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Characterization of virulence factor genes as well as antibiotic and biocide resistance genes in methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from European hares (*Lepus europaeus*) and companion animals originating from the German North Frisian Island Pellworm (European hares) as well as Austria (companion animals)

Diplomarbeit

Veterinärmedizinische Universität Wien

vorgelegt von

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Wien, im September 2020

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ACKNOWLEDGMENTS

I would like to sincerely thank everyone that assisted us to fulfill our Diploma Thesis project for their encouragement and valuable discussions throughout all the phases of this project.

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1 Introduction and hypothesis

1.1 Introduction:

1.1.1 *S. aureus* main characteristics

Staphylococcus aureus is a Gram-stain-positive, cocci-shaped, nonspore-forming bacterium belonging to the genus *Staphylococcus* of the family *Staphylococcaceae*. *Staphylococcus aureus* (*S. aureus)* was first described by Rosenbach in 1884 (Rosenbach et al., 1884) and has a size of 0.5-1.0 μm forming pairs and clusters. This bacterium can be found in a variety of matrixes. Usually *S. aureus* is considered a skin and mucous membranes colonizer not only in humans, but also in domestic animals and wildlife. It can be found in healthy humans and animals as a commensal on skin, glands and mucous membranes, where it can persist asymptomatically (Schleifer and Bell., 2015). Nevertheless *S. aureus* can be involved in a variety of infections of mammals and birds and additionally it holds an important role in nosocomial infections as antibiotic resistance pathogen.

One of the most frequent encounters of *S. aureus* in humans is the food-borne illness caused by the bacterium. *S. aureus* is considered to be quite aggressive due to the to the ability to product numerous virulence factors (Schleifer and Bell., 2015). It can grow in a vast temperature range, ranging from 7 to 48.5°C (optimum at 37°C), while it can withstand lower pH values (grows as low as a pH of 4.2). Furthermore this bacterium is considered halotolerant and can tolerate a concentration of NaCl up to 15% (Schleifer and Bell, 2015). It is quite clear that the bacterium can grow in various food matrixes, and when consumed by humans it is able to provoke the staphylococcal food-borne disease (Kadariya et al., 2014). Ingestion of *S. aureus* and its toxins results to numerous symptoms, with the first symptoms appearing at 3 to 5 hours after ingestion. Usual symptoms are hypersalivation, nausea, vomiting accompanied with abdominal cramps and diarrhea, with the latter two being recorded at a higher frequency in humans. Even though food-borne infections with *S. aureus* are quite common, usually they are not life-threatening

and they are self-limiting, but several cases have been recorded in the past, especially the elderly, infants and patients under immune-compromise (Balaban and Rasooly, 2000; Murray, 2005; Argudín et al., 2010).

Apart from the food-borne infections *S. aureus* which is considered a pathogen can result in infections also in humans and animals (Weese, 2005). Humans constitute an important reservoir for *S. aureus* with the bacterium being found on mucous membranes and the skin (Boucher and Corey, 2008). Recent estimates suggest that 30 % of adults are colonized by the *S. aureus,* while it is accepted that certain groups (health care providers, patients, and immunocompromised individuals) have higher rates of *S. aureus* colonization (Tong et al., 2015). As *S. aureus* can cause a huge range of infections in humans, such as skin and soft tissues, osteomyelitis and bursitis infection and may result in more serious conditions like endocarditis, pulmonary infection and/or infection of internal organs, if the bacterium accesses the bloodstream (Lakhundi and Zhang, 2018). Additionally, *S. aureus* in animals together with *S. intermedius* and *S. hyicus* is one of the most pathogenic species of the family (Hermans et al., 2004). It has been observed that *S. aureus* in can also cause pneumonia, joint infections, osteomyelitis as well as septicemia in poultry (McNamee and Smyth, 2000; Linares and Wigle, 2001; Alfonso and Barnes, 2006). Moreover, other studies have shown that even more species can get infected from *S.aureus* including subcutaneous abscesses, mastitis and pododermatitis in rabbits (Okerman et al., 1984; Hermans et al. 2003), dermatitis and cellulitis in horses (Middleton et al., 2005; Fjordbakk et al., 2008) as well as septicaemia in pigs (Devriese, 1990). However, the most important infection caused by *S. aureus* in animals is described to be the intramammary infections in cattle which can lead to major economic losses (Waage et al., 1999; Hermans et al., 2004; Tenhagen et al., 2006). Moreover, in numerous of studies *S. aureus* has been detected also in samples collected from wildlife. More specifically *S.aureus* has been detected by European brown hares (*Lepus europaeus*) (Loncaric et al., 2014), squirrels (Campbell et al., 1981; Simpson et al., 2010; Simpson et al.; 2013), hedgehogs (Monecke et al., 2013; Rasmussen et al.,2019) as well as by bats (Akobi et al., 2012).

Therefore, it is important to explain the pathogenicity of the bacterium, as well as to clarify the potential transmission from domestic and wild animals to humans and the other way around. For that purpose, it is important to shed light on the virulence characteristics of the bacterium.

1.1.2 *S. aureus* virulence characteristics

In order to be able to adapt in so many niches and exert pathogenicity, *S. aureus* owns a panoply of virulence factors that can be used to ensure bacterial survival (Richardson, 2018).
Generally it is considered that virulence gene regulation in *S. aureus* is centralized and often controlled by the *agr* (accessory gene regulator) locus, as well as the staphylococcal accessory regulator (*sarA, sae, sigB, arl*), which controls many of the cell-wall associated, as well as extra-cellular proteins of *S. aureus* (Yarwood and Schlievert, 2003; Novick and Geisinger, 2008 Pereira et al., 2009; Gomes-Fernandes et al., 2017). First of all, *S. aureus* is well known for its ability to produce proteins, such as the protein A, the chemotaxis-inhibitory protein, and the extracellular-adherence protein which enhance its survival within the host by blocking chemotactic signaling of the neutrophil recognition, as well as neutralizing the host opsonins (Foster, 2005; Rooijakkers et al., 2005; Sun et al., 2018).

Additionally, *S. aureus* produces a wide range of adhesins, which help the bacterium to adhere to the host cell surface. Major adhesins of *S. aureus* are the "microbial surface component recognizing adhesive matrix molecule" (MSCRAMM) (Foster and Höök, 1998). The most typical proteins of the family are the *Staphylococcus* protein A (*SpA*), the fibronectin-binding proteins A and B, as well as the collagen-binding protein and the clumping factor A and B proteins (Foster and Höök, 1998; Lowy, 1998). The proteins mentioned above work by recognizing and binding components of the blood plasma such as fibrinogen, fibronectin and collagens (Lowy, 1998; Cheung and Zhang, 2002; Haggar et al., 2004).
One of the major virulence characteristics of *S. aureus* is the production of exotoxins. The most well-known of these toxins are the toxic shock syndrome toxin 1, the *Staphylococcus* enterotoxins, and the exfoliative toxins (Lina et al., 2004). These toxins are responsible for toxic shock and food poisoning caused by *S. aureus*.
On the other hand the exfoliative toxins are involved mainly in skin syndromes caused by *S. aureus* (Melish and Glasgow, 1970). Additionally several *S. aureus* strains possess enzymes such as the staphylokinase or the hyaluronidase, which help the bacterium further survive and colonize the host (Bokarewa et al., 2006).

Finally, one of the most effective virulence factors of *S. aureus* is the ability to biofilm. Biofilms formation of *S. aureus* is usually complex and the characteristics of the biofilms are only recently elucidated (Lister and Horswill, 2014), nevertheless it seems that biofilms may play a role on the survival of the bacterium on the environment, as recent studies pinpoint that host-adapted mastitis isolated of *S. aureus* have a greater capacity of producing biofilms (Marbach et al., 2019).

1.1.3 Methicillin-resistant *S. aureus* (MRSA)

Increased misuse of antibiotics in terms of application and dosage, is an additional promoting factor for the development of antibiotic resistance. (Kimang'a, 2012; Nosanchuk et al., 2015).
S. aureus was firstly described as resistant to penicillin back in late 1950s (Stapleton and Taylor, 2002). This resistance of the bacterium to penicillin led to the discovery of penicillin derivative, methicillin in 1959, which was the first semisynthetic β-lactamase-resistant penicillin against β-lactamase-producing staphylococci (Stapleton and Taylor, 2002). The methicillin-resistant *S. aureus* (MRSA) has increasingly been reported since the 1960s, with the first case and isolate observed in animals back in the early 1970s, in cows with mastitis (Devriese et al., 1972).
Nowadays it has been observed that *Staphylococcus aureus* can not only evolve a methicillin-resistance but a lot of *S. aureus* strains are capable of acquiring resistance to nearly all antibiotic classes. (Loncaric et al., 2014; Schauer et al., 2018; Soimala et al., 2018).
De novo mutations in chromosomal genes as well as acquisition of horizontally transferred resistance determinants can lead to antibiotic resistance. (Vestergaard et al., 2019)
MRSA is a very common human and animal pathogen and causes approximately 170.000 infections in and more than 5000 fatalities in Europe each year (Köck et al., 2014) and is reported not only to affect humans, but also livestock, companion and wild animals.
Depending on the origin of the strains, MRSA can be categorized as: community-acquired MRSA (CA-MRSA), found in the environment, healthcare-associated MRSA (HA-MRSA) which is associated with nosocomial infections and livestock-associated MRSA (LA-MRSA).
Humans that are in contact with livestock are at higher risk of infection with LA-MRSA. (Anjum et al., 2019).

While nosocomial MRSA infections (HA MRSA) often occur in immune-compromised patients, the community-associated MRSA (CA-MRSA) is responsible for infections of the skin and soft tissues in healthy individuals without any prior disease and is associated with complications described before and connected with high morbidity and mortality. This differentiation on the pathogenic effect of HA-MRSA and CA-MRSA could be attributed to different pathogenicity mechanisms used by the different types of MRSA strains (Choo, 2017). The most important genes related to antibiotic resistance are the methicillin resistance genes such as *mecA* and *mecC* genes which are part of the staphylococcal cassette chromosome *mec* and encode the penicillin-binding protein 2a. That protein substitutes in the cell-wall synthesis when β-lactam antibiotics are present. And due to the low affinity to the β-lactam antibiotics, the bacterial cell wall construction is not interrupted (Pinho et al., 2001; Schwarz et al., 2017). The resistance to penicillin derivatives, as well as the resistance to various antibiotics is attributed to the presence of different antibiotic resistance genes. In the following table (Table 1) an overview of the genes encoding for antibiotic resistance can be seen.

