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Characteristics of hospital-associated infections in horses caused by methicillin-resistant *Staphylococcus aureus*

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List of abbreviations

CA-MRSA	-	community-associated methicillin-resistant S*taphylococcus aureus*
CC	-	clonal complex
CDC	-	Centers for Disease Control and Prevention
CEM	-	Centro de Epidemiologia Molecular initiative
ClfA	-	clumping factor A
DNA	-	deoxyribonucleic acid
DCEM	-	Department and Clinic of Equine Medicine
EHV-1	-	Equine Herpes Virus 1
EHV-4	-	Equine Herpes Virus 4
GPM	-	Gesellschaft für Pferdemedizin
HA-MRSA	-	hospital-associated methicillin-resistant S*taphylococcus aureus*
LA-MRSA	-	livestock-associated methicillin- resistant S*taphylococcus aureus*
MDR	-	multidrug resistance
MLST	-	multilocus sequence typing
MRE	-	multi-resistant organisms
MRSA	-	methicillin-resistant *Staphylococcus aureus*
MSSA	-	methicillin-sensitive *Staphylococcus aureus*
PBP2a	-	penicillin-binding protein 2a
PSM	-	phenol-soluble modulins
PVL	-	Panton-Valentine-leucocidin
SCC	-	staphylococcal cassette
SCCmecA	-	staphylococcal cassette, gene mecA
SCCmecC	-	staphylococcal cassette, gene mecC
SCINeq	-	equine staphylococcal complement inhibitor protein
SOP	-	standard operating procedure
SSI	-	surgical site infection
ST	-	sequence type
vWbp	-	von Willebrand factor binding protein
WGS	-	whole genome sequencing
wgMLST	-	whole genome multilocus sequence typing

1. Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA)-related illnesses have become more common in recent years, posing a significant problem for medical treatment. As typical commensals of the natural flora in equines, *Staphylococcus aureus* (*S. aureus*) possess the ability to induce infections under specific circumstances, especially after surgical interventions. These opportunistic bacterial infections are prevalent within healthcare settings; the growth of multidrug-resistant organisms (MRE) and nosocomial infections is primarily attributed to the misuse of antibiotics and inadequate compliance to preventive hygiene protocols. The therapy for these infections has become increasingly challenging. While most *S. aureus* strains do not pose an immediate threat to the host after colonization, some of them have increased pathogenic potential and are resistant to a variety of antimicrobial agents.

The resulting consequences include extended treatment durations, heightened financial burdens, and an increased risk of nosocomial transmission. Particularly, MRSA can enable cross-species infections between horses and humans, significantly impacting the staff of veterinary hospitals. In general, the processes of domestication and globalization within the livestock industry have significantly heightened the possibilities of bacterial exchange between human and animal populations. The evolutionary course of each MRSA strain is influenced by genetic variability, drug resistance, virulence, and host adaptive genes.

As early as the mid-19th century, the Hungarian doctor Ignaz Philipp Semmelweis († August 13, 1865) advocated for the introduction of hygiene regulations in clinics. He recognized the transmission of pathogens by doctors and hospital staff and its consequences. Implementing effective cleaning and disinfection procedures is necessary to prevent the transmission of these pathogens.

2. Introduction

Nosocomial infections are a major issue in equine clinics worldwide. These infections can be caused by a variety of microorganisms and can have significant consequences, such as infections that result in extended hospital stays for patients [1]. The main pathogens associated with nosocomial infections in equine clinics are Salmonella, MRSA, EHV-1, EHV-4 and Enterobacteriaceae [2].

In recent years, the emergence and spread of multidrug-resistant microorganisms have become a significant concern both in human and veterinary medicine [3]. A study revealed that horses are more likely to develop multidrug-resistant bacterial infections if they have a history of antibiotic usage, comorbidities or extended hospital stays [1]. This highlights the widespread prevalence of MRE infections in hospitalized horses. The inappropriate use of antibiotics in veterinary medicine contributes to the development of antibiotic resistances in bacteria and depending on the site of infection, the mortality rates of these disorders vary, with MRSA being the causative agent in several infections [1, 4].

