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Characterisation of phenotypic and genotypic antibiotic resistance with special consideration of macrolide, lincosamide and streptogramin B (MLS) antibiotics 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, European hares)

Diplomarbeit

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Contents

1. Introduction and hypothesis

Introduction:

Antibiotic resistance is the ability of microorganisms to respond, adapt and survive in the presence of an antibiotic. The rapidly increasing spread of antibiotic resistance imposes a major threat to public health worldwide (Munita and Arias, 2016). Multidrug resistant-microorganisms constitute a concerning phenomenon, as more and more microorganisms become resistant to one or more antibiotics and chempotherapeutic agents (Nikaido, 2009).

Antibiotic resistance can be categorized in intrinsic and acquired resistance. Intrinsic resistance is a condition where the resistance is pre-existent. The resistance gene(s) are found inside the bacterial genome. On the other hand, in acquired resistance new genetic material is acquired or mutations in the intrinsic gene(s) occur (Sandner-Miranda et al., 2018).

Antibiotic resistance can be achieved with or without genetic modification. In the genotypic antibiotic resistance scenario, the two major strategies bacteria use are: (i) gene mutations mostly associated with the mechanism of action of the compound and (ii) acquisition of foreign DNA via Horizontal Gene Transfer (HGT) (Munita and Arias, 2016).

In the scenario of no genetic changes the main bacteria strategies are: (i) the drug indifference, (ii) the growth in biofilms and (iii) the phenomenon of persistence. This is called phenotypic antibiotic resistance (Corona and Martinez, 2013).

Methicillin-resistant *Staphylococcus aureus* is a major human antimicrobial-resistant pathogen (Lakhundi and Zhang, 2018) responsible for a great number of nosocomial and community-associated infections worldwide (Khashei et al., 2018). In the last two decades an alarming number of livestock-associated infections have been reported. Livestock-associated MRSA genesis is believed to be related with pigs and spread to other species (Lakhundi and Zhang, 2018). It was first described in a Dutch study in 2005 (Voss et al., 2005). Methicillin-resistant *Staphylococcus aureus* can lead to a wide variety of life-threatening infections including skin, soft tissue, bone and joint infections, bacteremia and endocarditis (Turner et al., 2019).

Macrolide, lincosamide and Streptogramin B (MLSB) antibiotics constitute a treatment option for staphylococcal infections (Khashei et al., 2018). Each of the three antibiotic groups has a different chemical structure and is classified as a different antibiotic family, but they exhibit an analogous mechanism of action and antibacterial spectrum. Their effectiveness is based on protein synthesis inhibition. More specifically, they act on the 50S bacterial ribosomal subunit (Ungureanu, 2010).

Macrolides-lincosamides and streptogramins B (MLSB antibiotics) resistance is achieved through four different mechanisms and it is based on the acquisition of resistance genes (Schwarz et al., 2018). The following MLS resistance genes have been used in this study: *erma*(A), *erm*(B), *erm*(C), *lnu*(A), *msr*(A), *mef*(A), *mph*(C), *vat*(A), *vat*(B), *vga*(A), *vga*(A)(BM3327) and *vgb*(A).

Hypothesis:

The aim of this thesis is to compare the presence of macrolide, lincosamide, streptogramin B resistance genes between MRSA that was isolated from Austrian companion animals and wild animals from German North Frisian Island Pellworm and Lower Austria.

Concerning companion animals, 90 non-repetitive MRSA isolates were collected in a period of five years from Autumn 2013 to Autumn 2018. They were obtained from horses (n=62), cats (n=13), dogs (n=10), rabbits (n=2), a domestic canary, a zoo kept hammer-headed bat (*Hypsignathus monstrosus*) and a semi-captive northern bald ibis (*Geronticus eremita*).

Referring to the wild animals, 78 non-repetitive MRSA isolates were obtained. All of them originated from European hares (*Lepus europaeus*).

It was hypothesized that macrolide, lincosamide and streptogramin B resistance genes are less common in MRSA isolates recovered from wildlife than in those isolated from companion animals.

2. Literature:

2.1. Phenotypic and genotypic antibiotic resistance

In general, antibiotic resistance occurs when bacteria change in a manner that reduces or eliminates the effectiveness of drugs designed to cure them. It is the bacteria that become antibiotic-resistant, not humans, animals or plants. Thus, it leads to problems such as increased illness, suffering, death, as well as an increased cost of treatment. It can be developed either by mutation or by the horizontal transfer of resistance-conferring genes, often found in mobile genetic cassettes. The contribution of these factors depends on the kind of the antibiotic and the different bacterial genetic plasticity. *Mycobacterium tuberculosis,* for example, attains antibiotic resistance primarily via nucleotide changes, whereas hospital-acquired *Enterobacteriaceae* infections often possess both multi-drug resistance cassettes and nucleotide changes (Baquero and Blázquez, 1997).

Bacteria follow two genetic schemes to remodel the antibiotic treatment: (i) gene(s) mutations commonly associated with the mechanism of action of the compound and (ii) acquisition of foreign DNA that includes resistance genes via horizontal gene transfer (HGT) (Munita and Arias, 2016). In comparison to HGT vertical gene transfer does not play an important role in antibiotic resistance. It describes the transmission of genetic material from mother cell to daughter cell during Mitosis and Meiosis (Lorenzo-Diaz et al., 2017).

This is the most frequent type of antibiotic resistance as it is associated with genetic changes (gene acquisition or mutation) and, therefore, named as genotypic resistance (Munita and Arias, 2016).

Mutational Resistance: It is the phenomenon, which occurs when in a number of bacterial cells belonging to a susceptible population, mutations occur in their genome resulting in reduced drug effectiveness and a longer cell survival in the presence of the antibiotic. By the presence of a mutant, the antibiotic use will lead only to the death of the susceptible population while the resistant bacteria will survive. In many cases, mutational changes resulting in resistance can be crucial to cell homeostasis (i.e., decreased fitness) and their maintenance depends on the presence of the antibiotic. Antimicrobial resistance caused by mutations can modify the antibiotic

reaction through one of the subsequent processes: (i) alterations of the antimicrobial target (reducing the tendency for the drug), (ii) decrease in the drug uptake, (iii) triggering of efflux mechanisms to extrude the harmful molecule, or (iv) global changes in important metabolic pathways via modulation of regulatory networks (Munita and Arias, 2016).

HGT refers to the integration of foreign DNA material by bacteria. It is considered to be one of the most significant ways of bacterial evolution and it is often responsible for the development of antimicrobial resistance. Typically, bacteria obtain external genetic material through three basic paths as depicted in Fig. 1 (Wintersdorf et al., 2016): (i) transformation (incorporation of naked DNA), (ii) transduction (phage mediated) and (iii) conjugation (bacterial "sex"). Transformation is the simplest type of HGT. Although, only a few clinically relevant bacterial species can "naturally" integrate naked DNA to develop resistance, when it comes to conjugation almost always Mobile Genetic Elements (MGEs) are used as vehicles to share valuable genetic information. Additionally, direct transfer between chromosomes has also been identified. The two major MGEs are plasmids and transposons (Munita and Arias, 2016).