Table 1: Genes encoding for antibiotic resistance in MRSA[1]

Encoding genes	*mecA*	*mecC*	*blaZ*	*blaI*	*blaR*	*aacA-aphD*
Resistance	β-lactam methicillin	β-lactam methicillin	β-lactam penicillin	β-lactam penicillin	β-lactam penicillin	Aminoglycosids (gentamicin, kanamycin, tobramycin, amikacin)
Resistance mechanism	Target site replacement (alternative penicillin binding proteins)	Target site replacement (alternative penicillin binding proteins)	Enzymatic inactivation (β-lactamase)	Enzymatic inactivation (β-lactamase)	Enzymatic inactivation (β-lactamase)	Enzymatic inactivation (acetyltransferase and phospotransferase)
Encoding genes	*aadD*	*aph-A3*	*sat*	*mph*(C)	*vat*(B)	*vga*(A)
Resistance	Aminoglycosids (neomycin, kanamycin, tobramycin)	Aminoglycosids (neomycin, kanamycin, amikacin)	Streptothricin	Macrolides	Strepto-gramin A	Lincosamides, pleuromutilines, streptogramin A
Resistance mechanism	Enzymatic inactivation (adenyltransferase)	Enzymatic inactivation (phospotransferase)	Enzymatic inactivation (acetyltransferase)	Enzymatic inactivation (phospotransferase)	Enzymatic inactivation (acetyltransferase)	Target site protection (ribosome protective)

[1] Information on the table collected from (Ross et al., 1990; Schwarz et al., 1992, 1998, 2018; Roberts, 1996; Derbise et al., 1996; Allignet and El Solh, 1997; Matsuoka et al., 1998; Schnellmann et al., 2006; Lüthje and Schwarz, 2006; O'Neill et al., 2007; Norström et al., 2009; Hauschild and Schwarz, 2010; Perreten et al., 2010; Zakour et al., 2011; Gómez-Sanz et al., 2011; Wendlandt et al., 2013; Soimala et al., 2018; Anjum et al., 2019).

Table 1 (Continued): Genes encoding for antibiotic resistance in MRSA

Encoding genes	*vgb*	*tet*(K)	*tet*(M)	*msr*(A)	*fusC*	*erm*(A)
Resistance	Streptogramin B	Tetracyclines	Tetracyclines	Macrolides, Streptogrmin B	Fusidic acid	Macrolides, Lincosamides, streptogramin B
Resistance mechanism	Enzymatic inactivation (hydrolase)	Active efflux (major facilitator superfamily)	Active efflux (major facilitator superfamily)	Target site protection (ribosome protective ABC-F protein)	Target site protection (ribosome protective protein)	Target site modification (rRNA methylase)

Encoding genes	*erm*(B)	*erm*(C)	*cfr*	*lnu*(A)	*mupA*
Resistance	Macrolides, Lincosamides, streptogramin B	Macrolides, Lincosamide, streptogramin B	Phenicols, linosamides, pleuromutilines, Strepto-gramin A	Lincosamides	Mupirocin
Resistance mechanism	Target site modification (rRNA methylase)	Target site modification (rRNA methylase)	Target site modification (rRNA methylase)	Enzymatic inactivation (nucleotidyltransferase)	Target replacement (mupirocin-insensitive isoleucyl-tRNA synthase

1.1.4 Biocides and *S. aureus*

Biocides are chemical agents that have been used for centuries as external decontamination agents. The term biocides cover a wide range of substances, with the most useful in veterinary medicine being antiseptics and disinfectants. The first are used for minimizing the risk for contamination before operation and for eliminating residual bacterial activity after washing, while the others are used mostly for objects in order to ensure bacterial sterility.
(Wijesinghe et al., 2010; Conceição et al., 2015; Gupta et al., 2018). The susceptibility of the bacteria to these chemical agents differs between the bacteria species. (Ducel et al., 2002).
Biocides exert their biocidal action using different mechanisms, for example chlorine-releasing biocides such as sodium hypochlorite, or hypochlorous acid are oxidizing agents which destroy the cellular activity of proteins, while iodine compounds alternate the proteins and fatty acids composition of bacteria leading to cell death (Gupta et al., 2018).
Two of the most frequently used disinfectants are quaternary ammonium compounds (QACs) and alcohol derivatives. The first one affect the bacterial cytoplasmic membrane leading to cell damage, while the later ones result in a denaturation of proteins and nucleic acids (Block, 2001). Due to the increased use of biocides such as quarternary ammonium compounds, alcohols and aldehydes as tool to reduce the resistant bacterial pathogens increased concerns have been observed about its impact on bacterial resistance (Morrissey et al., 2014). Therefore, the importance of the screening of bacterial isolates for decreased biocide susceptibility increases. The determination of biocide susceptibility is defined by its minimum inhibitory concentrations (MICs). MICs are the lowest antimicrobial concentrations needed to inhibit the macroscopic visible growth of a microorganism after overnight incubation. MICs are assumed to be the ‘gold standard’ for the determination of the susceptibility to antimicrobials and are mainly used to confirm resistance and to test the invitro activity of new antimicrobials (Andrews, 2001). Additionally, in a row of two recent studies is presented a newly developed broth microdilution method for biocide susceptibility testing (Feßler et al., 2018; Schug et al., 2020).
Bacteria usually inherit resistance to bactericides through genes, which usually encode for efflux pumps, proteins which reduce cellular impermeability and cell wall damage and enzymes (Fraise, 2002; Costa et al., 2013; Conceição et al., 2016). Just like with antibiotic resistance, the misuse and overuse of biocides resulted in an increase of the antiseptic resistance of *S.aureus* against various antiseptic substances (Conceição et al., 2016).

One of the main mechanisms with which biocides resistance is acquired, is through efflux pumps. Efflux pumps are used by bacterial cells in order to draw out of the cell potentially toxic compounds such as antibiotics, heavy metals, or chemical compounds, like the biocides (as seen in Fig. 1). In *Staphylococcus* species, the encoding genes for the multidrug efflux pump are called *qac* genes (Conceição et al., 2016). These gene encode six different QAC efflux pump proteins, with QacA and QacB being among the ones that are conserved within the *Staphylococcus* species (Wassenaar et al. 2015). The regulation of these proteins is under the control of numerous genes, which are involved in the expression of efflux pumps or other biocide resistance molecules. The most notorious genes are *qacA, qacB, smr, norA, lmrS, mepA*, and *sepA*, which have been detected in clinical *S. aureus* isolates from animals, humans and environment (Bjorland et al., 2001; Noguchi et al., 2005; Conceição et al., 2016). Interestingly previous studies have indicated that there could be a potential association between β-lactam resistance and QAC resistance in *S. aureus* (Akimitsu et al., 1999; Conceição et al., 2016).

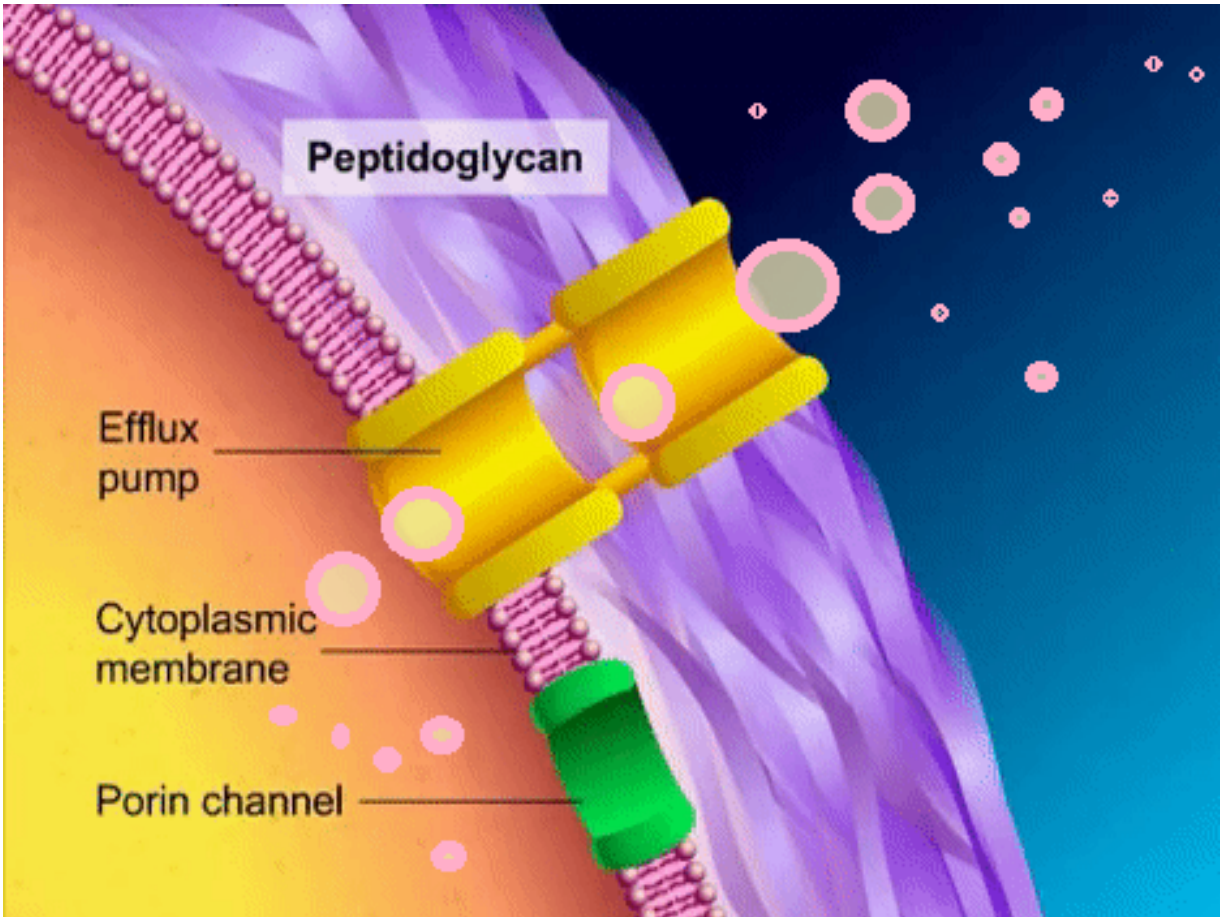

Figure 1: Animation of efflux pump action, efflux pump draws out molecules such as antibiotics, or biocides when they enter the bacterial cell.

Adapted and modified from mechanismsinmedicine.com

1.2 Hypothesis

Through this study a comparison of the presence and prevalence of virulence factor genes as well as antibiotic and biocide resistance genes between MRSA isolates from companion animals from Austria and wild animals from the German North Frisian island of Pellworm, took place.

The hypothesis was: Virulence factor genes as well as antibiotic and biocide resistance genes are less common in MRSA isolates recovered from wildlife than in those isolated from companion animals.

2. Material and methods

2.1. Isolation of MRSA and antimicrobial susceptibility testing

The collected MRSA strains consisted a considerable pool of isolates from different sources. Samples originating from companion animals (cats, dogs, horses, rabbits, and exotic birds) were collected over a period of five years from 2013 to 2018. In total 90 non-repetitive MRSA isolates were collected, 62 isolates originated from horses (68.9 %), 13 from cats (14.4 %), 10 from dogs (11.0 %), two from rabbits (2.2 %), and one each of the remaining samples from a domestic canary, a zoo-kept hammer-headed bat (*Hypsignathus monstrosus*) and a semi-captive northern bald ibis (*Geronticus eremita*) (n =1, 1.1% of the total samples each).
Samples isolated from from wild European hares (*Lepus europaeus*) from the Pellworm islands, located in northern Germany (n = 78,non-repetitive MRSA isolates). The MRSA isolates have been collected from various bodily sites such as the nasal cavity (n = 49; 62,8 %) , the intestine (n = 20; 25,6 %), abscesses (n = 3;3,8 %), the liver (n = 1; 1,2 %) and the eye (n = 1; 1,2 %). The origin of four isolates (n = 4; 5,1 %) was not reported.