Nosocomial infections caused by MRE bacteria are increasingly becoming a challenge in equine clinics. Currently, it is believed that comparable issues could arise in equine managements outside hospitals [5], emphasizing the importance of regulating the use of antibiotics [3]. Newly discovered antimicrobial agents are effective against a large number of isolates that exhibit multidrug resistance. To avoid the occurrence of new resistances, these agents must be used with extreme caution in horses and other animal species due to their high value in human medicine [1, 6]. Furthermore, rediscovery of antiseptic procedures may be necessary to prevent infections and antibiotic usage [7]. Apart from that, patients with clinical infections have limited other alternatives for treatment [3].

In conclusion, research shows that nosocomial infections have a substantial negative impact on horse clinics, and issues related to antibiotic resistance and infection management must be addressed. Placing a particular focus on preventative measures and newly available treatment options is an intention to further investigate the understanding of nosocomial infections in horse clinics as it stands today. In this work, nosocomial infections caused by methicillin-resistant *Staphylococcus aureus* were retrospectively investigated to better understand the risk factors and characteristics of related diseases in the equine clinic environment.

3. Materials and Methods

This section describes the approach used for conducting a narrative literature review. A comprehensive search strategy was implemented to collect relevant scientific articles, books, and other scholarly resources. Various electronic databases were systematically searched, including PubMed, Google Scholar, and specific academic journals relevant to the subject matter. Criteria for inclusion and exclusion were defined to ensure the selection of appropriate research. Therefore, it was essential to focus on the significance of nosocomial infections in equine clinics and find examples in human and small animal medicine to make meaningful comparisons. Moreover, information about epidemiological aspects was included, with key points covering the microbiological properties of *Staphylococcus aureus* and typing aspects. Furthermore, thematic analysis and comparative assessment were applied to structure and classify the information derived from the literature. The objective of this method is to provide a comprehensive understanding of existing knowledge and identify gaps in current research and explore prevention and outlook in this field.

4. Overview of the pathogen

Staphylococcus aureus (*S. aureus*) is a gram-positive and facultative anaerobe, non–motile, cocci-formed bacterium. It can be found as natural component of the skin and mucous membrane flora in both human and animals [8, 9]; approximately 30% of the global human population becomes resident to *Staphylococcus aureus* [8, 10]. While the membranes of the upper respiratory tract are the typical site of colonization, the mucous membranes of the lower urinary and digestive tracts can also be colonized. Despite its normal habitat, pathogenic infiltration of tissues by *S. aureus* can develop and results in the formation of several diseases; ranging from minor skin infections to more serious invasive infections such as sepsis, pneumonia and endocarditis [9]. Usually, *S. aureus* is a pus-forming pathogen and may infect almost any part of the body [11].

Methicillin, a penicillinase-stable ß-lactam antibiotic, became publicly accessible in 1959 firstly [8]. Since then, the development of MRSA strains that are resistant to many kinds of antimicrobial agents, including ß-lactams and even the most recent antibiotics, followed shortly after [8, 12]. This presents a problem for veterinary medicine nowadays [9]. Consequently, it is essential to understand the microbiological properties of the bacteria in order to prevent transmission from veterinary professionals to animal patients.

Among Staphylococci, *S. aureus subspecies aureus* is widely regarded as the most significant human pathogen [13]. Mucous membranes and moist parts of the skin, such as axillae and perineal area, are typically the places where pathogenic staphylococci are carried. According to studies, 20% of humans have *S. aureus* in their noses permanently [9]. In horses and other animals, the mucous membranes of the nares also represent the major site of carriage [14]. Interspecies spreading of staphylococcal strains between animals and people is rare but noteworthy [8, 15]. This is an important aspect for the transmission of antibiotic-resistant bacteria, as it will be discussed in the following [9].

One common way *S. aureus* infections arise is through the spread from the nasal vestibule to other parts of the body that do not normally host it as commensal organism. Predisposing factors, such as trauma or immunosuppression, can increase the risk of colonisation and tissue invasion [9]. As mentioned previously, transmission from human to human or animal to human, and vice versa is rare but possible and must be considered, especially in medical care facilities [13, 16]. In these settings, *S. aureus* infections affect patients with underlying

illnesses, leading to higher rates of morbidity and mortality compared to the general population [13].