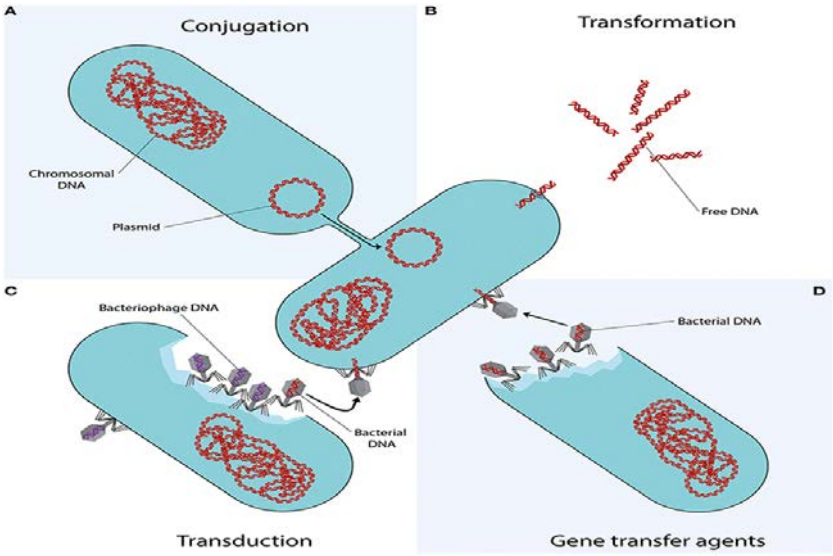

Fig. 1: The three basic paths that bacteria acquire genetic material: A) conjugation (bacterial "sex"), B) transformation (incorporation of naked DNA), C) transduction (phage mediated) and D) gene transfer agents (Wintersdorf et al., 2016).

However, in some cases resistance can occur without any genetic modification. This type of resistance is called phenotypic resistance (Corona and Martinez, 2013).

Bacterial phenotypic resistance is associated to specific processes that might occur during infection such as the drug indifference, the growth in biofilms and the phenomenon of persistence (Fig. 2, Corona and Martinez, 2013). These situations are not usually taken into consideration in classical susceptibility tests at microbiology laboratories. Also, changes in the bacterial metabolism and global metabolic regulators play a very important role since they can affect the susceptibility to antibiotics and modulate the phenotype. Moreover, bacterial metabolic state can also affect their susceptibility to antibiotics (Corona and Martinez, 2013).

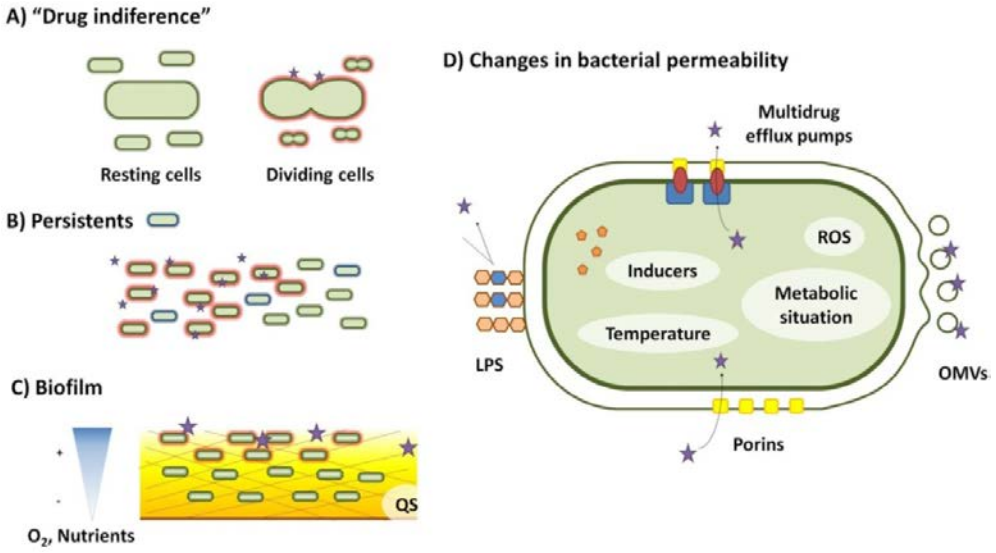

Fig. 2: The four major mechanisms for the acquisition of phenotypic resistance: A) Drug indifference, B) Persistens, C) Biofilm and D) Changes in bacterial permeability (Corona and Martinez, 2013).

As depicted in Fig. 2, the four major mechanisms for the acquisition of phenotypic resistance are:

A) Drug indifference: antibiotic treatment is only successful when the bacteria are in a specific physiological condition (Corona and Martinez, 2013).

B) Persistens: when the antibiotic treatment is effective the largest part of the bacterial population is killed, but there is still a sub-population which is not killed named as persistens. When the growth of the persistens population resumes they become susceptible to antibiotics, indicating that the resistant phenotype is not permanent and it is not the result of a genetic change (Corona and Martinez, 2013).

C) Biofilm: when bacteria adhere to surfaces they usually grow forming biofilms and under these circumstances they become more resistant to antibiotics. The different gradients of nutrients and oxygen concentration may cause a different bacterial metabolic state depending on the depth inside the biofilm. This situation may be crucial, affecting the susceptibility to antibiotics. Furthermore, matrix compounds can diminish the diffusion of the antibiotic or even bind the drug resulting in a decreased free concentration (Corona and Martinez, 2013). Manandhar investigated in 2018 the detection of *in vitro* biofilm production from *S. aureus*. More specifically, 161 *S. aureus* isolates were collected from tertiary hospitals in Nepal. 131 were methicillin-resistant *S. aureus* and 30 methicillin-sensitive *S. aureus* (MSSA). MRSA isolates demonstrated thicker biofilms and, therefore, increased possibility of antibiotic resistance. 18 MRSA and one MSSA produced strong (+++) biofilms, 27 MRSA and 15 MSSA produced moderate (++) biofilms and 86 MRSA and 14 MSSA produced weak (+) biofilms. This study showed the high prevalence of MRSA produced biofilms.

D) Changes in bacterial permeability: antibiotic susceptibility can be affected by a number of environmental and internal factors such as temperature, the presence of specific inducers, Reactive Oxygen Species (ROS) or specific metabolic situations. Bacteria use two main strategies to accomplish it. First, a reduction of the antibiotic binding can be achieved through modification of the lipopolysaccharide (LPS) or via building outer membrane vesicles, which expand the surface and reduce the effective amount of the antibiotic per cell. Second, a modification of a number or type of aquaporins used by the antibiotic to penetrate the bacteria cells or through expression of efflux pumps, which can excrete the antibiotic when it has entered the cell. Both strategies result in a decreased antibiotic concentration and in a decreased effectiveness (Corona and Martinez, 2013).

2.2 **Macrolide, lincosamide and streptogramin B (MLS) antibiotics**

Macrolide antibiotics:

Macrolides are natural products which consist of a large microcyclic lactone ring and one or more deoxy sugars. Commonly used macrolides have a 14-membered (clarithromycin, dirithromycin, erythromycin and roxithromycin) or a 15-membered (azithromycin) lactone ring. Also 16-membered ring macrolides (josamycin, midecamycin, miocamycin, rokitamyin, spiramycin and tyolsin) exist in certain countries (Leclercq, 2002). In the 1960s, spiramycin was introduced as the first macrolide for food animal use. In the next decade, also tylosin and erythromycin were introduced. Macrolides were used as growth promoters until 1998 in the European Union. Tilmicosin, tulathromycin, gamithromycin have been used for this purpose (Pyörälä et al., 2014). Tilmicosin is a 16-membered macrolide and is synthetized from tylosin (Kirst, 1997) while tulathromycin and gamithromycinare are 15-membered macrolides (Pyörälä et al., 2014).

The spectrum of activity includes gram-positive cocci (mostly staphylococci and streptococci but also enterococccci and *Trueperella*), gram-negative organisms such as *Actinobacillus*, *Haemophilus*, *Histophilus*, *Mannheimia*, *Pasteurella*, *Moraxella*, *Bordetella*, *Campylobacter* and *Lawsonia*. Anaerobes: *Fusobacterium*, *Clostridium* and *Bacteroides species* are in most cases also susceptible. Additionally, they are effective against *Mycoplasma* and sphirochaetes such as *Leptospira* and *Brachyspira* (Pyörälä et al., 2014).