All samples mentioned above have been cultivated, species-characterised and tested for antibiotic resistance by using the agar disk diffusion method. All MRSA isolates were stored at −80°C until further examination. The exact determination of the bacterial species has been achieved by using Matrix-Assisted Laser Desorption/ Ionization- Time of Flight (MALDI-TOF) mass spectrometry. (Spergser et al., 2019)

Agar disk diffusion was performed according to the recommendations given in the Clinical and Laboratory Standards Institute (CLSI). The following disks have been used (Beckton Dickinson, Heidelberg, Germany): penicillin (PEN, 10 IU), cefoxitin (FOX, 30µg), gentamicin (GEN, 10µg), erythromycin (ERY, 15µg), clindamycin (CLI, 2µg), tetracycline (TET, 30µg), ciprofloxacin (CIP, 5µg), trimethoprim sulfamethoxazole (SXT, 1.25/ 23.75 µg), chloramphenicol (CHL, 30 µg), and linezolid (LZD, 30 µg). The reference strain *S. aureus* ATCC®25923 served as a quality control strain (Loncaric et al., 2019).

2.2. Molecular characterization of MRSA

Prior to DNA extraction, isolates were grown on BD Columbia III agar with 5% sheep blood (Beckton Dickinson) and incubated overnight at 37 °C. Bacterial cells were enzymatically lysed and DNA extraction was performed using commercially available spin columns (GenEluteTM Mammalian Genomic DNA Miniprep Kits, Sigma-Aldrich, Vienna, Austria) as previously described (Loncaric et al., 2014).

Methicillin resistance was confirmed by PCRs with primers targeting *mecA* and *mecC* as described elsewhere (Loncaric et al., 2019). A DNA microarray (*S. aureus* Genotyping Kit 2.0, Alere, Jena, Germany) was used for the identification of more than 330 species-specific, virulence-associated, and resistance genes (Monecke et al., 2008; Loncaric et al., 2019). Microarray provides a basis for genotyping thousands different loci at the same time, which can be used for association and linkage studies to isolate chromosomal regions which are related to a particular gen or disease (Govindarajan et al., 2012). For visualization of the diversity between the DNA microarray results the program SplitsTree4 was used (Huson and Bryant, 2006; Coombs et al., 2010; Loncaric et al., 2019). The presence of antimicrobial resistance and virulence genes was extracted from WGS data via comparison with the Comprehensive Antibiotic Resistance Database (CARD) (Jia et al., 2017; Loncaric et al., 2019) or based on Alere Microarray data (Strauß et al., 2016; Loncaric et al., 2019).

Further Details about the Materials and Methods used can be found in the published Papers or after contact with the supervisor of the project, Dr. Loncaric.

2 Results

2.1 Prevalence of virulence factor genes and biocide resistance genes

In general differences in the prevalence of virulence factor genes between isolates from wildlife and companion animals were observed (Figure 2). Both *fnaA* and *fnaB* were found in much higher rates in isolates originating from companion animals. Additionally, the *cna* gene was also more prevalent in companion animals (76 positives from companion animals, while 7 positives from wildlife), while the *efb/fib* gene as found more frequently in wildlife isolates (73 positive wildlife isolates in comparison to 15 from companion animals). The Panton–Valentine leukocidin (PVL) genes *(lukF-PV, lukS-PV)* genes were exclusively found in isolates from companion animals, with 3/90 and 4/90 of the tested isolates being positive in lukF-PV and lukS-PV respectively. On the contrary the leukotoxin gene *lukD* was found mostly in isolates originating from wildlife (56 positives from wildlife, in comparison to 16 from companion animals). Small differences accompanied by statistical significance were noted in the prevalence of *lukY* gene (86 samples positive from companion animals and 68 from wildlife), while no statistically significant difference was noted in the prevalence of *lukE* and *lukX gene*. The virulence of *S.aureus* is attributed to the presence of different virulence resistance genes. In the following table (Table 2) an overview of the genes encoding for virulence factors can be seen.

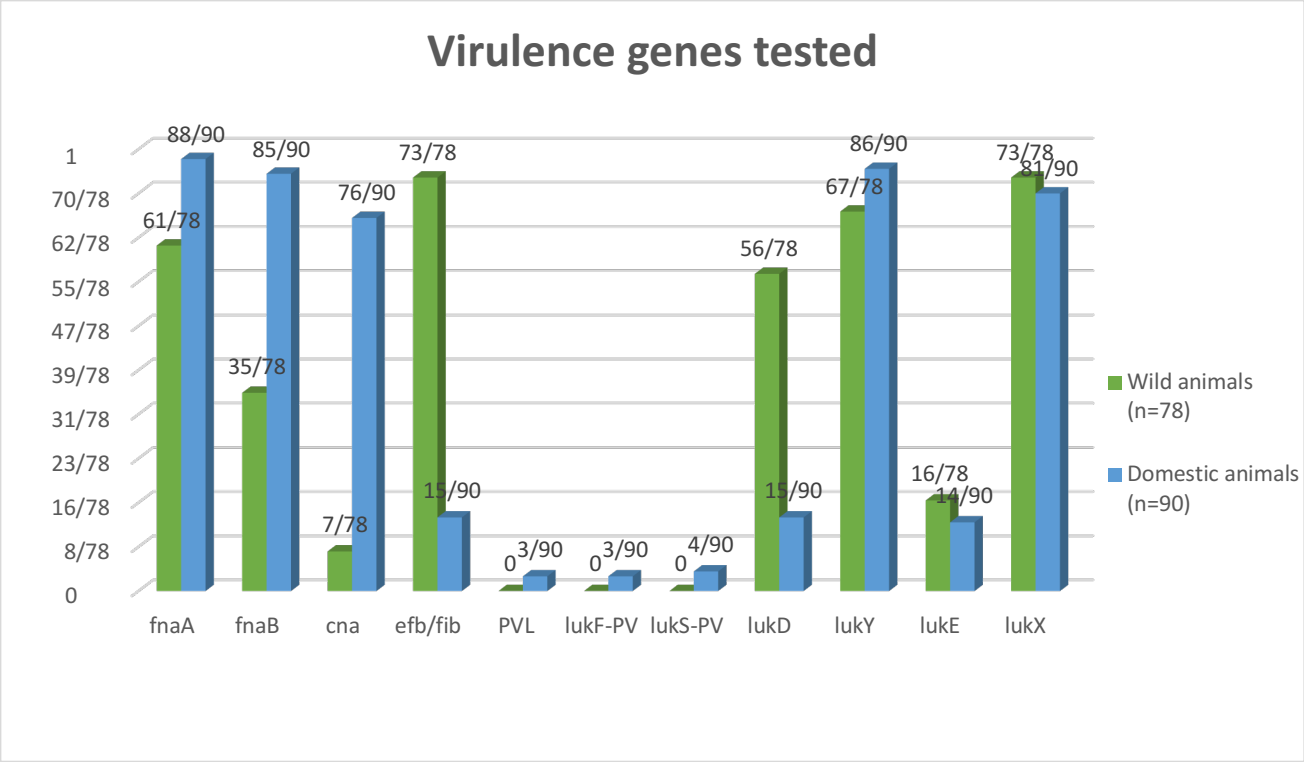

Figure 2: Comparison of the number of MRSA isolates between companion and wildlife animals which tested positive for various virulence resistance genes. On the current table are only represented the isolates showing significant differences in the prevalence.

Encoding gene	*fnbA*	*fnbB*	*efb/fib*
Virulence factor	Fibronectin binding Protein A	Fibronectin binding Protein B	Fibrinogen-binding proteins
Encoding gene	*cna*	*clfA*	*clfB*
Virulence factor	Collagen binding adhesin	Clumping factor A	Clumping factor B
Encoding gene	PVL	*lukF*-PV	*lukS*-PV
Virulence factor	Panton-Valentine leukocidin	F- component of PVL	S- component of PVL
Encoding gene	*hlgA*	*lukM,lukD,lukE, lukX,lukY*	*sea,seb,sec,sed,see, seh,sej,sek,sel,seq, ser,seg,sei*
Virulence factor	Gamma-γ-hemolysin A	Leukotoxin	SE- staphylococcal enterotoxins
Encoding gene	*selm, seln, selo, selu*	*egc*	
Virulence factor	SE-like, Staphylococcal like enterotoxins	egc-encoded Superantigens	

Table 2: Genes encoding for virulence factors of S.aureus have been compared in this Study.

Biocides have been used for centuries to control and supress the growth of pathological microorganisms on humans and animals (Conceição et al. 2015, Gupta et al 2018). Just like antibiotic resistance, the mis-/ overuse of biocides, has created a concern that such intense usage could lead to decreased bacterial susceptibility to a product and maybe to cross-resistance to unrelated antimicrobials (Conceição et al. 2015). In our study we tested the *qacC* and *qacA* genes which confer resistance to quaternary ammonium compounds, but there has been observed only a very low prevalence of the qacC gene (n=2 of 90) by companion animals, while both *qacC and qacA* were negative in all wildlife isolates tested.

2.2 Prevalence and differences of antibiotic resistance genes

In general differences between the prevalence of antibiotic resistance genes between the two isolates populations were observed (as seen in Figure 3). Regarding the methicillin resistance, *mecA* was found mainly in companion animals, with all of the tested strains from companion animals being positive, on the other hand *mecC* gene was only found in wildlife samples, with 71 samples collected from wildlife being positive. The contrary applies for the *blaZ, blaI* and *blaR* genes, all being found much more frequently in companion animal isolates rather to the ones originating from wildlife, while the difference in the prevalence of these genes between companion animals and wildlife was always highly statistically significant. Significant differences of the prevalence between wildlife and companion animals were also observed in other antibiotic resistance genes (Figure 3)

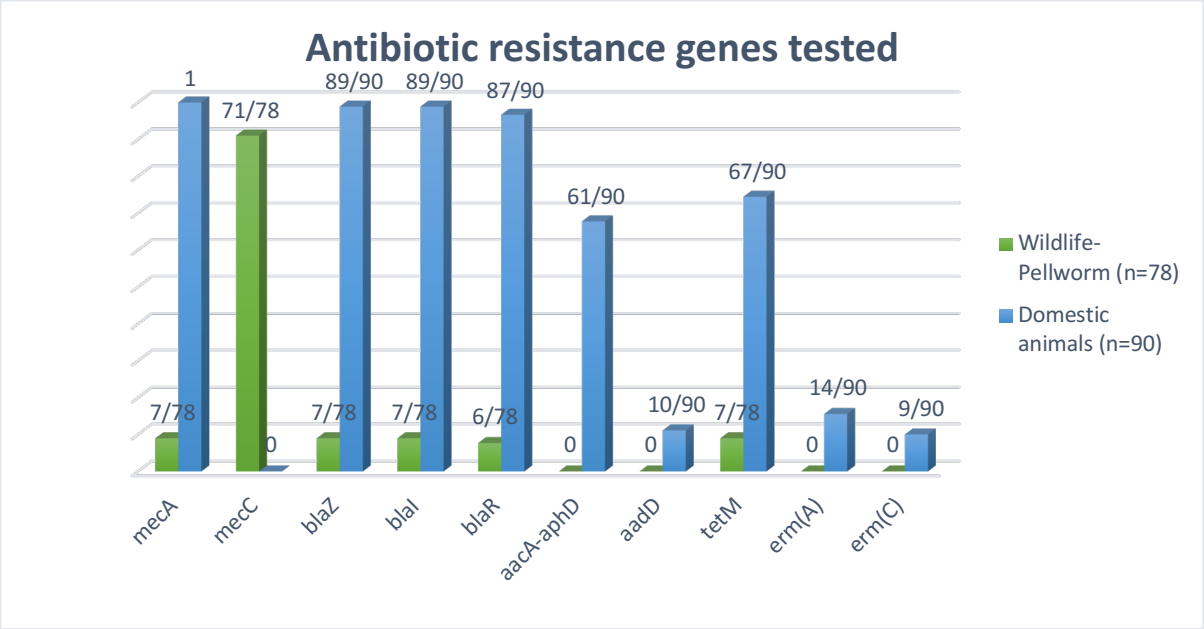

Figure 3: Comparison of the number of MRSA isolates between companion and wildlife animals which tested positive for various antibiotic resistance genes. On the current table are represented only the isolates showing significant differences in the prevalence.