4.1. Pathogenesis and virulence in MRSA

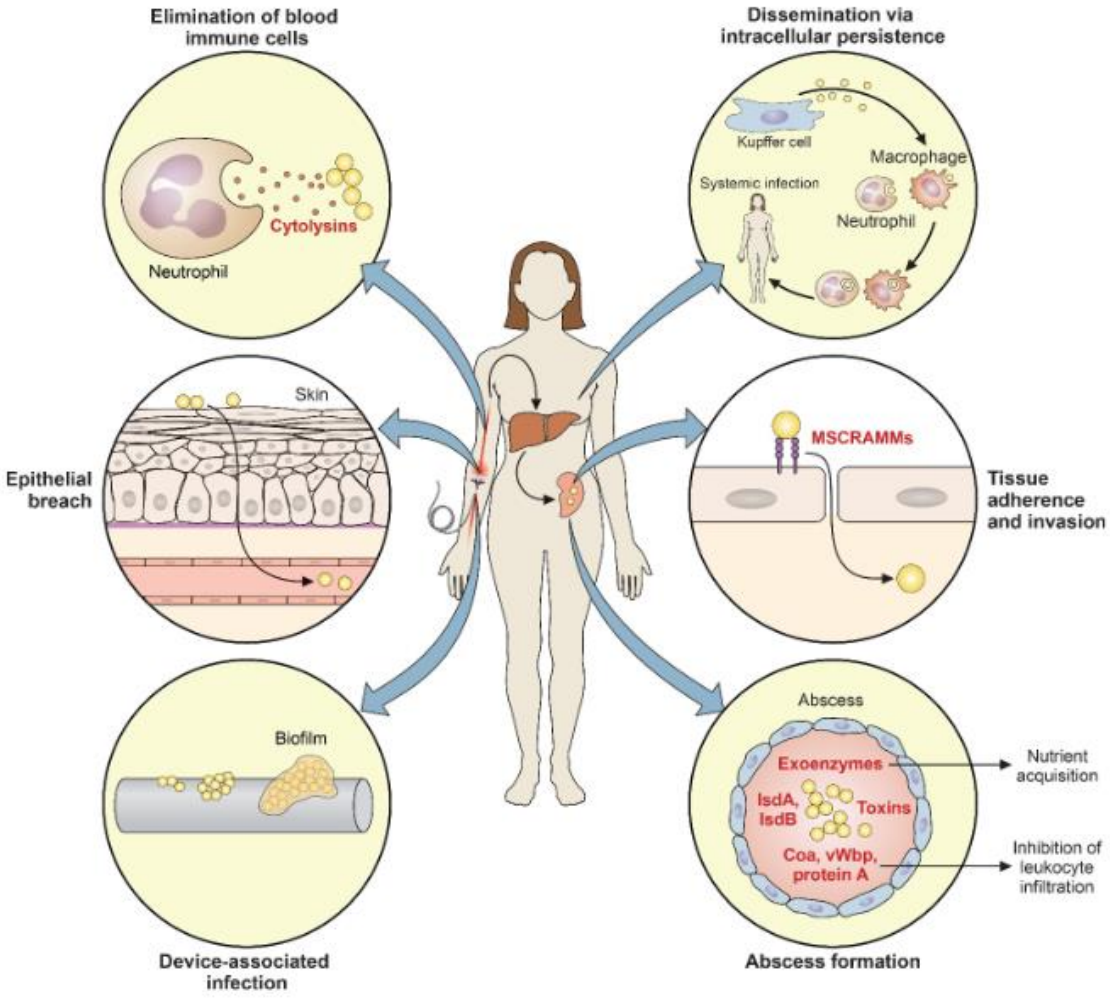

Figure 1: Phases in the development of a systemic infection caused by *S. aureus*, Gordon Y. C. Cheung [17]

Staphylococci show a variety of virulence factors that are responsible for their pathogenicity (Figure 1) [10] and their accompanied ability to evade the tissue and immune response of the infected organism (Figure 2) [9]. If natural barriers or defence systems, like skin or mucus membranes, are damaged or weakened, infections and disease outbreaks can occur [11]. Virulence factors enable the penetration of *S. aureus* for replication in non-phagocytic cells of the organism [11].

Generally, bacterial virulence factors include toxins and biofilm production as well as structural features of the cell wall that protect the bacteria against phagocytosis [9].

Neutrophils are part of the primary host defence against *S. aureus* [17] and typically, this cells are the targeted cells of bacterial virulence factors [10, 21]. Weakened host defence mechanisms are taken as advantage for invasion of pathogens and cofactors such as drugs, radiation, and exogenous pathogens may additionally damage this mechanism and dissolve the tight junctions of the epithelial layer, allowing bacteria to enter the interstitial space [9]. In the process of a physiologic antigen reaction, the epithelial cell of an organism recognizes pathogens and activates immune responses. If pathogenic invasion of microorganisms takes place, macrophages, neutrophil granulocytes, and other leucocytes cannot recognize the bacterial cell as antigen [9]. The bacteria can persist within the host due to a peptidoglycan layer that hinders the process of opsonization [10, 19].