Their mechanism of action is based on their ability to bind to the bacterial 50S ribosomal subunit. As a result, bacterial protein synthesis is inhibited. After binding, mRNA translation is blocked. Specifically, the growing peptide chain is affected by blocking the addition of the next amino acid by the tRNA (Patel and Hashmi, 2019).

Based on their mechanism of action macrolides can be classified as bacteriostatic as they only inhibit protein synthesis. However, at high doses, they can be bactericidal. Typically, they are used to treat infections like pneumonia, sinusitis, as well as pharyngitis and tonsillitis (Patel and Hashmi, 2019).

Lincosamide antibiotics:

Lincosamide originates from a natural product, lincomycin, and includes semisynthetic derivatives, clindamycin and pirlimycin. It consists of unusual amino acid, viz. trans-N-methyl-4-n-L-proline 54 (propylhygric acid) linked by a peptide bond with the sugar 6-amino-6, 8-dideoxy-1-thio-D55 erythro-α-D-galactopyranoside (methylthio-lincosamide). Natural and semisynthetic 56 lincosamides are lincomycins A, B, C, D, S, K, celesticetins A, B, C, D, desalicetin, 57 desalicetin D, and N-demethylcelesticetin. Clyndamycin a semi-synthetic derivative with 58 high biological activity is of great importance (Spížek and Řezanka, 2016). It is used as a broad-spectrum antibiotic (Rezanka et al., 2007), but it is mostly effective against gram-positive bacteria. It can also be used for some gram-negative anaerobes and protozoa (Schlünzen et al., 2001).

Their mechanism of action is based on the binding to the 50S subunit at a location which overlaps both the A and P sites on the ribosome, blocking the docking of charged tRNAs and their transport through the peptidyl transferase center. In this way the protein synthesis is inhibited (Sauberan and Bradley, 2018). .

Lincosamides are bacteriostatic. However, in higher concentrations (2-4 times the minimum inhibitory concentration) and against certain organisms can be bactericidal (Sauberan and Bradley, 2018). They are mostly used for the treatment of septicemia, intra-abdominal infections, lower respiratory infections, gynecological infections, bone and joint infections, skin infections, streptococcal pharyngitis, acne vulgaris, bacterial vaginosis and severe pelvic inflammatory disease (Patrick et al., 2019).

Streptogramin B antibiotics:

Steptogramines are natural products, extracted from Streptomyces strains. They can be categorised based on their chemical structure in two major groups (Group A and group B). Group A is composed of polyunsaturated cyclic peptolides. The most known are virginiamycin M1, pristinamycin IIA, pristinamycin IIB and dalfopristin (a pristinamycin IIB derivative). Group B members are cyclic hexadepsipeptides. The most important group B members are virginiamycin S and pristinamycins IA, IB, and IC (Soriano, 2010).

Their mechanism of action is analogous to macrolides and lincosamides as they inhibit the protein synthesis through binding to the peptidyl transferase at a similar location (Sauberan & Bradley, 2018).

Streptogramines are considered to be bacteriostatic. When group A and group B members are used together they act synergistically (Lee, 2007).

Streptogramin is a reserve antibiotic and is only indicated for infections with highly resistant Gram-positive bacteria (Padberg, 2015).

2.3. **MLS antibiotic resistance mechanism and resistance genes**

Macrolides, lincosamides and streptogramins B (MLS antibiotics) resistance is attributed to four major mechanisms: (i) methylation of rRNA (target modification), (ii) protection of the ribosome with ABC-F proteins, (iii) antibiotic efflux through Major Facilitator Superfamily (FMS) and (iv) enzymatic inactivation (Petinaki and Papagiannitsis, 2018; Fessler et al., 2018).

Ribosomal methylation: it represents the most frequent mechanism of resistance to MLS antibiotics in gram-positive bacteria, (including *Staphylococcus aureus*) and is highly dependent on the *erm* (erythromycin rRNA methylase) genes. A large number of microorganisms express the *erm* (erythromycin rRNA methylase) genes, which encode the erm proteins. Moreover, erm proteins are of great importance because they can play a crucial role in the binding of MLS antibiotics. Specifically, through methylation of 23S rRNA the binding of the MLS antibiotic is damaged, leading to cross-resistance (Petinaki and Papagiannitsis, 2018). It is proven that *erm*(A), *erm*(B) and *erm*(C) mediate a combined resistance to macrolides, lincosamides and streptogrammin B (Schwarz et al., 2018).

Today, there are four main categories of *erm* genes (*erm*(A)*, erm*(B)*, erm*(C)*, erm*(F)) and over 42 documented *erm* genes. *erm* genes are carried mostly by plasmids and transposons (Petinaki and Papagiannitsis, 2018). It has been found that *erm*(A) and *erm*(B) are connected with transposons, while *erm*(C) with small plasmids (Schwarz et al., 2018).

The *erm*(A) gene was identified in *Staphylococcus aureus* (*S. aureus*) (mostly MRSA) in different animal species.. In addition, it was found in poultry associated *Staphylococcus.* The *erm*(B) gene was identified in porcine *S. aureus* isolates, but also in livestock-associated MRSA from turkeys, chickens, pigs, cattle, a brown hare and a mink. The *erm*(C) gene was identified in *S. aureus* (some of them MRSA) from pigs, cattle, horses, dogs, cats, rabbits, goats, sheep and a wild boar. Extensive literature concerning these issues can be found in Schwarz et al., 2018.

Ribosome protection with ABC-F proteins: ABC-F proteins can effectively protect the bacterial ribosome from the antibiotic attack. These proteins are encoded from *msr* genes. It is experimentally demonstrated that *vga* and *lsa* genes encode proteins, which act in a similar way (Fessler et al., 2018). While *msr* genes can mediate resistance to macrolides and streptogramin B, *vga* genes lead to lincosamide, pleuromutilines and streptogramin A resistance (Schwarz et al., 2018).

The *msr*(A) gene has been detected in *S. aureus* from dogs, cats, poultry, cattle and horse. On the other hand, the *vga(A)* gene has been found in *S. aureus* (largest part MRSA) from pigs, cattle, chicken and turkeys (Schwarz et al., 2018).

Antibiotic efflux: Bacterial cells having high pump expression present enhanced antibiotic resistance in comparison to cells with low or no pump expression. Efflux pumps have the ability to export antibiotics out of the bacterial cell which can often lead to multidrug resistance. Resistance via active efflux in gram-positive bacteria is achieved through two major pumps. Both pumps are members of the ATP-binding-cassette (ABC) transporter superfamily and of the major facilitator superfamily (MFS) (Petinaki and Papagiannitsis, 2018). This can only be achieved through the encoding of *mef* genes which confer resistance only to macrolides. More research should be done about *mef* genes in staphylococci as we have little information about them (Fessler et al., 2018). Luna investigated in 2002 among others the distribution of *mef*(A) gene in different *staphylococcus* species from isolates which were collected from healthy children. In 1/11 of *S. aureus* the *mef*(A) gene was detected. Zmantar in 2011 examined along others the presence of *mef* genes in *S. aureus* isolated from auricular infections in a Tunisian hospital. *mef* genes were not identified in his study.