3 Discussion

3.1 Virulence factors and biocide resistance genes

Staphylococcus aureus is being characterised as the most pathogenic member of the *Staphylococcaceae* due to the large arsenal of virulence factors that are regulated by a series of transcription factors (Richardson, 2018). Differences in the prevalence of virulence factor genes in isolates originating from wildlife and companion animals were recorded in our study. The *fnaA* and *fnaB* genes which encode for the fibronectin-binding proteins were found both in wildlife and companion animals MRSA isolates. The prevalence of both genes ranged at 97.8 % and 78.2 % for *fnaA* at companion and wildlife respectively, while it ranged at 34.4 % and 44.9 % respectively for *fnaB*. This is higher than what it was described before for MRSA isolates originating from human specimens (Thompson and Brown, 2014) but in accordance with previous studies which report high prevalence of the genes in samples from dogs and cats (Asanin et al., 2019), as well as the veterinary clinic environment (Chen et al., 2020). The detection of the *cna* gene, which encodes for a collagen adhesion has been recovered mainly from MRSA isolates originating from companion animals. Previous studies has placed the prevalence of the *cna* gene at the MRSA population isolated from veterinary clinics specimens at 82.4 % (Chen et al., 2020), while it was found on 84.4 % of our samples of companion animal origin. This is also in accordance with a previous study conducted on companion animals where 16,6% (1/6) of the isolates tested, were *cna* positive (Asanin et al., 2019). Moreover, in a recent study the *cna* gene was absent from all wildlife animal (Wild Lagomorphs) isolates that have been tested (Moreno-Grua et al., 2020).

The *efb/fib* gene is responsible for the production of an extracellular fibrinogen binding protein in *S. aureus* and in our study, it was found more frequently in the MRSA samples isolated from wildlife animals, rather than companion animals. This comes in contrast with previous studies which indicate a high prevalence of the gene in isolates originating from dogs, cats and the veterinary clinic environment (Chen et al., 2020). Although, the *fib* gene is also being described to be detected on a wildlife animal study conducted in Spain (Moreno-Grua et al., 2020).
In our study differences in the prevalence of the Leukotoxin (*luk)* genes as well as in Panton-Valentine-Leukocidin (PVL) were found. Panton-Valentine Leukocidin (PVL) is a pore-forming toxin associated with the CA-MRSA and can lead to necrotizing pneumonia often with lethal ending (Rahman et al., 1991; Hoppe et al., 2019).

In this study the presence of *lukF*-PV *and lukS*-PV genes were only found in MRSA from domestic animals with 3% and 4% respectively, while there was no wild animal isolate tested positive for these genes. This is in accordance with several studies which indicate a high prevalence of the Panton-Valentine-toxins (*lukF*-PV and *lukS*-PV) on companion animal isolates (Rankin et al., 2005; Haenni et al., 2012; Gomez- Sanz et al., 2013) and the absence on all wild animal isolates tested (Monecke et al., 2013; Rasmussen et al., 2019; Moreno-Grua et al., 2020). However, the Leukotoxin gene *lukD* was isolated mostly from wildlife MRSA. On the contrary the *lukY* was more frequent in companion animals, while the prevalence of *lukE* and *lukX* genes was almost similar within the two MRSA populations. These results do not match with these of other studies, while it has been reported that *lukD* and *lukE* can mainly be found in companion animals (Gomez-Sanz et al., 2013; Chen et al., 2020). Concluding *lukF, lukS* and *lukY* genes were found mainly in MRSA isolates from companion animals. This is in accordance with previous studies which have recovered the aforementioned genes from all the tested human and animal origin samples (Jamrozy et al., 2012).

Additionally, in our study the Staphylococcal enterotoxin (SEs) encoding genes *sea, seb, sej, sei, seg, selm, seln, selo, selu* were present only in companion animal MRSA isolates. This comes in concordance with several studies showing the presence of these genes in companion animals (Haenni et al., 2012; Gomez-Sanz et al., 2013; Asanin et al., 2019) as well as their absence from wild animal isolates (Moreno-Grua et al., 2020).

In our study we also wanted to elucidate the presence the *qacC* and *qacA* genes which confer resistance to quaternary ammonium compounds and chlorhexidine, through encoding of efflux pumps. While no isolate irrespective from its origin was positive for the *qacA* gene, two of the MRSA isolates originating from companion animals were positive for the *qacC* gene. The low prevalence in which the gene was found in the pool of analyzed isolates is not a surprise. Previous studies have shown that the recovery rate of the gene is pretty low in MRSA samples originating from companion animals (Vincze et al., 2013; Haenni et al., 2017).

3.2 Antibiotic resistance genes

Antimicrobial resistance rises up as an emerging public health threat and continues until today to be a major problem in healthcare units. Antibiotic resistant bacteria usually result in increased time of hospital stays, and often in serious life threating complications in elderly, pregnant or immune-compromised patients, therefore increasing the morbidity and mortality (Kraker et al., 2011; Chang et al., 2011). Additionally recent studies have shown that long-term care facilities, such as nursing homes, may also be a major source for infection for the elderly (Rowan-Nash et al., 2020).

In numerous studies it has been reported that antimicrobial resistant (AMR) bacteria are commonly detected in healthcare institutions, which makes these facilities a common acquisition place (Rowan-Nash et al., 2020). Moreover patient-to-patient transmission of AMR isolates can occur because of bad hygiene conditions or environmental contamination (Struelens, 1998; Cookson, 2005; Paterson, 2006b; Mulvey and Simor, 2009; Rowan-Nash et al., 2020). It is estimated that 35% of health care facility residents are colonized with multidrug resistant microorganisms (Pop-Vicas et al., 2008; O'Fallon et al., 2009; O'Fallon et al., 2010;Cassone and Mody, 2015; Aliyu et al., 2017). The patient environment in nursing facilities is proved to harbor a reservoir of potentially harmful, and often lethal multidrug-resistant organisms (MDROs) (Chemaly et al., 2014). Apart from that, it is accepted that animals may act as a reservoir for the transmission of antibiotic resistant *S. aureus* to humans. (Guardabassi et al., 2004). Resistant bacterial strains might be acquired by humans through pathways such as person-to-person transmission, environmental exposure and direct exposure to animals (Guardabassi et al., 2004). Cats and dogs represent potential sources of spread of antimicrobial resistance due to the extensive use of antimicrobial agents in these animals and their close contact with humans and especially children. (Guardabassi et al., 2004). Moreover a potential of two-sided transmission route has been observed (Ferreira et al., 2011).

The colonisation of healthcare workers is a common phenomenon and it is assumed to play a role in the nosocomial transmission (Albrich and Harbarth 2008). MRSA at low prevalence has been described to be found also in healthy horses. Although in equine hospitals a much higher prevalence has been observed (Van Balen et al., 2014; Tiroshlevy et al., 2015). Conserningly is the fact that such a high prevalence of MRSA constitutes a major risk factor not only for the horses, but also for the veterinary personnel (Koop, 2016). It has been described in previous

studies that veterinarians are at an increased risk of carrying MRSA compared to the general population (Jordan et al., 2011, Cuny et al., 2016; Koop, 2016). Moreover, in a study conducted in an equine clinic a possible transmission from the vet to, the equine patients, has been described (Koop, 2016). An older study has indicated that the source of infection for companion animals in clinics may be the colonized nasal cavities of surgeons and nurses which could act as a reservoir for the bacterium. Even though this mechanism of transmission through this route is not totally clarified, still it cannot be disregarded (McLean and Ness, 2008).

Apart from the presence of MRSA in companion animals one should not disregard the presence of the bacterium in livestock and wild animals. Studies have already indicated that pig farming may be a potential source for LA-MRSA carryover from animals to humans and vice versa (Köck et al., 2009; Lozano et al., 2011). Moreover MRSA has been found in carcasses of pigs in slaughterhouses, therefore, hinting that there is also a potential of transmission of MRSA through the food chain (Ivbule et al., 2017). Additionally MRSA has been isolated from various wild animals in the past, such as red deer, ibex, and wild boars (Porrero et al., 2013).

In our study we found a high prevalence of the *mecA* gene in isolates originating from companion animals, with all the isolates (n=90 out of 90; 100%) testing positive for the gene. This comes in accordance with two other studies in which the *mecA* gene was present in all isolates originating from companion animals, such as dogs, cats and horses (Baptiste et al., 2005; Ruzauskas et al., 2015). Previous studies have also shown that the *mecA* is frequently recovered from companion animals in neighboring countries, e.g. Germany (Walther et al., 2012). On the other hand, the low prevalence of *mecA* gene detected in our study in isolates of European hares (*Lepus europaeus*) is not totally in accordance with previous studies. In our study we found the *mecA* gene in 9.0 % of the isolates originating from European hares (n = 7/78), while previous studies have indicated that MRSA and other methicillin resistant staphylococcal species (such as *S. sciuri, S. vitulinus* etc.) collected from wild hares (*Lepus granatensis*), all harbored the *mecA* gene (Silva et al., 2020). On this point it should be noted that all wildlife samples in our study were collected from a small island in northern Germany from only one animal species (European hares-*Lepus europaeus*), which means that a direct comparison with other studies is not absolutely accurate and hardly comparable.
On the other hand, we recorded a high prevalence of the *mecC* gene in the studied strains isolated from wildlife (n= 71 out of 78; 91.0 %), while no strain isolated from companion animals harbored the gene. These findings are in line with previous studies which describe the

presence of the *mecC* gene in isolates originating from wild animals, such as deers and wild boars (Concepción Porrero et al., 2014). On contrary to our results, the *mecC* gene has also been detected in isolates originating from companion animals such as dogs (Drougka et al., 2016). Additionally, other research groups have identified only one positive *mecC* isolate (which incidentally originated from a pet rabbit) in samples originating from companion animals (Ruzauskas et al., 2015).

4 Summary

Through this comparative study we tried to highlight the different potential of MRSA strains isolated from different origins. MRSA isolates originating from companion and wildlife animals, show different antibiotic resistance, as well as virulence factors profiles. Certain methicilline-resistance gene *mecA*, has been detected by far more frequently in MRSA samples originating from companion animals, while *mecC* gene was mainly found in wildlife MRSA isolates.

Additionally, the *qac* genes (*qacA, qacC*) were found at a low prevalence, with only two of the companion animal isolates (n=90) being positive to *qacC*, while both *qacA* and *qacC* were absent of all wildlife animal isolates (n=78). Moreover, virulence factor patterns differed between the wildlife/companion animal groups with *fnaA*, *fnaB* as well as *cna* gene being more prevalent in MRSA isolates from companion animals. In contrast the *efb/fib* gene was more frequent in wildlife MRSA isolates. The *luk* genes, showed variability in their prevalence with *lukD* and *lukE* genes being found in higher frequency in MRSA isolates from wildlife. The Panton-Valentine-Leukocidin genes, *lukF*-PV und *lukS*-PV have been only detected in companion animal isolates. Additionally, in our study Staphylococcal enterotoxin (SEs) genes were tested, with all of them (*sea, seb, sec, sed, see, seh, sej, sek, sel, seq, ser, seg and sei*) being absent in all wildlife animal isolates tested, and present some companion animal isolates.