In the context of skin infection, the primary role of phenol-soluble modulins (PSM) is to eliminate leukocytes, facilitating the evasion of *S. aureus* from the host's immune defence mechanisms [19]. PSMs are able to alter the natural state of cells, leading to cell lysis [10].

Also, the bacterial cytolytic enzyme hyaluronidase and the exotoxins, namely α-haemolysin and Panton-Valentine-leucocidin (PVL), damage host cell membranes and inhibit the physiologic in- and efflux of ions into the cytoplasm [9, 10]. α-haemolysin is a toxin that attaches to target sites to form pores in different kinds of cell types [10, 17]. *S. aureus* within a cell triggers tissue deterioration by initiating host cell death [10], and as a result, host cell membranes degrade, leading to the necrosis seen in a variety of skin disorders [10, 20].

The main adhesion virulence factor of *S. aureus,* clumping factor A (ClfA), allows the connection to host cells [10, 22], such as epithelial, fibroblast, and osteoblast cells. ClfA activates bacterial adhesion through to platelet aggregation and bacterial accumulation [10, 23].

Staphylococci are capable of releasing superantigens, which are exotoxins that challenge the adaptive immune response [9]. They induce tissue damage due to an excessive and uncoordinated release of pro-inflammatory cytokines [9]. Systemic effects such as fever, shock, and multiple organ failure can occur. The consequence of the interaction between pathogen and the host varies from asymptomatic infection to fatal diseases. The extent of bacterial virulence and the effectiveness of the host response determines the severity of the infection [9].

The genes responsible for these virulence factors are not expressed constitutively; instead, they are activated when needed [9] and the distribution of these genes is not identical in all strains. So, it is important to highlight that the majority of diseases caused by a given pathogen are caused by a limited number of clones. This indicates, that the relative virulence differs among clones and therefore, some lineages may be inherently more pathogenic than others [9].

Further complications can occur due to the formation of a biofilm in chronic wounds. These so-called "antimicrobial barriers", formed by various microorganisms, significantly complicate therapeutic intervention [2, 24]. The development of new therapeutic approaches to effectively treat established *S. aureus* biofilm-associated infections has been the focus of a recent study [10, 25]. Biofilms cause higher resistance of bacteria to both the body's defences and antibiotics. The treatment's goal here is to remove these conglomerates, and only then can further therapeutic steps be taken [2]. In order to address these challenging illnesses, this study has resulted in the creation of phytochemicals, enzymes, sulfhydryl compounds, nanoparticles, antibodies, and metal chelator [10, 25].