Enzymatic inactivation: The inactivation of an enzyme is a chemical process which involves a number of phenomena taking place synchronously and leads to resistance

to antibiotics with similar structure. More specifically, *ere* and *mph*(C) genes encode esterases and phosphotransferases and furnish resistance to erythromycin and other 14- and 15-membered macrolides. On the other hand, *lnu*(A) (formerly *lin*A) and *lnu*(B) (formerly *lin*B) genes encode lincosamide nucleotidyl transferases in *staphylococci* and confer resistance only to lincosamides (Petinaki and Papagiannitsis, 2018). Moreover the *vgb*(A) and *vgb*(B) genes encode hydrolases which can hydrolyze streptogramin B and lead to resistance solely to streptogramin B. Also, *vat* genes have the ability to encode tranferases. It has been proven that *vat*(A) and *vat*(B) genes mediate resistance only to streptogramin A (Schwarz et al., 2018). The *mph(C)* gene was identified in *S. aureus* from dogs (Lüthje and Schwarz, 2007), while, the *lnu(A)* was detected in *S. aureus* (some of them MRSA) from pigs, dairy cattle and a turkey (Schwarz et al., 2018). Regarding *vat* genes, they have not been identified in *S. aureus* or MRSA isolates from companion animals and wild animals, to the author's best
knowledge.

2.4. **Methicillin-resistant *Staphylococcus aureus* (MRSA)**

Alexander Fleming observed in 1929 that a mold called Penicillium was able to kill bacteria, including some *staphylococci*. He named it penicillin. Only a year after the first medical use of penicillin, resistant isolates of *S. aureus* were observed while *S. aureus* continued to develop resistance to other antibiotics. By the 1960s, penicillin-resistant strains of *S. aureus* were considered a pandemic. The first reports of methicillin resistance, which was introduced in 1959 as an antibiotic against *S. aureus* were in 1961 from the United Kingdom. Methicillin-resistant *Staphylococcus aureus* (MRSA) isolates were also found later in Japan, Australia, and USA. Today, MRSA is a serious problem worldwide. More than 170.000 nosocomial infections were reported in 2013 in the European Union (Köck et al., 2014), but according to the European Center for Disease Prevention and Control (ECDC) between 2013 and 2016 there was a significant decrease of the MRSA infections. The mean MRSA percentage in 2014 was 19.6% and 16.9% in 2017 as it was reported in ECDC. In the United States over 119.000 infections and almost 20.000 deaths were recorded in 2019 (Kavanagh, 2019).

Healthcare-associated MRSA strains, also known as HA-MRSA are the most frequent cause of hospital-acquired infections (Green et al., 2012). MRSA is one of the most serious problems among healthcare associated infections in humans since decades. In the early 1990s the first non-hospital associated MRSA infections were recorded. They occurred in the community and they were named community-associated methicillin -resistant *S. aureus* (CA-MRSA) (Deurenberg and Stobberingh, 2008). In addition, in the last decades isolation of MRSA from livestock (livestock -associated, methicillin resistant *S. aureus*, LA-MRSA) and companion animals have also been reported (Vincze et al., 2014). HA-MRSA and CA-MRSA affect mostly humans and do not play a significant role in livestock infections. However, LA-MRSA could be harbored by humans if there is close contact with affected livestock (Cuny et al., 2015). Between the different MRSA reservoirs overlaps have been documented, involving hospital infections by CA -MRSA (Moore et al., 2009) and isolation of LA-MRSA in hospitals (Van Rijen et al., 2008). The theory of invasive HA-MRSA infection appears to be in recession (Rossolini et al. 2014), while a marked increase of CA-MRSA infections has been documented in the society (CDC 2013). CA-MRSA has been identified in companion animals, livestock animals and wild animals (Aires-de-Sousa, 2017). Therefore, the traditional epidemiological MRSA classification into HA-MRSA, CA-MRSA and LA-MRSA may be no more valid due to the overlaps between these groups (Sergelidis and Angelidis, 2017). There is only little information about MRSA in wild animals as only a limited number of studies were focused on wild animals. Although, the presence of MRSA was reported in wild animals with no direct contact with antibiotics (Silva et al., 2020).

According to ECDC the percentages of MRSA in Austrian isolates were 7.8% in 2014, 7.5% in 2015, 7.1% in 2016 and 5.9% in 2017. In addition, the Austrian Agency for Health and Food Safety Ltd. (AGES) reported that in 2008 the percentage of MRSA which was isolated from pigs was 5.3% while, in 2016 the percentage of MRSA which was isolated from raw chicken meat was 1.4%.

MRSA is a term, which refers to strains of *Staphylococcus aureus* which are methicillin-resistant despite the fact, that they may be also resistant to other antibiotics too (Green et al., 2012). *S. aureus* is able to develop resistance to almost all antibiotic groups, creating occasionally a multidrug resistance pattern (Loncaric et al., 2014, Schauer et al., 2018, Soimala et al., 2018). More specifically, the non-

susceptibility of an isolate to a minimum one agent in three or more different antimicrobial classes tested is referred as multidrug resistance of *S. aureus* while resistance to at least one agent in all but one or two antimicrobial classes is defined as extensive drug resistance. By pan drug resistance, we can observe a non-susceptibility to all agents of all antimicrobial classes (Sweeney et al., 2018). Methicillin-resistant *S. aureus* lifespan is considered to be many months in hostile environments and, therefore, a transmission from surfaces after a long period is possible.

MRSA strains can also be resistant to antibiotics such as macrolides, lincosamides, fluoroquinolones, aminoglycosides and trimethoprim-sulfamethoxazole (Green et al., 2011).

The mechanism of resistance to methicillin is based on the production of a modified penicillin-binding protein (PBP2a), which is encoded by the *mecA* gene (Stefani et al., 2012) and exhibits a poor affinity to penicillins and other β-lactam antibiotics for β-lactams (Lakhundi and Zhang, 2018). This modified penicillin-binding protein (PBP2a) is not present in susceptible strains and it is believed that it has been acquired from a distantly related species (Enright et al., 2002). Based on the multiple studies which were carried out, *mecA* was developed from a harmless core gene named *mecA1* which was able to encode the penicillin-binding protein D (PbpD) from *Staphylococcus Sciuri* group because of the β-lactam overuse in human created environments. (Miragaia, 2018)

mecA gene is carried on the staphylococcal cassette chromosome *mec* (SCC*mec*), from which 13 different types have been described in *S. aureus* that differ in size and genetic composition (Stryjewski and Corey, 2014; Lakhundi and Zhang, 2018).

SCC*mec* is a mobile genetic element that carries the *mec* gene complex and the cassette chromosome recombinase (*ccr*) gene complex. The mec gene complex is constituted from *mecA*, the insertion sequences and the regulatory genes. It can be classified in: A, B, C1, C2, D and E. On the other hand, *ccr* genes encode recombinaces that are responsible for the integration and excision of SCC*mec* into the genome of *S. aureus*. Also some other genes including transposons, insertion sequences and plasmids are contained in the SCC*mec* (Saber et al., 2017). Funaki reported in 2019 that SCCmec types between CA-MRSA and HA-MRSA are not identical.

In addition *mecC* gene can also encode a PBP2a. *mecC* gene function was described by Kim in 2012. Although, there are biochemistry differences between *mecA* and *mecC* encoded PBP2a it has been proven that *mecC* can also mediate methicillin resistance (Paterson et al., 2014). Gene *mecC* was firstly isolated from a bulk tank milk sample in England and his DNA sequence is 69% identical to *mecA*. *mecC* gene has been identified in livestock animals, companion animals and wild animals from different European countries (Paterson et al., 2014). Loncaric identified in 2014 the *mecC* gene in Austrian companion animals and in 2013 in Austrian goats. He also detected in 2013 the *mecC* gene in wild animals from Austria. More specifically, from three European brown hares, one European otter, one European hedgehog and one Eurasian lynx.