5 Zusammenfassung

Durch diese Vergleichsstudie haben wir versucht, das unterschiedliche Potenzial von MRSA-Stämmen hervorzuheben, die aus Begleits- und Wildtieren stammten. MRSA-Isolate, die von Begleit- und Wildtieren stammen, zeigen unterschiedliche Virulenzfaktorprofile sowie unterschiedliche Antibiotikaresistenzen. Bestimmte Methicillin-Resistenzgene, wie *mecA* wurden weitaus häufiger in MRSA-Proben von Haustieren nachgewiesen, während das *mecC*-Gen hauptsächlich in MRSA-Isolaten von Wildtieren gefunden wurde.

Zusätzlich zeigten die qac-Gene (*qacA, qacC*) eine geringe Prävalenz, wobei nur zwei der Haustierisolaten (n = 90) positiv für *qacC* gefunden wurde, während sowohl *qacA* als auch *qacC* bei allen Isolaten von Wildtieren (n=78) abwesend waren.

Darüber hinaus unterschieden sich die Muster der Virulenzfaktoren zwischen den Wildtier- und Begleittiergruppen, wobei die *fnaA, fnaB und cna*-Gene in MRSA-Isolaten von Begleittieren häufiger vorkamen. Im Gegensatz dazu kamm das *efb / fib*-Gen in MRSA-

Wildtierisolaten häufiger vor. Die luk-Gene zeigten Variabilität in ihrer Prävalenz. Die *lukD*- und *lukE*-Gene wurden in MRSA-Isolaten aus Wildtieren häufiger gefunden, während die Panton-Valentine-Leukocidin *lukF*-PV und *lukS*-PV- Gene lediglich bei Beigleittieren detektiert worden sind.

Zusätzlich wurden in unserer Studie Staphylokokken-Enterotoxin (SEs) -Gene getestet, wobei alle (*sea, seb, sec, sed, see, seh, sej, sek, sel, seq, ser, seg* und *sei*) in allen Wildtierisolaten abwesend waren, während sie bei einigen Begleittierisolaten nachgewiesen worden sind.

6 Abbreviations

AMR- antimicrobial resistant

CA-MRSA - Community-associated methicillin-resistant *S. aureus*

EthBr- Ethidium Bromide

fnaA- fibronectin-binding protein A

fnaB- fibronectin-binding protein B

HA-MRSA - Healthcare Associated MRSA strains

LA-MRSA - Livestock –associated methicillin-resistant *S. aureus*

MRSA – Methicillin-resistant *S. aureus*

MSCRAMM- microbial surface component recognizing adhesive matrix molecule

MSSA- Methicillin-sensitive *S.aureus*

PVL-Panton–Valentine leukocidin

SEs- Staphylococcal-Enterotoxin

PBP2a-penicillin-binding protein 2a

QAC- quarternary ammonium compounds

MICs-minimum inhibitory concentrations

7 References

Akimitsu, N., Hamamoto, H., Inoue, R.I., Shoji, M., Takemori, K., Hamasaki, N., Sekimizu, K., 1999. Increase in resistance of methicillin-resistant *Staphylococcus aureus* to β-lactams caused by mutations conferring resistance to benzalkonium chloride, a disinfectant widely used in hospitals [1]. Antimicrob. Agents Chemother. doi:10.1128/aac.43.12.3042

Akobi B, Aboderin O, Sasaki T, Shittu A (2012) Characterization of *Staphylococcus aureus* isolates from faecal samples of the straw-coloured fruit bat (Eidolon helvum) in Obafemi Awolowo University (OAU), Nigeria. BMC Microbiol 12: 279 10.1186/1471-2180-12-279 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

Allignet, J., El Solh, N., 1997. Characterization of a new staphylococcal gene, *vgaB*, encoding a putative ABC transporter conferring resistance to streptogramin A and related compounds. Gene 202, 133–138. doi:10.1016/S0378-1119(97)00464-2

Alfonso, M., & Barnes, H. J. (2006). Neonatal osteomyelitis associated with *Staphylococcus aureus* in turkey poults. Avian diseases, 50(1), 148–151. https://doi.org/10.1637/7298-110104R.1

Andrews, J.M., 2001. Determination of minimum inhibitory concentrations. J. Antimicrob. Chemother. 48, 5–16. doi:10.1093/jac/48.suppl_1.5

Anjum, M.F., Marco-Jimenez, F., Duncan, D., Marín, C., Smith, R.P., Evans, S.J., 2019. Livestock-associated methicillin-resistant *Staphylococcus aureus* from animals and animal products in the UK. Front. Microbiol. doi:10.3389/fmicb.2019.02136

Argudín, M.Á., Mendoza, M.C., Rodicio, M.R., 2010. Food poisoning and *Staphylococcus aureus* enterotoxins. Toxins (Basel). doi:10.3390/toxins2071751

Asanin, J., Misic, D., Aksentijevic, K., Tambur, Z., Rakonjac, B., Kovacevic, I., Spergser, J., Loncaric, I., 2019. Genetic profiling and comparison of human and animal methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from Serbia. Antibiotics 8. doi:10.3390/antibiotics8010026

Aslantaş, Ö., & Demir, C. (2016). Investigation of the antibiotic resistance and biofilm-forming ability of *Staphylococcus aureus* from subclinical bovine mastitis cases. Journal of Dairy Science, 99(11), 8607–8613. https://doi.org/10.3168/jds.2016-11310

Balaban, N., Rasooly, A., 2000. Staphylococcal enterotoxins. Int. J. Food Microbiol. doi:10.1016/S0168-1605(00)00377-9

Baptiste, K.E., Williams, K., Willams, N.J., Wattret, A., Clegg, P.D., Dawson, S., Corkill,

J.E., O'Neill, T., Anthony Hart, C., 2005. Methicillin-resistant Staphylococci in companion animals. Emerg. Infect. Dis. 11, 1942–1944. doi:10.3201/eid1112.050241

Benito, D., Lozano, C., Gómez-Sanz, E., Zarazaga, M., & Torres, C. (2013). Detection of methicillin-susceptible *Staphylococcus aureus* ST398 and ST133 strains in gut microbiota of healthy humans in Spain. Microbial ecology, 66(1), 105–111. https://doi.org/10.1007/s00248-013-0240-1

Bjorland, J., Sunde, M., Waage, S., 2001. Plasmid-borne smr gene causes resistance to quaternary ammonium compounds in bovine *Staphylococcus aureus*. J. Clin. Microbiol. 39, 3999–4004. doi:10.1128/JCM.39.11.3999-4004.2001

Block, S., 2001. Disinfection, sterilization, and preservation, 5th ed. ed. Lippincott Williams & Wilkins, Philadelphia PA.

Bokarewa, M.I., Jin, T., Tarkowski, A., 2006. *Staphylococcus aureus*: staphylokinase. Int. J. Biochem. Cell Biol. doi:10.1016/j.biocel.2005.07.005

Boucher, H.W., Corey, G.R., 2008. Epidemiology of methicillin-resistant *Staphylococcus aureus*. Clin. Infect. Dis. 46. doi:10.1086/533590

Campbell G, Kosanke S, Toth D, White G (1981) Disseminated staphylococcal infection in a colony of captive ground squirrels (Citellus lateralis). J Wildl Dis 17: 177–181. [PubMed] [Google Scholar]

Chanchaithong, P., Perreten, V., Schwendener, S., Tribuddharat, C., Chongthaleong, A., Niyomtham, W., Prapasarakul, N., 2014. Strain typing and antimicrobial susceptibility of methicillin-resistant coagulase-positive staphylococcal species in dogs and people associated with dogs in Thailand. J. Appl. Microbiol. 117, 572–586. doi:10.1111/jam.12545

Chang, H. J., Hsu, P. C., Yang, C. C., Kuo, A. J., Chia, J. H., Wu, T. L., & Lee, M. H. (2011). Risk factors and outcomes of carbapenem-nonsusceptible *Escherichia coli* bacteremia: a matched case-control study. Journal of microbiology, immunology, and infection = Wei mian yu gan ran za zhi, 44(2), 125–130. https://doi.org/10.1016/j.jmii.2010.06.001

Chemaly, R. F., Simmons, S., Dale, C., Jr, Ghantoji, S. S., Rodriguez, M., Gubb, J., Stachowiak, J., & Stibich, M. (2014). The role of the healthcare environment in the spread of multidrug-resistant organisms: update on current best practices for containment. Therapeutic advances in infectious disease, 2(3-4), 79–90. https://doi.org/10.1177/2049936114543287

Chen, L., Tang, Z.Y., Cui, S.Y., Ma, Z.B., Deng, H., Kong, W.L., Yang, L.W., Lin, C.,

Xiong, W.G., Zeng, Z.L., 2020. Biofilm production ability, virulence and antimicrobial resistance genes in *Staphylococcus aureus* from various veterinary hospitals. Pathogens 9, 264. doi:10.3390/pathogens9040264

Cheung, A.L., Zhang, G., 2002. Global regulation of virulence determinants in *Staphylococcus aureus* by the SarA protein family. Front. Biosci. doi:10.2741/a882

Choo, E.J., 2017. Community-Associated Methicillin-Resistant *Staphylococcus aureus* in Nosocomial Infections . Infect. Chemother. 49, 158. doi:10.3947/ic.2017.49.2.158

Conceição, T., Coelho, C., De Lencastre, H., Aires-De-Sousa, M., 2016. High prevalence of biocide resistance determinants in *Staphylococcus aureus* isolates from three african countries. Antimicrob. Agents Chemother. 60, 678–681. doi:10.1128/AAC.02140-15

Concepción Porrero, M., Valverde, A., Fernández-Llario, P., Díez-Guerrier, A., Mateos, A., Lavín, S., Cantón, R., Fernández-Garayzabal, J.F., Domínguez, L., 2014. *Staphylococcus aureus* carrying *mecC* gene in animals and urban wastewater, Spain. Emerg. Infect. Dis. doi:10.3201/eid2005.130426

Coombs, G.W., Monecke, S., Ehricht, R., Slickers, P., Pearson, J.C., Tan, H.L., Christiansen, K.J., O'Brien, F.G., 2010. Differentiation of clonal complex 59 com- munity-associated methicillin-resistant *Staphylococcus aureus* in Western Australia. Antimicrob. Agents Chemother. 54, 1914–1921.