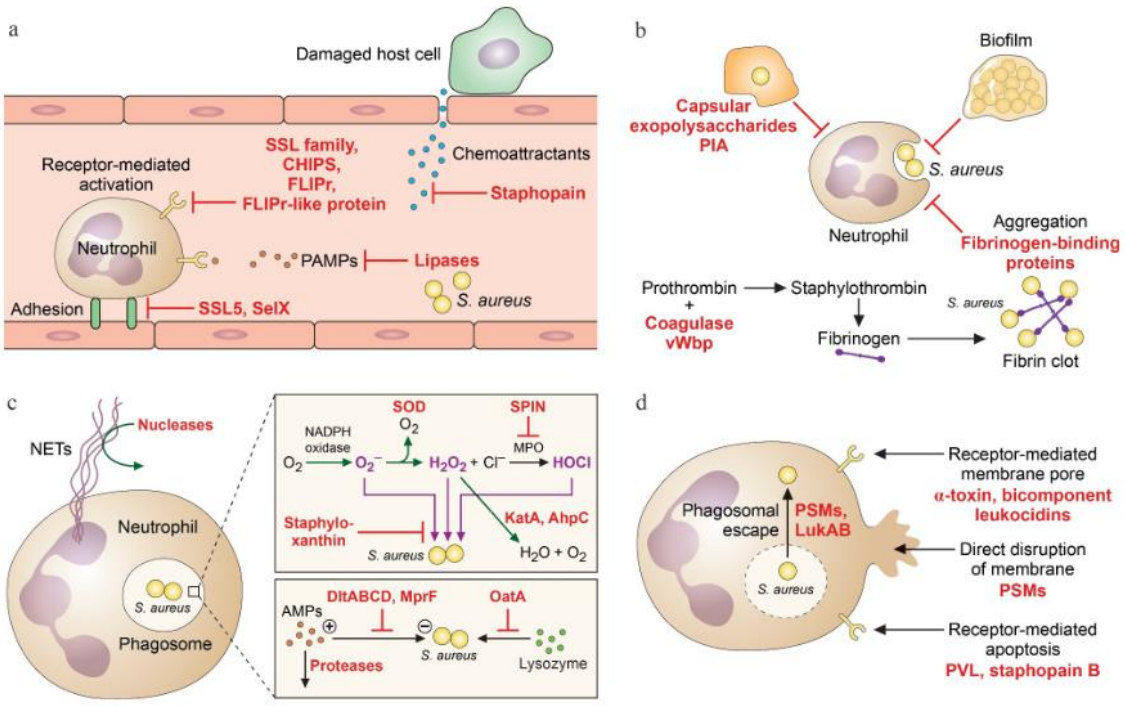

Figure 2: Immune evasion by *S. aureus*, Gordon Y. C. Cheung [17]

5. Epidemiology of MRSA

5.1. HA-, CA-, LA- associated MRSA

Each MRSA variant arises from genetic changes, such as the acquisition of resistance genes, virulence, and host adaptation genes. These changes, combined with selective pressures like antibiotic use, allow clones to proliferate in healthcare, community, and livestock settings [24].

Hospital-associated (HA-MRSA) "refers to cases where MRSA is identified more than 48 hours after admission to a healthcare facility, or in individuals with a history of MRSA infection or colonization, as well as a history of admission to a healthcare facility, dialysis, surgery, or insertion of indwelling devices in the previous year's", epidemiological definition by the Centers for Disease Control and Prevention (CDC) [24]. The prevalence of HA-MRSA has been steadily rising since the early 1980s, becoming a growing public health concern [25]. The emergence of multidrug-resistant HA-MRSA strains are common and often have resistance to numerous antibiotics. The initial documentation of MRSA infections in animals dates back to the early 1970s, with a reported case of bovine mastitis in Belgium [27, 28].

Community-acquired MRSA (CA-MRSA) infections in humans, which are not associated with hospitalization, are a significant problem worldwide [9]. CA-MRSA "refers to cases where MRSA is identified in the outpatient setting or within 48 hours following hospital admission in an individual with no prior medical history of MRSA infection or colonization, admission to a healthcare facility, dialysis, surgery, or indwelling devices in the past years" (CDC) [24]. CA-MRSA may cause skin and soft tissue infections more often and [25] it is noteworthy that CA-MRSA strains differ from HA-MRSA strains in essential ways [8]. Phenotypic and molecular characterization of CA-MRSA isolates revealed differences from the original HA-MRSA clones [25]; CA-MRSA strains have a different set of virulence factors than HA-MRSA isolates, and multidrug resistance is more common [13, 29]. The isolates exhibit increased virulence, often produce Panton-Valentine-leucocidin, and exhibit higher agr activity, which is a regulator that controls the expression of most genes encoding *S. aureus* [25]. Nevertheless, currently there are beliefs that the conventional classification of MRSA into HA-MRSA and CA-MRSA is no longer applicable. Due to the remarkable overlap of identical clones within these groups, the classical differentiation is no longer

meaningful. It has been suggested that these CA-MRSA clones may replace HA-MRSA strains [24].

The growing recognition of human MRSA infections caused by livestock-associated (LA-MRSA) is evident, and these lineages are distinct from CA- and HA-MRSA based on their genome [27, 30]. “LA-MRSA does not have a precise definition but is usually of the CC398 lineage in Europe, often CC9 in Asia” (CDC) [24]. Acquisition is generally through occupational contact with animals in husbandry [26, 31]. Infections from LA-MRSA can manifest in people who work with farm animals in industrial farming settings. Particularly within pig and poultry production systems, such as farmers, veterinarians, and slaughterhouse workers. The level of risk is determined by both the extent and intensity of contact [24].

In general, to prevent and treat MRSA infections, a thorough understanding of the differences between these strains is required [13]. In response to the emergence and global spread of MRSA international surveillance systems, such as the Centro de Epidemiologia Molecular initiative (CEM), have been established [25].