For the investigation of MRSA epidemiology a plethora of phenotypic and genotypic methods can be used. These methods vary significantly in their discriminatory power (the ability to distinguish between different strains), reproducibility of the results, and the cost and efforts required. A typing technique is considered successful when it is simple, inexpensive, and reproducible, with sufficient discriminatory power and is widely available (Lakhundi and Zhang, 2018).

2.5. **MLS resistance in *S. aureus* and methicillin-resistant *S. aureus* spread**

MLS-resistant *staphylococci* rates are different among countries and animal species. In a Japanese study, it was published that almost 97% of MRSA and 34.6% of MSSA were resistant to one or more MLS agents (Otsuka et al., 2007). In a Turkish hospital the rate of resistance to MLS antibiotics was 38.5% (Cetin et al., 2008), while in another Turkish hospital in Ismir 79% of the isolates exhibited resistance to erythromycin (Uzun et al., 2014). In another study, which took place in a tertiary Greek hospital, the rate of MLS resistance was 44% (Vallianou et al., 2014). Also, a study in Cyprus showed that 67.61% of *S. aureus* were resistant to erythromycin (Petinaki and Papagiannitsis, 2018). Sedaghat reported that 43.8% of *S.aureus* isolates were resistant to erythromycin. The samples were obtained from hospitals in Isfahan.

Hendriksen reported in 2008 among others the percentage of resistance to erythromycin in *S. aureus* that was isolated from bovine mastitis in France, England,

Denmark, Netherlands, Spain and other European countries for the years 2002, 2003 and 2004. The highest percentages were identified in France with a 11.4% resistance in Erythromycin in 2002 and 7% in 2003. In 2004 the percentage of erythromycin resistant *staphylococci* was 2% in England, 1.8% in Spain and 0% in Netherlands. Bahraminia reported in 2017 that 56.9% of *S. aureus* isolates which were obtained from bovine mastitis were resistant to tylosin. Bierowiec examined in 2016 among others the prevalence of erythromycin resistance in *S. aureus* isolated from pet cats and feral cats. 4.17% of *S. aureus* isolated pet cats were resistant to erythromycin while, 12.5% of *S. aureus* isolated from feral cats were resistant to erythromycin.

Among different countries significant differences in the resistance rate to MLS antibiotics have been recorded. These variations might mirror the differences in the drug usage, gene carriage and the clonality of strains (Otsuka et al., 2007)

3. Material and methods

3.1. Population study and collected samples.

90 non-repetitive MRSA isolates were collected from companion animals in a period of five years from Autumn 2013 to Autumn 2018. All 90 isolates come from Austria. Companion animals MRSA isolates (Tab. 1) originated from horses (n=62, 68.9%), cats (n=13, 14.4%), dogs (n=10, 11.1%), rabbits (n=2, 2.2%), a domestic canary (1.1%), a zoo-kept hammer-headed bat (Hypsignathus monstrosus) (1.1%) and a semi-captive northern bald ibis (Geronticus eremita) (1.1%) (Tab. 1). All 90 isolates come from Austria. They have been taken from wounds (n=44), noses (n=6), fistulas (n=5), ears (n=3), tracheal lavage (n=3), eyes (n=3), urine (n=2), urine bladders (n=2), abscesses (n=2), horse sinuses (n=2), skin (n=2), mouth (n=1), uterus (n=1), vein content (n=1), fibrin (n=1), synovia (n=1), exudate (n=1), feces (n=1), ascites material (n=1), joint (n=1), lung (n=1), osteosynthesis (n=1), subcutaneous tissue (n=1), screw post-operative (n=1), urinary calculi (n=1), claw fracture (n=1) and knee (n=1).

78 non-repetitive MRSA isolates were obtained from wild animals. All of them were obtained from European hares (*Lepus europaeus*). 72 originated from the German North Frisian Island Pellworm and 6 from Lower Austria. The MRSA isolates have been obtained from the nasal cavity (n=49), the intestine (n=20), abscesses (n=3), the liver (n=1) and the eye (n=1). The origin of four isolates was not reported.

The aim of this study is to compare the presence of macrolide, lincosamide, streptogramin B resistance genes between MRSA that was isolated from Austrian companion animals and wild animals from German North Frisian Island Pellworm and Lower Austria.

3.2. **MRSA isolation and testing for antimicrobial susceptibility**

All samples have been cultivated, species-characterised and tested for antibiotic resistance.

All MRSA isolates have been kept at -80 °C until further examination (Loncaric et al., 2019). The exact determination of the bacterial species has been achieved by using Matrix-Assisted Laser Desorption/ Ionisation-Time of Flight (MALDI-TOF) mass spectrometry (Spergser et al., 2019). The agar disk diffusion process was carried out according to the recommendations given in the CLSI document M100 (28th ed.) (Clinicaland Laboratory Standards Institute (CLSI), 2018) using the following disks (Beckton Dickinson, Heidelberg, Germany): penicillin (PEN, 10 IU), cefoxitin (FOX, 30 µg), gentamicin (GEN, 10 µg), erythromycin (ERY, 15 µg), clindamycin (CLI, 2 µg), tetracycline (TET, 30 µg), ciprofloxacin (CIP, 5 µg), 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 (Loncaric et al., 2019).

3.3. **MRSA molecular characterisation**

DNA extraction was performed after isolates were grown on BD Columbia III agar with 5% sheep blood (Beckton Dickinson) and incubated overnight at 37°C. Enzymatical lysis of bacterial cells followed and then DNA was extracted by using commercially available spin columns (GenElute™ Mammalian Genomic DNA Miniprep Kits, Sigma-Aldrich, Vienna, Austria). The DNA extraction was achieved through the following steps:

Step 1. A2 tubes were centrifuged for a couple of seconds.

Step 2. Resuspend cells: In tubes A1 material from the blood agar plates is resuspended with 200µl of Lyse A. Then, homogenised through centrifugation and incubated for 60 minutes at 37°C and at 550rpm.

Step 3. Lyse cells: after the incubation, 200µl of Lysis Solution and 40µl of proteinase K is added. After thoroughly vortexing the mix is incubated for 60-90 minutes at 55°C. Then at 70°C for ten minutes in order to deactivate proteinase K.

Step 4. Column preparation: 500µl of the column preparation solution is added to each pre-assembled GenElute™ Miniprep Binding Column and centrifuged at 42000 rpm for one minute. The flow-through liquid is discarded.

Step 5. Prepare for binding: For the binding preparation 200µl of ethanol (95–100%) is added to the lysate and then mixed thoroughly by vortexing for 5–10 seconds.

Step 6. Load lysate: the entire content of the tubes is transferred to the binding columns from step 4 and centrifuged for 2 minutes at 14000rpm. Consequently, the collection tube containing the flow-through liquid is discarded and the binding column is placed in a new 2ml collection tube.

Step 7. First wash: 500µl of the Wash Solution Concentrate with ethanol is added. The mix is centrifuged for two minutes at 14000rpm. Next, the collection tube containing the flow-through liquid is discarded and the binding column is placed in a new 2ml collection tube.

Step 8. Second wash: this step is exactly the same as step 7 except that the mix is centrifuged for three minutes instead of two. Then, the new 2ml collections tubes with the binding column are centrifuged for one minute at 14000rpm. After the centrifugation the binding column is placed in a new 2ml collection tube.

Step 9. Elute DNA: 200µl of the Elution Solution is added in the center of the binding column. Then, centrifuged for one minute at 12500rpm. After centrifugation the binding columns are discarded and the collection tubes are incubated for 5 minutes in room temperature.

Primers targeting *mecA* and *mecC* were used in order to detect methicillin resistance. The MLS resistance genes were identified by using a DNA microarray (*S. aureus* Genotyping Kit 2.0, Alere, Jena, Germany) (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).

For further details, please contact the supervisor of the project, Dr. I. Loncaric.