Costa, S.S., Viveiros, M., Amaral, L., Couto, I., 2013. Multidrug efflux pumps in *Staphylococcus aureus*: an Update. Open Microbiol. J. 7, 59–71. doi:10.2174/1874285801307010059

Couto, N., Monchique, C., Belas, A., Marques, C., Gama, L.T., Pomba, C., 2016. Trends and molecular mechanisms of antimicrobial resistance in clinical staphylococci isolated from companion animals over a 16 year period. J. Antimicrob. Chemother. 71, 1479–1487. doi:10.1093/jac/dkw029

Davis, J.A., Jackson, C.R., Fedorka-Cray, P.J., Barrett, J.B., Brousse, J.H., Gustafson, J., Kucher, M., 2014. Carriage of methicillin-resistant staphylococci by healthy companion animals in the US. Lett. Appl. Microbiol. 59, 1–8. doi:10.1111/lam.12254

de Kraker, M. E., Davey, P. G., Grundmann, H., & BURDEN study group (2011). Mortality and hospital stay associated with resistant *Staphylococcus aureus* and *Escherichia coli* bacteremia: estimating the burden of antibiotic resistance in Europe. PLoS medicine, 8(10), e1001104. https://doi.org/10.1371/journal.pmed.1001104

Derbise, A., Dyke, K.G.H., El Solh, N., 1996. Characterization of a *Staphylococcus aureus* transposon, Tn5405, located within Tn5404 and carrying the aminoglycoside resistance

genes, aphA-3 and aadE. Plasmid 35, 174–188. doi:10.1006/plas.1996.0020

Drougka, E., Foka, A., Koutinas, C. K., Jelastopulu, E., Giormezis, N., Farmaki, O., Sarrou, S., Anastassiou, E. D., Petinaki, E., & Spiliopoulou, I. (2016). Interspecies spread of *Staphylococcus aureus* clones among companion animals and human close contacts in a veterinary teaching hospital. A cross-sectional study in Greece. Preventive veterinary medicine, 126, 190–198. https://doi.org/10.1016/j.prevetmed.2016.02.004

Devriese, L.A., Van Damme, L.R., Fameree, L., 1972. Methicillin (Cloxacillin)-Resistant *Staphylococcus aureus* strains Isolated from bovine mastitis cases. Zentralblatt für Veterinärmedizin R. B 19, 598–605. doi:10.1111/j.1439-0450.1972.tb00439.x

Devriese L. A. (1990). Staphylococci in healthy and diseased animals. Society for Applied Bacteriology symposium series, 19, 71S–80S. https://doi.org/10.1111/j.1365-2672.1990.tb01799.x

Ferreira, J.P., Anderson, K.L., Correa, M.T., Lyman, R., Ruffin, F., Reller, L.B., Fowler, V.G., 2011. Transmission of MRSA between companion animals and infected human patients presenting to outpatient medical care facilities. PLoS One 6, 26978. doi:10.1371/journal.pone.0026978

Feßler, A. T., Schug, A. R., Geber, F., Scholtzek, A. D., Merle, R., Brombach, J., Hensel, V., Meurer, M., Michael, G. B., Reinhardt, M., Speck, S., Truyen, U., Schwarz, S., & Biocide susceptibility study group (2018). Development and evaluation of a broth macrodilution method to determine the biocide susceptibility of bacteria. Veterinary microbiology, 223, 59–64. https://doi.org/10.1016/j.vetmic.2018.07.006

Fjordbakk, C. T., Arroyo, L. G., & Hewson, J. (2008). Retrospective study of the clinical features of limb cellulitis in 63 horses. The Veterinary record, 162(8), 233–236. https://doi.org/10.1136/vr.162.8.233

Foster, T.J., 2005. Immune evasion by staphylococci. Nat. Rev. Microbiol. doi:10.1038/nrmicro1289

Foster, T.J., Höök, M., 1998. Surface protein adhesins of *Staphylococcus aureus*. Trends Microbiol. doi:10.1016/S0966-842X(98)01400-0

Fraise, A.P., 2002. Biocide abuse and antimicrobial resistance - A cause for concern? J. Antimicrob. Chemother. 49, 11–12. doi:10.1093/jac/49.1.11

Gomes-Fernandes, M., Laabei, M., Pagan, N., Hidalgo, J., Molinos, S., Villar Hernandez, R., Domínguez-Villanueva, D., Jenkins, A., Lacoma, A., & Prat, C. (2017). Accessory gene regulator (Agr) functionality in *Staphylococcus aureus* derived from lower respiratory tract infections. PloS one, 12(4), e0175552.

https://doi.org/10.1371/journal.pone.0175552

Gómez-Sanz, E., Torres, C., Benito, D., Lozano, C., & Zarazaga, M. (2013). Animal and human *Staphylococcus aureus* associated clonal lineages and high rate of *Staphylococcus pseudintermedius* novel lineages in spanish kennel dogs: predominance of *S. aureus* ST398. Veterinary microbiology, 166(3-4), 580–589. https://doi.org/10.1016/j.vetmic.2013.07.014

Gómez-Sanz, E., Torres, C., Lozano, C., Sáenz, Y., Zarazaga, M., 2011. Detection and characterization of methicillin-resistant *Staphylococcus pseudintermedius* in healthy dogs in La Rioja, Spain. Comp. Immunol. Microbiol. Infect. Dis. 34, 447–453. doi:10.1016/j.cimid.2011.08.002

Govindarajan, R., Duraiyan, J., Kaliyappan, K., & Palanisamy, M. (2012). Microarray and its applications. *Journal of pharmacy & bioallied sciences*, *4*(Suppl 2), S310–S312. https://doi.org/10.4103/0975-7406.100283

Guardabassi, L., Schwarz, S., & Lloyd, D. H. (2004). Pet animals as reservoirs of antimicrobial-resistant bacteria. The Journal of antimicrobial chemotherapy, 54(2), 321–332. https://doi.org/10.1093/jac/dkh332

Gupta, P., Bhatia, M., Gupta, P., & Omar, B. J. (2018). Emerging biocide resistance among multidrug-resistant bacteria: myth or reality? A pilot study. Journal of pharmacy & bioallied sciences, 10(2), 96–101. https://doi.org/10.4103/JPBS.JPBS_24_18

Haenni, M., Châtre, P., Dupieux-Chabert, C., Métayer, V., Bes, M., Madec, J.-Y., Laurent, F., 2017. Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* in horses, cats, and dogs over a 5-year period in France. Front. Microbiol. 8, 2493. doi:10.3389/fmicb.2017.02493

Haenni, M., Saras, E., Châtre, P., Médaille, C., Bes, M., Madec, J. Y., & Laurent, F. (2012). A USA300 variant and other human-related methicillin-resistant *Staphylococcus aureus* strains infecting cats and dogs in France. The Journal of antimicrobial chemotherapy, 67(2), 326–329. https://doi.org/10.1093/jac/dkr499

Haggar, A., Ehrnfelt, C., Holgersson, J., Flock, J.I., 2004. The extracellular adherence protein from *Staphylococcus aureus* inhibits neutrophil binding to endothelial cells. Infect. Immun. 72, 6164–6167. doi:10.1128/IAI.72.10.6164-6167.2004

Hauschild, T., Schwarz, S., 2010. Macrolide resistance in *Staphylococcus spp.* from free-living small mammals. Vet. Microbiol. doi:10.1016/j.vetmic.2010.06.017

Hermans, K., Devriese, L. A., & Haesebrouck, F. (2003). Rabbit staphylococcosis: difficult solutions for serious problems. Veterinary microbiology, 91(1), 57–64.

https://doi.org/10.1016/s0378-1135(02)00260-2

Hoppe, P. A., Holzhauer, S., Lala, B., Bührer, C., Gratopp, A., Hanitsch, L. G., Humme, D., Kieslich, M., Kallinich, T., Lau, S., Leistner, R., Niebank, M., Pokrywka, A., Ringe, H., Schaper, A. S., Schröder, J. T., Schwarz, C., Staab, D., Stegemann, M. S., Thee, S., … Krüger, R. (2019). Severe infections of panton-valentine leukocidin positive *Staphylococcus aureus* in children. Medicine, 98(38), e17185. https://doi.org/10.1097/MD.0000000000017185

Huson, D.H., Bryant, D., 2006. Application of phylogenetic networks in evolutionary studies. Mol. Biol. Evol. 23, 254–267.

Ivbule, M., Miklaševičs, E., Čupane, L., Berziņa, L., Balinš, A., Valdovska, A., 2017. Presence of methicillin-resistant *Staphylococcus aureus* in slaughterhouse environment, pigs, carcasses, and workers. J. Vet. Res. 61, 267–277. doi:10.1515/jvetres-2017-0037

Jamrozy, D.M., Fielder, M.D., Butaye, P., Coldham, N.G., 2012. Comparative genotypic and phenotypic characterisation of methicillin-resistant *Staphylococcus aureus* ST398 isolated from animals and humans. PLoS One 7, 40458. doi:10.1371/journal.pone.0040458

Jia, B., Raphenya, A.R., Alcock, B., Waglechner, N., Guo, P., Tsang, K.K., Lago, B.A., Dave, B.M., Pereira, S., Sharma, A.N., Doshi, S., Courtot, M., Lo, R., Williams, L.E., Frye, J.G., Elsayegh, T., Sardar, D., Westman, E.L., Pawlowski, A.C., Johnson, T.A., Brinkman, F.S., Wright, G.D., McArthur, A.G., 2017. CARD 2017: expansion and model-centric curation of the comprehensive antibiotic resistance database. Nucleic Acids Res. 45 (D1), D566–D573.

Kadariya, J., Smith, T.C., Thapaliya, D., 2014. *Staphylococcus aureus* and staphylococcal food-borne disease: an ongoing challenge in public health. Biomed Res. Int. doi:10.1155/2014/827965

Kadlec, K., Ehricht, R., Monecke, S., Steinacker, U., Kaspar, H., Mankertz, J., Schwarz, S., 2009. Diversity of antimicrobial resistance pheno- and genotypes of methicillin-resistant *Staphylococcus aureus* ST398 from diseased swine. J. Antimicrob. Chemother. 64, 1156–1164. doi:10.1093/jac/dkp350

Kimang'a A. N. (2012). A situational analysis of antimicrobial drug resistance in Africa: are we losing the battle?. Ethiopian journal of health sciences, 22(2), 135–143.

Köck, R., Becker, K., Cookson, B., Van Gemert-Pijnen, J.E., Harbarth, S., Kluytmans, J., Mielke, M., Peters, G., Skov, R.L., Struelens, M.J., Tacconelli, E., Witte, W., Friedrich, A.W., 2014. Systematic literature analysis and review of targeted preventive measures to

limit healthcare-associated infections by meticillin-resistant *Staphylococcus aureus.* Eurosurveillance. doi:10.2807/1560-7917.es2014.19.29.20860

Köck, R., Harlizius, J., Bressan, N., Laerberg, R., Wieler, L.H., Witte, W., Deurenberg, R.H., Voss, A., Becker, K., Friedrich, A.W., 2009. Prevalence and molecular characteristics of methicillin-resistant *Staphylococcus aureus* (MRSA) among pigs on German farms and import of livestock-related MRSA into hospitals. Eur. J. Clin. Microbiol. Infect. Dis. 28, 1375–1382. doi:10.1007/s10096-009-0795-4

Koop G. (2016). MRSA transmission between horses and vets: who's doing the infecting?. The Veterinary record, 178(19), 471–472. https://doi.org/10.1136/vr.i2531

Lakhundi, S., Zhang, K., 2018. Methicillin-resistant *Staphylococcus aureus*: molecular characterization, evolution, and epidemiology. Clin. Microbiol. Rev. doi:10.1128/CMR.00020-18

Lina, G., Bohach, G.A., Nair, S.P., Hiramatsu, K., Jouvin-Marche, E., Mariuzza, R., 2004. Standard nomenclature for the superantigens expressed by *Staphylococcus.* J. Infect. Dis. 189, 2334–2336. doi:10.1086/420852

Linares, J. A., & Wigle, W. L. (2001). *Staphylococcus aureus* pneumonia in turkey poults with gross lesions resembling aspergillosis. Avian diseases, 45(4), 1068–1072.