5.2. Molecular typing

The *S. aureus* population has a clonal structure dominated by clonal complexes (CC), each containing a variety of clonal lineages or sequence types [13]. This system is used for assigning CCs in multilocus sequence typing (MLST) [25]. Integrating whole genome sequencing (WGS) technology as whole genome-MLST (wgMLST) into routine health diagnostics offers the potential to rapidly provide information on drug resistance, virulence, host adaptation, and outbreak details. This improvement could be important for patient management, infection control, and biosecurity measures [24].

Although, staphylococcal antibody serological testing has been used in the past, it does not provide precise specificity for the diagnosis of the majority of staphylococcal diseases. As a result, cultivation and species-level identification of the pathogen is the gold standard diagnostic method for staphylococcal infections [13]. Moreover, this technique allows the comparison of data between different geographical regions, which can be valuable for evaluating transmission patterns [25].

The choice of subtyping technique is based on the specific data that needs to be subtyped, as well as the available technology. MLST has demonstrated its reliability among various

subtyping techniques [13]. Presently, it is the preferred sequence-based method to evaluate the genetic relatedness of *S. aureus* isolates, which involves sequencing segments of seven housekeeping genes to generate a sequence type (ST) [13, 32]. Isolates that are identical in at least five out of seven alleles are classified into clonal complexes (CC) [8, 33].

In the so-called spa typing, point mutations in region X on the cell wall protein A are detected, which is a region of the spa gene [8, 11, 32]. This technique is also considered to be one of the most preferred typing technique due to its low cost, fast execution, and effective selectivity [8].

Isolates obtained from sterile sites, like blood and aspirated pus, are generally considered clinically significant because the contamination of samples under aseptic conditions is rare [13].

MRSA arose from the methicillin-sensitive *Staphylococcus aureus* (MSSA) lineage through the acquisition of the chromosomal component of the staphylococcal cassette (SCC) [24]. MRSA has acquired the SCC containing a specific gene (mecA or mecC) that is necessary for the methicillin resistance [2, 35]. The resistance is mediated by the presence of the mecA gene, which encodes a penicillin-binding protein 2a (PBP2a). It is an additional transpeptidase, that takes over the essential cross-linking step, substituting for the inhibition of the original enzyme [11] and ß-lactam antibiotics cannot bind to this protein [2].

This SCCmec gene type can be used to distinguish strains belonging to the same MLST or spa type [13]. The elements of the SCCmec are large segments of DNA containing a variety of genes, including those encoding resistance to antibiotics aside from methicillin [26, 36]. In instances where MRSA isolates share a common genetic heritage but are thought to have diverse epidemiological origins, subtyping of SCCmec could provide further information [13]. So far, SCCmec variants I to XI have been reported [26, 36]. SCCmec II and III confer multidrug resistance, meaning resistance to at least one antibiotic in more than three classes, whereas types I, IV, and V only confer resistance against ß-lactam antibiotics. SCC IV is the most common, likely due to its small size and resulting better transferability from MSSA [11].

CA-MRSA isolates carry smaller SCCmec types, which allows them to spread more easily due to their enhanced capacity to colonize multiple body sites [13]. The clones of CA-MRSA commonly possess SCCmec elements of type IV or V and frequently test positive for the PVL toxin [13, 26].

5.3. Carriage rates in- and outside the equine clinics, ways of transmission and role of staff

One objective of this literature review is to analyse the epidemiology of MRSA, with a specific emphasis on the contribution of equine clinics to the carriage and dissemination of its strains. Evaluating data is crucial for developing efficient measures to manage the transmission of MRSA within human and animal populations. This part will involve assessing the carriage rates of MRSA within and outside of clinics, pointing out the duration of colonization and carriage rates, as well as reviewing recent studies on antimicrobial resistance characteristics.

Infections caused by MRSA in equine clinics are similar to those that occur in human hospitals, and it can also appear in the community among people with no risk factors for MRSA [8, 27]. Within the setting of an equine hospital, strains can persist for several years, with staff and environmental factors primarily contributing to their spread [8, 37, 38]. The potential for transmission of MRSA between horses and personnel is fundamentally the same as in human and small animal medicine. Colonization typically occurs unnoticed generally without the manifestation of clinical symptoms [2, 39–41]. Strains that are found in equine clinics may only be sporadically sampled in the general horse population and among person who interact with horses outside of the hospital setting [8, 42, 43]. Special attention is given to livestock-associated MRSA CC398, which is primarily associated with occupational exposure [25, 42] but is highly pathogenic [25].

