg A, Stamm I, Kopp PA, Wieler LH, Walther B, Tagung der DVG-Fachgruppe "Bakteriologie und Mykologie" Leipzig – 27.-29.06.2012, ISBN: 978-3-941703-70-4

The frequency of Livestock-associated MRSA of CC398 in clinical samples from small animals and horses is on the rise
Vincze S ,Lübke-Becker A, Stamm I, Kopp PA, Abou-Elnaga Y, Semmler T, Wieler LH, Walther B. 63. Jahrestagung der Deutschen Gesellschaft für Hygiene und Mikrobiologie (DGHM) Essen – 25.-28.09.2011, ISSN: 1438-4221

Comparative genetic analysis of canine and human *S. aureus* isolates reveals a substantial proportion of extended host spectrum genotypes (EHSG)
Vincze S, Lübke-Becker A, Monecke S, Semmler T, Stamm I, Kopp PA, Wieler LH, Walther B. National Symposium on Zoonosis Research Berlin – 06.-07.10.2011

Poster presentations

Risk factors associated with MRSA infections in companion animals: a Germany-wide survey

Vincze S, Brandenburg AG, Espelage W, Stamm I, Wieler LH, Kopp PA, Lübke-Becker A, Walther B: Tagung DVG-Fachgruppe „Bakteriologie und Mykologie“ Freising – 26.-28.052014, ISBN:978-3-86345-206-3

Infection series with methicillin-resistant (MRSP) and methicillin-susceptible *S. pseudintermedius* (MSSP) in a canine patient over five years

Vincze S, Walther B, Wieler LH, Kohn B, Brunnberg L, Lübke-Becker A: Tagung DVG-Fachgruppe „Bakteriologie und Mykologie“ Freising – 26.-28.05.2014, ISBN:978-3-86345-206-3

Identification of risk factors associated with MRSA infections in companion animals

Vincze S, Brandenburg AG, Espelage W, Stamm I, Wieler LH, Kopp PA, Lübke-Becker A, Walther B: Jahrestagung der Deutschen Gesellschaft für Hygiene und Mikrobiologie (DGHM) Dresden – 05.-08.10.2014, ISSN: 0947-0867

Recurrent methicillin-resistant and –susceptible *Staphylococcus pseudintermedius*-infections in a dog over a five-year period

Vincze S, Walther B, Wieler LH, Kohn B, Brunnberg L, Lübke-Becker A. Jahrestagung der Deutschen Gesellschaft für Hygiene und Mikrobiologie (DGHM) Rostock – 22.-25.09.2013, ISSN 1438-4221

Alarmingly high MRSA proportions in wound infections of dogs, cats and horses: A Germany-wide survey.

Vincze, S.; Stamm, I.; Kopp, P. A.; Wieler, L.; Lübke-Becker, A.; Walther, B. (2013).; Gordon Research Conference on Staphylococcal Diseases, Waterville Valley, NH – 28.07.-02.08.2013.

A case report on a canine long term patient suffering from recurrent infections due to indistinguishable methicillin-resistant and –susceptible *S. pseudintermedius* genotypes.

Vincze, S.; Walther, B.; Wieler, L. H.; Kohn, B.; Brunnberg, L.; Lübke-Becker, A. (2013): 3rd ASM-ESCMID Conference on Methicillin-resistant Staphylococci in Animals: Veterinary and Public Health Implications, Copenhagen, Denmark – 04.11.-07.11.2013.

Whole genome sequencing of two equine CC398-MRSA reveals genomic diversity.
Vincze S; Kopp PA; Semmler T; Wieler LH; Lübke-Becker A; Walther, B. National Symposium on Zoonosis Research, Berlin, Germany – 19.09.-20.09.2013.

Proportion and genetic background among MRSA from wound swabs of companion animal origin in Germany
Vincze S, Stamm I, Kopp PA, Wieler LH, Lübke-Becker A, Walther B. National Symposium on Zoonosis Research, Berlin, Germany – 11.-12.10.2012

Proportion of MRSA in wound specimens from dogs, cats and horses: A Germany-wide survey
Vincze S, Stamm I, Kopp PA, Wieler LH, Lübke-Becker A, Walther B. Jahrestagung der Deutschen Gesellschaft für Hygiene und Mikrobiologie (DGHM) Hamburg – 30.09.-03.10.2012, ISSN: 1438-4221

Insights into extended host spectrum genotypes (EHSG) among clinical *S. aureus* of human and canine origin
Vincze S, Lübke-Becker A, Monecke S, Semmler T, Stamm I, Kopp PA, Wieler LH, Walther B. 2nd ASM-ESCMID Conference on Methicillin-resistant Staphylococci in Animals: Veterinary and Public Health Implications, Washington D.C. – 08.-11.09.2011

12. Danksagung

An dieser Stelle möchte ich allen Menschen danken, die durch ihre fachliche und insbesondere ihre moralische Unterstützung zum Gelingen dieser Arbeit beigetragen haben.

Zuerst möchte ich mich ganz herzlich bei Prof. Wieler für die Überlassung des interessanten Themas sowie für seine Hilfsbereitschaft und Unterstützung während meiner Doktorarbeit bedanken. Mein besonderer Dank gilt auch Birgit Walther und Antina Lübke-Becker für ihre sehr engagierte Betreuung, die vielen konstruktiven Gespräche und wissenschaftlichen Anregungen.

Prof. Osterrieder möchte ich für die Betreuung im Rahmen der Dahlem Research School danken. Allen Beteiligten der Dahlem Research School danke ich für die Möglichkeit, dass ich während meiner Doktorandenzeit eine Vielzahl verschiedenster sehr interessanter Kurse besuchen konnte.

Bei dem Team der Bakteriologie von Vet Med Labor Ludwigsburg – insbesondere Ivonne Stamm – möchte ich mich besonders für die stets unkomplizierte Zusammenarbeit bedanken. Ohne eure Hilfe wäre diese Arbeit nicht möglich gewesen.

Bei allen Kollegen des Instituts für Mikrobiologie und Tierseuchen bedanke ich mich für ihre Hilfsbereitschaft und Freundlichkeit. Diese einzigartige Arbeitsatmosphäre hat mir auch in Krisenzeiten viel Kraft gegeben und wesentlich zum Gelingen dieser Arbeit beigetragen.

Ein großes Dankeschön auch an alle meine Freunde, die mich erinnert haben, dass es ein Leben neben der Doktorarbeit gibt und mich in schwierigen Zeiten immer aufgemuntert haben.

Herzlichst bedanken möchte ich mich bei meinen Eltern und meiner Schwester, die mich seit jeher unterstützen und mir bedingungslosen Rückhalt zukommen lassen.

Schließlich danke ich Sebastian für sein geduldiges Zuhören, sein nahezu grenzenloses Verständnis und seine Unterstützung in guten wie in schlechten Zeiten.

13. Selbstständigkeitserklärung

Hiermit bestätige ich, dass ich die vorliegende Arbeit selbständig, ohne unzulässige fremde Hilfsmittel und nur unter Verwendung der angegebenen Literatur und Hilfsmittel angefertigt habe.

Szilvia Vincze

Berlin, 15.09.2014