Lister, J.L., Horswill, A.R., 2014. *Staphylococcus aureus* biofilms: recent developments in biofilm dispersal. Front. Cell. Infect. Microbiol. 4. doi:10.3389/fcimb.2014.00178

Loncaric, I., Kübber-Heiss, A., Posautz, A., Stalder, G. L., Hoffmann, D., Rosengarten, R., & Walzer, C. (2014). *mecC*- and *mecA*-positive meticillin-resistant *Staphylococcus aureus* (MRSA) isolated from livestock sharing habitat with wildlife previously tested positive for *mecC*-positive MRSA. Veterinary dermatology, 25(2), 147–148. https://doi.org/10.1111/vde.12116

Loncaric, I., Künzel, F., Licka, T., Simhofer, H., Spergser, J., Rosengarten, R., 2014. Identification and characterization of methicillin-resistant *Staphylococcus aureus* (MRSA) from austrian companion animals and horses. Vet. Microbiol. 168, 381–387. doi:10.1016/j.vetmic.2013.11.022

Loncaric, I., Tichy, A., Handler, S., Szostak, M.P., Tickert, M., Diab-Elschahawi, M., Spergser, J., Künzel, F., 2019. Prevalence of methicillin-resistant *Staphylococcus spp.* (MRS) in different companion animals and determination of risk factors for colonization with MRS. Antibiotics 8. doi:10.3390/antibiotics8020036

Lowy, F.D., 1998. Medical progress: *Staphylococcus aureus* infections. N. Engl. J. Med. doi:10.1056/NEJM199808203390806

Lozano, C., Aspiroz, C., Lasarte, J.J., Gómez-Sanz, E., Zarazaga, M., Torres, C., 2011. Dynamic of nasal colonization by methicillin-resistant *Staphylococcus aureus* ST398 and ST1 after mupirocin treatment in a family in close contact with pigs. Comp. Immunol. Microbiol. Infect. Dis. 34. doi:10.1016/j.cimid.2010.06.006

Lüthje, P., Schwarz, S., 2006. Antimicrobial resistance of coagulase-negative staphylococci from bovine subclinical mastitis with particular reference to macrolide-lincosamide resistance phenotypes and genotypes. J. Antimicrob. Chemother. 57, 966–969. doi:10.1093/jac/dkl061

Marbach, H., Mayer, K., Vogl, C., Lee, J.Y.H., Monk, I.R., Sordelli, D.O., Buzzola, F.R., Ehling-Schulz, M., Grunert, T., 2019. Within-host evolution of bovine *Staphylococcus aureus* selects for a SigB-deficient pathotype characterized by reduced virulence but enhanced proteolytic activity and biofilm formation. Sci. Rep. 9, 1–12. doi:10.1038/s41598-019-49981-6

Matsuoka, M., Endou, K., Kobayashi, H., Inoue, M., Nakajima, Y., 1998. A plasmid that encodes three genes for resistance to macrolide antibiotics in *Staphylococcus aureus* . FEMS Microbiol. Lett. 167, 221–227. doi:10.1111/j.1574-6968.1998.tb13232.x

Mcdonnell, G., Russell, A.D., 1999. Antiseptics and disinfectants: Activity, action, and resistance. Clin. Microbiol. Rev. doi:10.1128/cmr.12.1.147

McLean, C.L., Ness, M.G., 2008. Meticillin-resistant *Staphylococcus aureus* in a veterinary orthopaedic referral hospital: staff nasal colonisation and incidence of clinical cases: Paper. J. Small Anim. Pract. 49, 170–177. doi:10.1111/j.1748-5827.2007.00529.x

McNamee, P. T., & Smyth, J. A. (2000). Bacterial chondronecrosis with osteomyelitis ('femoral head necrosis') of broiler chickens: a review. Avian pathology : journal of the W.V.P.A, 29(4), 253–270. https://doi.org/10.1080/03079450050118386

Melish, M.E., Glasgow, L.A., 1970. The staphylococcal scalded-skin syndrome: development of an experimental model. N. Engl. J. Med. 282, 1114–1119. doi:10.1056/NEJM197005142822002

Middleton, J. R., Fales, W. H., Luby, C. D., Oaks, J. L., Sanchez, S., Kinyon, J. M., Wu, C. C., Maddox, C. W., Welsh, R. D., & Hartmann, F. (2005). Surveillance of *Staphylococcus aureus* in veterinary teaching hospitals. Journal of clinical microbiology, 43(6), 2916–2919. https://doi.org/10.1128/JCM.43.6.2916-2919.2005

Monecke, S., Gavier-Widén, D., Hotzel, H., Peters, M., Guenther, S., Lazaris, A., Loncaric, I., Müller, E., Reissig, A., Ruppelt-Lorz, A., Shore, A.C., Walter, B., Coleman, D.C., Ehricht, R., 2016. Diversity of *Staphylococcus aureus* isolates in european wildlife.

PLoS One 11, 168433. doi:10.1371/journal.pone.0168433

Monecke S, Gavier-Widen D, Mattsson R, Rangstrup-Christensen L, Lazaris A, Coleman DC, et al. (2013) Detection of *mecC*-positive *Staphylococcus aureus* (CC130-MRSA-XI) in diseased European hedgehogs (Erinaceus europaeus) in Sweden. PLoS ONE 8: e66166 10.1371/journal.pone.0066166[PMC free article] [PubMed] [CrossRef] [Google Scholar]

Monecke, S., Slickers, P., Ehricht, R., 2008. Assignment of *Staphylococcus aureus* isolates to clonal complexes based on microarray analysis and pattern recognition. FEMS Immunol. Med. Microbiol. 53, 237–251.

Moreno-Grúa, E., Pérez-Fuentes, S., Viana, D., Cardells, J., Lizana, V., Aguiló, J., Selva, L., & Corpa, J. M. (2020). Marked presence of methicillin-resistant *Staphylococcus aureus* in wild lagomorphs in Valencia, Spain. Animals : an open access journal from MDPI, 10(7), 1109. https://doi.org/10.3390/ani10071109

Murray, R.J., 2005. Recognition and management of *Staphylococcus aureus* toxin-mediated disease. Intern. Med. J. 35, S106–S119. doi:10.1111/j.1444-0903.2005.00984.x

Nesin, M., Svec, P., Lupski, J.R., Godson, G.N., Kreiswirth, B., Kornblum, J., Projan, S.J., 1990. Cloning and nucleotide sequence of a chromosomally encoded tetracycline resistance determinant, *tetA*(M), from a pathogenic, methicillin-resistant strain of *Staphylococcus aureus*. Antimicrob. Agents Chemother. 34, 2273–2276. doi:10.1128/AAC.34.11.2273

Noguchi, N., Suwa, J., Narui, K., Sasatsu, M., Ito, T., Hiramatsu, K., Song, J.H., 2005. Susceptibilities to antiseptic agents and distribution of antiseptic-resistance genes *qac*A/B and *smr* of methicillin-resistant *Staphylococcus aureus* isolated in Asia during 1998 and 1999. J. Med. Microbiol. 54, 557–565. doi:10.1099/jmm.0.45902-0

Norström, M., Sunde, M., Tharaldsen, H., Mørk, T., Bergsjø, B., Kruse, H., 2009. Antimicrobial resistance in *Staphylococcus pseudintermedius* in the Norwegian dog population. Microb. Drug Resist. 15, 55–59. doi:10.1089/mdr.2009.0865

Nosanchuk, J. D., Lin, J., Hunter, R. P., & Aminov, R. I. (2014). Low-dose antibiotics: current status and outlook for the future. Frontiers in microbiology, 5, 478. https://doi.org/10.3389/fmicb.2014.00478

Novick, R. P., & Geisinger, E. (2008). Quorum sensing in staphylococci. Annual review of

genetics, 42, 541–564. https://doi.org/10.1146/annurev.genet.42.110807.091640

O'Neill, A.J., McLaws, F., Kahlmeter, G., Henriksen, A.S., Chopra, I., 2007. Genetic basis of resistance to fusidic acid in staphylococci. Antimicrob. Agents Chemother. 51, 1737–1740. doi:10.1128/AAC.01542-06

Okerman L, Devriese LA, Maertens L, Okerman F, Godard C. Cutaneous staphylococcosis in rabbits. Vet Rec. 1984;114(13):313-315. doi:10.1136/vr.114.13.313

Olsen, J.E., Christensen, H., Aarestrup, F.M., 2006. Diversity and evolution of *blaZ* from *Staphylococcus aureus* and coagulase-negative staphylococci. J. Antimicrob. Chemother. 57, 450–460. doi:10.1093/jac/dki492

Pereira, V., Lopes, C., Castro, A., Silva, J., Gibbs, P., Teixeira, P., 2009. Characterization for enterotoxin production, virulence factors, and antibiotic susceptibility of *Staphylococcus aureus* isolates from various foods in Portugal. Food Microbiol. 26, 278–282. doi:10.1016/j.fm.2008.12.008

Perreten, V., Kadlec, K., Schwarz, S., Andersson, U.G., Finn, M., Greko, C., Moodley, A., Kania, S.A., Frank, L.A., Bemis, D.A., Franco, A., Iurescia, M., Battisti, A., Duim, B., Wagenaar, J.A., van Duijkeren, E., Weese, J.S., Fitzgerald, J.R., Rossano, A., Guardabassi, L., 2010. Clonal spread of methicillin-resistant *Staphylococcus pseudintermedius* in Europe and North America: An international multicentre study. J. Antimicrob. Chemother. 65, 1145–1154. doi:10.1093/jac/dkq078

Pinho, M.G., 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. doi:10.1073/pnas.191260798

Porrero, M.C., Mentaberre, G., Sánchez, S., Fernández-Llario, P., Gómez-Barrero, S., Navarro-Gonzalez, N., Serrano, E., Casas-Díaz, E., Marco, I., Fernández-Garayzabal, J.F., Mateos, A., Vidal, D., Lavín, S., Domínguez, L., 2013. Methicillin resistant *Staphylococcus aureus* (MRSA) carriage in different free-living wild animal species in Spain. Vet. J. 198, 127–130. doi:10.1016/j.tvjl.2013.06.004

Pournaras, S., Slavakis, A., Polyzou, A., Sofianou, D., Maniatis, A.N., Tsakris, A., 2001. Nosocomial spread of an unusual methicillin-resistant *Staphylococcus aureus* clone that is sensitive to all non-β-lactam antibiotics, including tobramycin. J. Clin. Microbiol. 39, 779–781. doi:10.1128/JCM.39.2.779-781.2001

Qu, Y., Zhao, H., Nobrega, D. B., Cobo, E. R., Han, B., Zhao, Z., Li, S., Li, M., Barkema, H. W., & Gao, J. (2019). Molecular epidemiology and distribution of antimicrobial resistance genes of staphylococcus species isolated from Chinese dairy cows with

clinical mastitis. Journal of dairy science, 102(2), 1571–1583. https://doi.org/10.3168/jds.2018-15136

Rahman, A., Izaki, K., Kato, I., Kamio, Y., 1991. Nucleotide sequence of leukocidin S-component gene (*lukS*) from methicillin resistant *Staphylococcus aureus*. Biochem. Biophys. Res. Commun. 181, 138–144. doi:10.1016/S0006-291X(05)81392-0

Rankin, S., Roberts, S., O'Shea, K., Maloney, D., Lorenzo, M., & Benson, C. E. (2005). Panton valentine leukocidin (PVL) toxin positive MRSA strains isolated from companion animals. Veterinary microbiology, 108(1-2), 145–148. https://doi.org/10.1016/j.vetmic.2005.02.013

Rasmussen, S. L., Larsen, J., van Wijk, R. E., Jones, O. R., Berg, T. B., Angen, Ø., & Larsen, A. R. (2019). European hedgehogs (*Erinaceus europaeus*) as a natural reservoir of methicillin-resistant *Staphylococcus aureus* carrying *mecC* in Denmark. PloS one, 14(9), e0222031. https://doi.org/10.1371/journal.pone.0222031