4. Experimental results and analysis

4.1. **MLS resistance genes**

The 12 MLS resistance genes, which were used in this experiment, were:

1) *erm*(A) (rRNA methyltransferase), 2) *erm*(B) (rRNA methyltransferase), 3) *erm*(C) (rRNA methyltransferase), 4) *lnu*(A) (lincosamide nucleotidyltransferase), 5) *msr*(A) (macrolide efflux pump), 6) *mef* (macrolide efflux protein A), 7) *mph*(C) (macrolide phosphotransferase II), 8) *vat*(A) (virginiamycin A acetyltransferase), 9) *vat*(B) (acetyltransferase), 10) *vga*(A) (ABC transporter), 11) *vga*(A) (BM3327) (*vga*(A) allele from strain BM3327), 12) *vgb*(A) (virginiamycin B hydrolase).

Their mechanisms of action and resistance profiles have been previously described in section 1.4.

4.2. **Comparison of the presence of MLS resistance genes between MRSA isolated from companion animals and wild animals**

The genes that were identified in companion animals as depicted in Tab. 1 are: *erm*(A) in 15.5% (n=14) of isolates, *erm*(B) in 2.2% (n=2) of isolates, *erm*(C) in 10% (n=9) of isolates, *lnu*(A) in 1.1% (n=1) of isolates, *msr*(A) in 2.2% (n=2) of isolates, *mph*(C) in 2.2% (n=2) of isolates and *vga*(A)(BM3327) in 1.1% (n=1) of isolates.

Genes *mef*(A), *vat*(A), *vat*(B), *vga*(A) and *vgb*(A) were not identified in companion animals in the present study.

The only gene that was detected in wild animals as depicted in Tab. 2 is *erm*(B). It was detected only in 5.1% (n=4) of the isolates.

Genes *erm*(A), *erm*(C), *lnu*(A), *msr*(A), *mef*, *mph*(C), *vat*(A), *vat*(B), *vga*(A), *vga*(A) (BM3327) and *vgb*(A) were not identified in wild animals in the present study.

From Tab. 2, it should be noticed that genes *lnu*(A) and *mph*(C) were not reported for the isolates 3683, 10PEMRSA, 17P, 1PEMRSA, 22P, 438AB, 438M, 7n, AC955, AC957, PE10-14, PE17-14, PE20-14, PE2-14, PE8-14.

Also from the same table, gene *vga*(A) (BM3327) was not reported for the following isolates: 3683, 10PEMRSA, 13P-WH, 17P, 19P, 1PEMRSA, 22P, 438AB, 438M, 7n, AC1082, AC955, AC957, P23-17, PE10-14, PE10-14K, PE17-14, PE17-14N, PE18-17, PE20-14, PE21-17, PE2-14, PE22-14, PE23b-17, PE24-17, PE33-17, PE33N-16, PE34b-16, PE34N-16, PE4N-16, PE8-14, PE9N-14.

The data of Tab. 1 and Tab. 2 was obtained from the Institute of Microbiology of the Veterinary Medicine University of Vienna.

Isolates	**Host**	*erm*(A)	*erm*(B)	*erm*(C)	*lnu* (A)	*msr*(A)	*mph*(C)	*vga*(A)(BM 3327)
2202	Horse	Negative	Negative	Positive	Negative	Negative	Negative	Negative
3855	Horse	Negative	Negative	Positive	Negative	Negative	Negative	Negative
2281	Dog	Negative	Negative	Positive	Negative	Negative	Negative	Negative
3214	Horse	Negative	Negative	positive	Negative	Negative	Negative	Negative
38	Horse	Negative	Negative	Negative	Negative	Negative	Negative	Positive
1012	Cat	Positive	Negative	Positive	Negative	Negative	Negative	Negative
1034	Dog	Positive	Negative	Negative	Negative	Negative	Negative	Negative
1435	Cat	Positive	Negative	Positive	Negative	Negative	Negative	Negative
1758	Horse	Negative	Positive	Negative	Negative	Negative	Negative	Negative
4023	Horse	Negative	Positive	Negative	Negative	Negative	Negative	Negative
2824	Cat	Negative	Negative	Positive	Negative	Negative	Negative	Negative
3278	Cat	Negative	Negative	Positive	Negative	Negative	Negative	Negative
593	Cat	Negative	Negative	Negative	Positive	Negative	Negative	Negative
306	Dog	Positive	Negative	Negative	Negative	Negative	Negative	Negative
1846	Cat	Positive	Negative	Negative	Negative	Negative	Negative	Negative
1847	Dog	Positive	Negative	Negative	Negative	Negative	Negative	Negative
2901	Dog	Positive	Negative	Negative	Negative	Negative	Negative	Negative
2984	Cat	Positive	Negative	Negative	Negative	Negative	Negative	Negative
3268	Dog	Positive	Negative	Negative	Negative	Negative	Negative	Negative
3376	Dog	Positive	Negative	Negative	Negative	Negative	Negative	Negative
3525	Horse	Positive	Negative	Negative	Negative	Negative	Negative	Negative
2838	Dog	Positive	Negative	Negative	Negative	Negative	Negative	Negative
2756	Cat	Positive	Negative	Negative	Negative	Negative	Negative	Negative
112	Dog	Negative	Negative	Positive	Negative	Negative	Negative	Negative
1038	Dog	Negative	Negative	Negative	Negative	Positive	Positive	Negative
1048	Cat	Negative	Negative	Negative	Negative	Positive	Positive	Negative
4454	Cat	Positive	Negative	Negative	Negative	Negative	Negative	Negative

Tab. 1: Presence of MLS resistance genes in companion animals. In this Tab. are depicted all isolates which were carrying at least one MLS resistance gene.

Isolates	Host	*erm*(B)
17P	European Hare	Positive
22P	European Hare	Positive
7n	European Hare	Positive
PE2014	European Hare	Positive

Tab. 2: Presence of MLS resistance genes in wild animals.

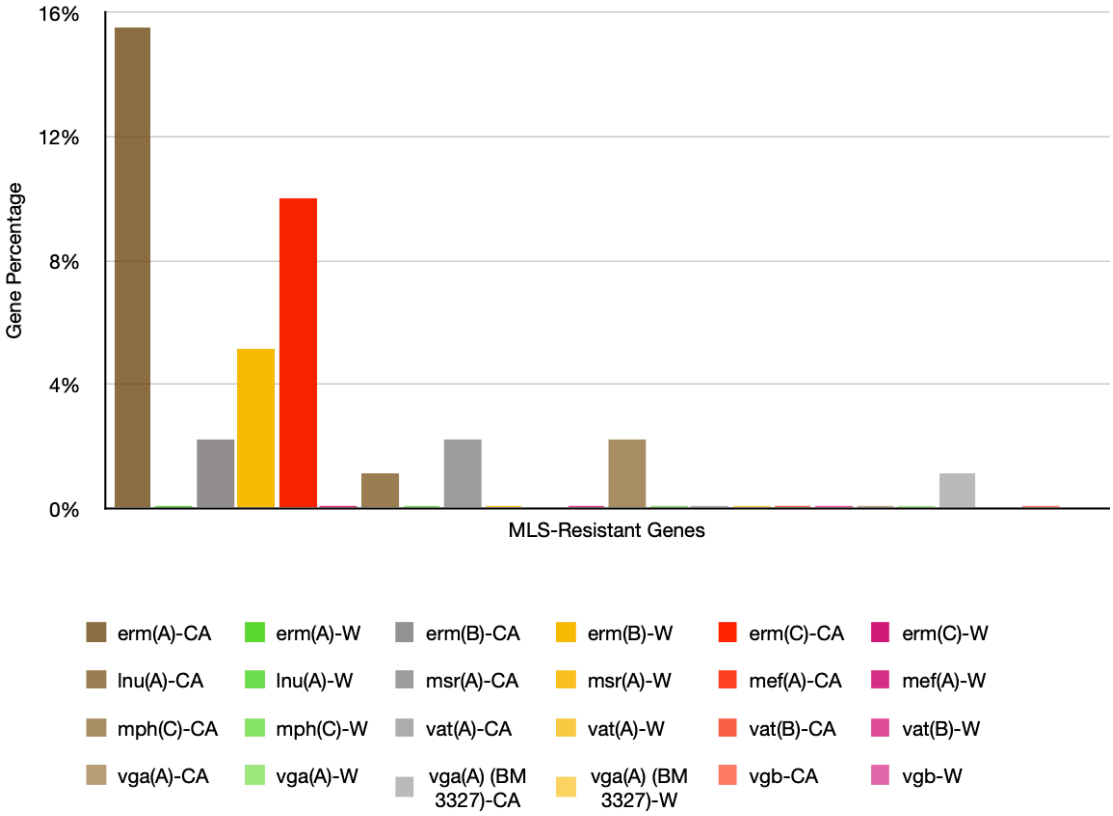

Fig. 3: Comparisons Graph of the presence of MLS resistance genes between companion animals (Tab. 1) and wild animals (Tab. 2). The very small percentages are not possible to be displayed on the graph. CA is referred to companion animals and W to wild animals.

Comparison of the presence of MLS resistance genes between companion animals (Tab. 1) and wild animals (Tab. 2) are depicted in Fig. 3. More specifically:

erm(A) gene was positive in 15.5% of companion animals and 0% of wild animals. *erm*(B) gene was positive in 2.2% of companion animals and 5.1% of wild animals. *erm*(C) gene was positive in 10% of companion animals and 0% of wild animals. *lnu*(A) gene was positive in 1.1% of companion animals and 0% of wild animals. *msr*(A) gene was positive in 2.2% of companion animals and 0% of wild animals. *mef*(A) gene was positive in 0% of companion animals and 0% of wild animals. *mph*(C) gene was positive in 2.2% of companion animals and 0% of wild animals. *vat*(A) gene was positive in 0% of companion animals and 0% of wild animals. *vat*(B) gene was positive in 0% of companion animals and 0% of wild animals. *vga*(A) gene was positive in 0% of companion animals and 0% of wild animals. *vga*(A) (BM3327) gene was positive in 1.1% of companion animals and 0% of wild animals.
vgb(A) gene was positive in 0% of companion animals and 0% of wild animals.

Regarding the companion animals: the 14 *erm*(A) gene positive hosts were: 50% dogs (n=7), 42.8% cats (n=6) and 7.2% horses (n=1). They are resistant to macrolides, lincosamides and streptogramin B. The two *erm*(B) positive hosts were: 100% horses (n=2). They are resistant to macrolides, lincosamides and streptogramin B. The nine *erm*(C) positive hosts were: 44.4% cats (n=4), 33.3% horses (n=3) and 22.2% dogs (n=3). They are resistant to macrolides, lincosamides and streptogramin B. The one *lnu*(A) positive host was a cat. The cat is resistant to lincosamides. The two *msr*(A) positive hosts were one dog and one cat. They are resistant to macrolides and streptogramin B. The two *mph*(C) positive MRSA isolates were obtained from one dog and one cat. They are resistant to macrolides. The one *vga*(A) (BM3327) positive MRSA isolate was obtained from a horse. The horse is resistant to lincosamides.

The initial hypothesis was almost entirely confirmed by the experimental results since the presence of MLS resistance genes was higher in companion animals than in wild animals. Genes *erm*(A), *erm*(C), *lnu*(A), *msr*(A), *mph*(C) and *vga*(A)(BM3327) were found in higher percentages in companion animals in comparison to wild animals. On the other hand, only gen *erm*(B) was found in a higher percentage in wild animals compared to companion animals. Four European hares were resistant to macrolides, lincosamides and streptogramin B.

5. Discussion

The present study depicts the comparison of the presence of the MLS resistance genes between MRSA isolates from Austrian companion animals and wild animals from German North Frisian Island Pellworm and Lower Austria. Different genes code for different resistance mechanisms, which can mediate resistance to macrolides, lincosamides and streptogramin B.

All 90 companion animal MRSA isolates were *mecA* positive and *mecC* negative whereas only seven wild animal MRSA isolates were *mecA* positive and *mecC* negative. The rest 71 wild animals were *mecC* positive and *mecA* negative.

Strommenger et al. (2005) reported the presence of *erm(C)* gene in 16 MRSA isolates which were isolated sporadically in a period of 12 months and obtained from dogs and cats with different type of infections. They were stationary patients at the School of Veterinary Medicine in Hanover. All isolates were resistant to macrolides, lincosamides and streptogramin B, as all were carrying the *erm*(C) gene. The percentage was 100%. This percentage is significant higher than the one found in this study where the *mec*(C) gene was identified in 30.7% (n=4) in cats and 33.3% (n=3) in dogs.

Lüthje and Schwarz (2007), among others, investigated the genetic basis of macrolide and/or lincosamide resistance of 248 coagulase-positive and coagulase-variable *staphylococci* which were selected from dogs, cats and pigs. All were originated from Germany during the period 2004–2006. Resistant *staphylococci* tested for the resistance genes *erm*(A), *erm*(B), *erm*(C), *erm*(TR), *msr*(A), *msr*(D), *mef*(A), *mph*(C), *lnu(A)*, *lnu(B)* and *lnu(C)*. The *erm* genes were identified in *staphylococci*, alone or in different combinations. The *erm*(B) gene was the most found gene in *Staphylococcus intermedius* while gene *msr*(A) and the genes *mph*(C) and *lnu*(A) were identified in single staphylococcal isolates. The prevalence of erythromycin resistance varied between 22.8% and 26.7% with the exception of porcine isolates from genitourinary trac (13.0%). Among the erythromycin-resistant *staphylococci*, mainly the methylase genes *erm*(A), *erm*(B) and *erm*(C) were detected. The gene *erm*(B) was the most widespread among *S. intermedius*, whilst *S. aureus* and *S. hyicus* displayed a more variable profile of resistance genes.

Kadlec et al. (2009) examined 54 methicillin-resistant *Staphylococcus aureus* ST398 isolates which were obtained from pigs with various diseases were investigated for their antimicrobial resistance phenotypes and genotypes. All of them came from Germany. Analytically 5.5% (n=5) were positive for *erm*(A), 11.1% (n=6) for *erm*(B) and 22.2% (n=12) for *erm*(C). One isolate was positive for both *erm*(A) and *erm*(B) and another for *erm*(A) and *erm*(C). In comparison to our study the stated percentage of *erm*(A) positive isolates was lower but, percentages were higher for *erm*(B) and *erm*(C) genes.

Coelho et al. (2011) described among others the molecular detection of methicillin-resistant *Staphylococcus aureus* in 54 healthy dogs. All samples were taken from the nasal cavity and 16 of the 54 samples were MRSA positive. The *erm*(B) gene was detected in 62.5% (n=10) of the MRSA positive isolates while *erm*(C) in 87.5% (n=14) and *msr(A)* in 31.2% (n=5). In this research the percentages of all three MLS genes detected were higher compared to those found in the study of this thesis. Specifically, regarding only the dogs, the percentages found are: 0% (n=0), 33.3% (n=3), 10% (n=1) for the genes *erm*(B), *erm*(C), *msr*(A), respectively. A larger sample of MRSA isolates obtained from dogs could provide more accurate numerical results.