Richardson A. R. (2019). Virulence and Metabolism. Microbiology spectrum, 7(2), 10.1128/microbiolspec.GPP3-0011-2018. https://doi.org/10.1128/microbiolspec.GPP3-0011-2018

Roberts, M.C., 1996. Tetracycline resistance determinants: mechanisms of action, regulation of expression, genetic mobility, and distribution. FEMS Microbiol. Rev. 19, 1–24. doi:10.1111/j.1574-6976.1996.tb00251.x

Rooijakkers, S.H.M., Van Kessel, K.P.M., Van Strijp, J.A.G., 2005. staphylococcal innate immune evasion. Trends Microbiol. doi:10.1016/j.tim.2005.10.002

Rosenbach, F.J., 1884. Mikroorganismen bei den wund-infections-krankheiten des Menschen. J.F. Bergmann, Wiesbaden. doi:10.5962/bhl.title.22955

Ross, J.I., Eady, E.A., Cove, J.H., Cunliffe, W.J., Baumberg, S., Wootton, J.C., 1990. Inducible erythromycin resistance in staphlyococci is encoded by a member of the ATP-binding transport super-gene family. Mol. Microbiol. 4, 1207–1214. doi:10.1111/j.1365-2958.1990.tb00696.x

Rouch, D.A., Byrne, M.E., Kong, Y.C., Skurray, R.A., 1987. The *aacA-aphD* gentamicin and kanamycin resistance determinant of Tn4001 from *Staphylococcus aureus*: expression and nucleotide sequence analysis. J. Gen. Microbiol. 133, 3039–3052. doi:10.1099/00221287-133-11-3039

Rowan-Nash, A.D., Araos, R., D'Agata, E.M.C., Belenky, P., 2020. Antimicrobial resistance gene prevalence in a population of patients with advanced dementia is related to specific pathobionts. iScience 23. doi:10.1016/j.isci.2020.100905

Ruzauskas, M., Couto, N., Kerziene, S., Siugzdiniene, R., Klimiene, I., Virgailis, M., Pomba, C., 2015. Prevalence, species distribution and antimicrobial resistance patterns of methicillin-resistant staphylococci in Lithuanian pet animals. Acta Vet. Scand. 57, 27. doi:10.1186/s13028-015-0117-z

Schauer, B., Krametter-Frötscher, R., Knauer, F., Ehricht, R., Monecke, S., Feßler, A.T., Schwarz, S., Grunert, T., Spergser, J., Loncaric, I., 2018. Diversity of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from Austrian ruminants and new world camelids. Vet. Microbiol. 215, 77–82. doi:10.1016/j.vetmic.2018.01.006

Schleifer, K.-H., Bell, J.A., 2015. *Staphylococcus*, in: Bergey's manual of systematics of archaea and bacteria. John Wiley & Sons, Ltd, Chichester, UK, pp. 1–43. doi:10.1002/9781118960608.gbm00569

Schnellmann, C., Gerber, V., Rossano, A., Jaquier, V., Panchaud, Y., Doherr, M.G., Thomann, A., Straub, R., Perreten, V., 2006. Presence of new *mecA* and *mph*(C) variants conferring antibiotic resistance in *Staphylococcus spp.* isolated from the skin of horses before and after clinic admission. J. Clin. Microbiol. 44, 4444–4454. doi:10.1128/JCM.00868-06

Schug, A. R., Bartel, A., Scholtzek, A. D., Meurer, M., Brombach, J., Hensel, V., Fanning, S., Schwarz, S., & Feßler, A. T. (2020). Biocide susceptibility testing of bacteria: development of a broth microdilution method. Veterinary microbiology, 248, 108791. Advance online publication. https://doi.org/10.1016/j.vetmic.2020.108791

Schwarz, S., Cardoso, M., Wegener, H.C., 1992. Nucleotide sequence and phylogeny of the *tet*(L) tetracycline resistance determinant encoded by plasmid pSTE1 from *Staphylococcus hyicus.* Antimicrob. Agents Chemother. 36, 580–588. doi:10.1128/AAC.36.3.580

Schwarz, S., Feßler, A.T., Loncaric, I., Wu, C., Kadlec, K., Wang, Y., Shen, J., 2018. Antimicrobial resistance among staphylococci of animal origin, in: antimicrobial resistance in bacteria from livestock and companion animals. American Society of Microbiology, pp. 127–157. doi:10.1128/microbiolspec.arba-0010-2017

Schwarz, S., Roberts, M.C., Werckenthin, C., Pang, Y., Lange, C., 1998. Tetracycline resistance in *Staphylococcus spp.* from domestic animals. Vet. Microbiol. 63, 217–227. doi:10.1016/S0378-1135(98)00234-X

Scott, G.M., Thomson, R., Malone-Lee, J., Ridgway, G.L., 1988. Cross-infection between animals and man: possible feline transmission of *Staphylococcus aureus* infection in humans? J. Hosp. Infect. 12, 29–34. doi:10.1016/0195-6701(88)90119-3

Silva, V., Pereira, J.E., Maltez, L., Ferreira, E., Manageiro, V., Caniça, M., Capelo, J.L., Igrejas, G., Poeta, P., 2020. Diversity of methicillin-resistant Staphylococci among wild *Lepus granatensis*: first detection of *mecA*-MRSA in hares. FEMS Microbiol. Ecol. 96. doi:10.1093/femsec/fiz204

Simpson VR, Davison NJ, Kearns AM, Pichon B, Hudson LO, Koylass M, et al. (2013) Association of a *lukM*-positive clone of *Staphylococcus aureus* with fatal exudative dermatitis in red squirrels (*Sciurus vulgaris*). Veterinary Microbiology 162: 987–991. 10.1016/j.vetmic.2012.10.025 [PubMed] [CrossRef] [Google Scholar]

Simpson VR, Hargreaves J, Everest DJ, Baker AS, Booth PA, Butler HM, et al. (2010) Mortality in red squirrels (*Sciurus vulgaris*) associated with exudative dermatitis. Veterinary Record 167: 59–62. 10.1136/vr.b4887 [PubMed] [CrossRef] [Google Scholar]

Soimala, T., Lübke-Becker, A., Schwarz, S., Feßler, A.T., Huber, C., Semmler, T., Merle, R., Gehlen, H., Eule, J.C., Walther, B., 2018. Occurrence and molecular composition of methicillin-resistant *Staphylococcus aureus* isolated from ocular surfaces of horses presented with ophthalmologic disease. Vet. Microbiol. 222, 1–6. doi:10.1016/j.vetmic.2018.06.009

Souza, G., de Almeida, A. C., Xavier, M., da Silva, L., Sousa, C. N., Sanglard, D. A., & Xavier, A. (2019). Characterization and molecular epidemiology of *Staphylococcus aureus* strains resistant to beta-lactams isolated from the milk of cows diagnosed with subclinical mastitis. Veterinary world, 12(12), 1931–1939. https://doi.org/10.14202/vetworld.2019.1931-1939

Stapleton, P.D., Taylor, P.W., 2002. Methicillin resistance in *Staphylococcus aureus*: mechanisms and modulation. Sci. Prog. doi:10.3184/003685002783238870

Strauß, L., Ruffing, U., Abdulla, S., Alabi, A., Akulenko, R., Garrine, M., Germann, A., Grobusch, M.P., Helms, V., Herrmann, M., Kazimoto, T., Kern, W., Mandomando, I., Peters, G., Schaumburg, F., von Müller, L., Mellmann, A., 2016. Detecting *Staphylococcus aureus* virulence and resistance genes: a comparison of whole-genome sequencing and DNA microarray technology. J. Clin. Microbiol. 54, 1008–1016.

Struelens, M.J., 1998. The epidemiology of antimicrobial resistance in hospital acquired infections: problems and possible solutions. Br. Med. J. doi:10.1136/bmj.317.7159.652

Sun, Y., Emolo, C., Holtfreter, S., Wiles, S., Kreiswirth, B., Missiakas, D., & Schneewind, O. (2018). Staphylococcal protein a contributes to persistent colonization of mice with *Staphylococcus aureus*. Journal of bacteriology, 200(9), e00735-17.

https://doi.org/10.1128/JB.00735-17

Tenhagen, B. A., Köster, G., Wallmann, J., & Heuwieser, W. (2006). Prevalence of mastitis pathogens and their resistance against antimicrobial agents in dairy cows in Brandenburg, Germany. Journal of dairy science, 89(7), 2542–2551. https://doi.org/10.3168/jds.S0022-0302(06)72330-X

Thompson, T., Brown, P.D., 2014. Comparison of antibiotic resistance, virulence gene profiles, and pathogenicity of methicillinresistant and methicillin-susceptible *Staphylococcus aureus* using a caenorhabditis elegans infection model. Pathog. Glob. Health 108, 283–291. doi:10.1179/2047773214Y.0000000155

Tomlin, J., Pead, M.J., Lloyd, D.H., Howell, S., Hartmann, F., Jackson, H.A., Muir, P., 1999. Methicillin-resistant *Staphylococcus aureus* infections in 11 dogs. Vet. Rec. 144, 60–64. doi:10.1136/vr.144.3.60

Tong, S.Y.C., Davis, J.S., Eichenberger, E., Holland, T.L., Fowler, V.G., 2015. *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. Clin. Microbiol. Rev. 28, 603–661. doi:10.1128/CMR.00134-14

Vestergaard, M., Frees, D., & Ingmer, H. (2019). Antibiotic resistance and the MRSA problem. Microbiology spectrum, 7(2), 10.1128/microbiolspec.GPP3-0057-2018. https://doi.org/10.1128/microbiolspec.GPP3-0057-2018

Vincze, S., Stamm, I., Monecke, S., Kopp, P.A., Semmler, T., Wieler, L.H., Lübke-Becker, A., Walther, B., 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. doi:10.1128/AEM.02704-12

Waage, S., Mørk, T., Røros, A., Aasland, D., Hunshamar, A., & Odegaard, S. A. (1999). Bacteria associated with clinical mastitis in dairy heifers. Journal of dairy science, 82(4), 712–719. https://doi.org/10.3168/jds.S0022-0302(99)75288-4

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

Weese, J.S., 2005. Methicillin-resistant *Staphylococcus aureus*: an emerging pathogen in small animals. J. Am. Anim. Hosp. Assoc. 41, 150–157. doi:10.5326/0410150

Wendlandt, S., Lozano, C., Kadlec, K., Gómez-Sanz, E., Zarazaga, M., Torres, C., Schwarz, S., 2013. The enterococcal ABC transporter gene *lsa*(E) confers combined resistance to

lincosamides, pleuromutilins and streptogramin A antibiotics in methicillin-susceptible and methicillinresistant *Staphylococcus aureus*. J. Antimicrob. Chemother. doi:10.1093/jac/dks398

Wipf, J.R.K., Perreten, V., 2016. Methicillin-resistant *Staphylococcus aureus* isolated from dogs and cats in Switzerland. Schweiz. Arch. Tierheilkd. 158, 443–450. doi:10.17236/sat00070

Yarwood, J.M., Schlievert, P.M., 2003. Quorum sensing in *Staphylococcus* infections. J. Clin. Invest. 112, 1620–1625. doi:10.1172/jci20442

Zakour, N.L.B., Bannoehr, J., van den Broek, A.H.M., Thoday, K.L., Fitzgerald, J.R., 2011. Complete genome sequence of the canine pathogen *Staphylococcus pseudintermedius.* J. Bacteriol. doi:10.1128/JB.00137-11

8 Figure and table references