Zhang et al. (2011) investigated among others the antibiotic resistance of 22 MRSA isolates which were obtained from dogs, cats and from a member of a veterinary clinic. All 22 isolates were resistant to macrolides and lincosamides as in all of them gene *erm*(B) and *lun*(A) were detected. The prevalence of both genes is much higher in comparison to our study.

Ho et al. (2012) researched the clonality and antimicrobial susceptibility of *S. aureus* and methicillin-resistant *S. aureus* isolates from food and other animals. His study was based on 3081 animals including 609 cats, 660 chickens, 589 dogs, 310 cattle, 305 pigs, and 608 rodents. He identified 65 MRSA isolates originating from pigs and one from a chicken. Gene *erm*(C) was identified in 90.7% (n=59) of pigs and gene *erm*(A) in only 1.5% (n=1) of pigs resulting in 92.3% resistance to erythromycin. *erm*(A), *mef*(A) and *mef*(E) genes were not identified in his study. Compared to our study the gene *erm*(C) was detected in a significant higher percentage, while, gene *erm*(A) was recorded in a lower percentage.

Loncaric et al. (2014) investigated the identification and characterisation of Austrian methicillin-resistant *Staphylococcus aureus* in dogs, cats and horses, one donkey and a pet rabbit. 89 non-repetitive MRSA isolates have been collected from 2004 to 2013. The MLS antibiotic resistance was investigated through the detection of *erm*(A), *erm*(C) and *msr*(A) genes. The largest part (93.3%) was not resistant to clindamycin and erythromycin. In comparison to current study the percentages of the susceptible to clindamycin and erythromycin isolates are higher, since in 15.5% and 10% of the companion animals er*m*(A) and *erm*(C) genes were identified, respectively.

Monecke et al. (2016) identified the *erm*(B) gene in MRSA which was isolated from a European hare (Lepus europaeus). In this Austrian study the diversity of *S. aureus* isolates in European wildlife was examined. 14 MRSA isolates were collected from hares, hedgehogs, one fox and a fallow deer. The prevalence of *erm*(B) gene is higher (7.1%) compared to our study. It is important to mention that the MRSA isolates in Monecke's study were obtained from different animals. Monecke also reported the identification *erm*(C) gene in a *S. aureus* isolate from a Thuringian wild boar.

Nowakiewicz et al. (2016) identified the *msr*(A) gene in an MRSA isolate originating from a wild animal (a marten). In comparison to our study gene *msr*(A) was not identified in wild animals (European hares) but detected in two companion animals.

Bortolami et al. (2017) described among others the antibiotic resistance of four MRSA isolates which were obtained from a zoo in the United Kingdom. One MRSA isolate originated from a dead yellow mongoose and the other three from a colony of dwarf mongooses. All four isolates were tested for the presence of *erm*(A), *erm*(B) and *erm*(C) genes. None of them was identified in all four MRSA isolates. MLS resistance was not detected in these wild animals in comparison to our study where 5.5% of the wild animals were resistant to MLS antibiotics.

As it was reported by Loncaric et al. (2019) in a publication which was based on the same companion animal dataset as the set used in this thesis, genes *lnu*(A), *msr*(A) and *mph*(C) were identified for the first time in MRSA isolates from companion animals.

Ma et al. (2019) examined in among others the MLS resistance in 10 MRSA isolates which were obtained from eight dogs. All MRSA isolates were susceptible to MLS

antibiotics and resistant only to β-lactams. In comparison to this study, the MRSA isolates originating from Austrian dogs exhibit a significant higher percentage of resistance to MLS antibiotics.

Finally, Silva et al. (2020) reported that in all three MRSA isolates obtained from wild hares in north Portugal, gene *erm*(C) was identified, while gene *erm*(B) in only one. Gene *mph*(C) was identified in two isolates. In our study *erm*(C) and *mph*(C) gene were not detected in wild animals (*Lepus Europaeus*). It should be noticed that the MRSA isolates sample size is not large enough.

Up to now the *lnu*(A) gene was detected in *S. aureus* including MRSA from pigs, dairy cattle and a turkey while the *msr*(A) in *S. aureus* from horses, poultry, cattle, dogs and cats. The *mph*(C) gene was identified in *S. aureus* originating from a dog (Schwarz et al., 2018).

6. Summary

In this Diploma Thesis the presence of macrolide, lincosamide, streptogramin B resistance genes between MRSA that was isolated from Austrian companion animals and wild animals from German North Frisian Island Pellworm and Lower Austria were investigated. The experimental procedure was based on the following data set. Companion animals: 90 non-repetitive MRSA isolates collected over a period of five years (Autumn 2013 to Autumn 2018). These were obtained from horses (n=62), cats (n=13), dogs (n=10), rabbits (n=2), a domestic canary, a zoo kept hammer-headed bat (*Hypsignathus monstrosus*) and a semi-captive northern bald ibis (*Geronticus eremita).* Wild animals: 78 non-repetitive MRSA isolates obtained, all of them originated from European hares (*Lepus europaeus*). It is has been shown that the presence of macrolide, lincosamide, streptogramin B resistance genes obtained from MRSA isolated from wild animals is lower than the corresponding of companion animals. Relative similar works are commented. As a future direction for research, the expansion of the data set with data obtained from other geographical regions are expected to enlighten and improve quantitative comparisons.

7. Zusammenfassung

In der vorliegenden Diplomarbeit wurde das Vorhandensein von Makrolid-, Lincosamid- und Streptogramin B-Resistenzgenen bei MRSA untersucht, das von österreichischen Haustieren und Wildtieren aus der deutschen Nordfriesischen Insel Pellworm und Südösterreich, isoliert wurde. Das experimentelle Verfahren basierte auf dem folgenden Datensatz, der verwendet wurde. Haustiere: 90 nicht-repetitive MRSA Isolate, die über einen Zeitraum von fünf Jahren (Herbst 2013 bis Herbst 2018) gesammelt wurden. Diese wurden erhalten von Pferden (n=62), Katzen (n=13), Hunden (n=10), Kaninchen (n=2), einem Hauskanarienvogel, einer Hammerkopf-Fledermaus (*Hypsignathus monstrosus*) und einem halbwilden Nordkahlen Ibis (*Geronticus eremita*). Wildtiere: 78 nicht-repetitive MRSA-Isolate wurden erhalten, die alle von europäischen Hasen (*Lepus europaeus*) stammten. Es wurde gezeigt, dass das Vorhandensein von Makrolid-, Lincosamid- und Streptogramin B-Resistenzgenen, die aus Wildtieren isoliertem MRSA erhalten wurden, geringer ist als das entsprechende von Haustieren. Studien, die sich mit dem gleichen wissenschaftlichen Thema befassen werden kommentiert. Sie stimmen hinsichtlich der Ergebnisse dieser Studie überein, und es werden vergleichende numerische Ergebnisse angegeben. Eine zukünftige Forschungsrichtung wäre die Erweiterung des Datensatzes mit Daten aus anderen geografischen Regionen, die quantitative Vergleiche aufklären und verbessern würden.

8. Abbreviations

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

HA-MRSA - Healthcare Associated MRSA strains

HGT - Horizontal Gene Transfer

LPS - Lipopolysaccharide

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

MGEs - Mobile genetic elements

MFS - Major Facilitator Superfamily

MLS - Macrolide, lincosamide and streptogramin B

MLSB - Macrolide, lincosamide and streptogramin B

MLST - Multilocus sequence typing

MRSA - Methicillin-resistant *S. aureus*

MSSA- Methicillin-sensitive *S. aureus*

ROS - Reactive Oxygen Species

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10. Figure and table references

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- Figure 3: Created by the author.
- Table 1: Extracted from the data set of the microbiology Institute of the University of Veterinary Medicine, Vienna.
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