Equine strains of MRSA were first reported to colonize the nasal passages of veterinary personnel in 1999 in a North Carolina veterinary teaching hospital [45, 46]. It is likely that certain STs and CCs have an animal reservoir, but these strains seem to have less host specificity and can colonize a variety of species [45, 47]. So, the transmission through nasal colonisation of humans caring for horses is also possible [43]. Consequently, animals carrying MRSA belonging to these CCs serve a reservoir for zoonotic infections in humans. MRSA strain CC398 can be transmitted via animal-to-animal contact or via iatrogenic route due to inadequately disinfected hands of veterinarians and veterinary staff.

The findings suggest that MRSA is transmittable between horses and humans, and both parties can be asymptomatic carrier [8]. Some MRSA strains from humans are able to adapt to new animal hosts, either by eliminating virulence factors that are not needed in the host, or by acquiring new genetic elements [25].

As mentioned earlier, people working in animal husbandry are at higher risk of exposure to MRSA. Therefore, it is essential to acknowledge the potential occupational health risks for veterinary personnel and other workers within the units [25]. Although horse practitioners have a lower rate of MRSA carriage compared to farm workers, it is essential to prioritize biosecurity [25]. This should include a specific focus on maintaining personal hygiene while handling patients at risk of carrying MRSA to prevent zoonotic infections.

A study published in 2009 revealed that MRSA can be carried by the personnel working in horse clinics, with carriage rates between 9.4-27.8%, and even higher rates among staff members in regular contact with horses [14]. The most common equine lineage among 13,756 human-originated MRSA isolates is CC398, accounting for 3.9% of those analysed in this survey. The carriage rates of CC398 among employees can reach up to 60 to 80% [8, 48, 49]. However, the infection is typically transient, and spontaneous decolonisation occurs [8, 50]. Comparable incidence rates are also observed among veterinarians outside the equine hospital environment [8, 42].

It is also possible that MRSA may be transmitted from person to person, supported by evidence of infections in humans without any animal contact and cases of transmission from veterinarians to their family members [45, 51]. Moreover, it is crucial to mention that staff can transmit MRSA between patients, not only within a single clinic but also between different healthcare institutions [52, 53]. For this reason, veterinary personnel must exercise caution and follow strict protocols to prevent further transmission to limit the spread of MRSA and protect human individuals. Even though decolonizing infected staff members may appear to be a practicable solution, studies have demonstrated its unreliability due to the rapid recurrence of colonization following treatment [39, 52]. Implementing strategies such as the use of disposable gloves, regular hand washing and disinfection between patients can significantly reduce the incidence of cases once MRSA has become established in a healthcare setting [52, 54].

5.4. Equine MRSA infection

Staphylococcus aureus, particularly methicillin-resistant *Staphylococcus aureus*, is known to cause a spectrum of infections in horses, including skin and soft tissue infections, septic arthritis, osteomyelitis, implant- and catheter-related infections, and also pneumonia [53].

The first reports of MRSA isolates causing nosocomial infections in horses were documented in Japan and the USA in 1997 [45, 56, 57]. Subsequently, investigations at an Austrian university veterinary hospital revealed MRSA strain CC398 in 2006 [52, 58], and similar cases have been described in the Netherlands, Germany, and in other European countries such as Belgium, Switzerland, Spain and in Hungary [43].

According to the only available publication on the matter, the median duration of carriage of MRSA in horses after infection is approximately 143 days, but it varied widely, ranging from 55 days to as long as 711 days. Even though some horses may test negative between two to four months after infection, some horses can develop persistent colonisation, meaning they can continue to carry and spread MRSA bacteria even after symptoms have resolved [8, 59].

Horse-associated staphylococcal strains have been shown to encode different, rather host-adapted versions of certain virulence genes. Such virulence factors in equine MRSA are, for instance, the "von Willebrand factor binding protein" (vWbp), which interferes with the coagulation cascade, the equine-related version of staphylococcal complement inhibitor protein (SCINeq), and various exotoxins that share structural similarity to the human-associated virulence factors [8, 60]. These virulence factors in MRSA increase the efficiency of the pathogen and their existence indicates steps towards a better host adaptation of MRSA [8].

Typing MRSA CC398 strains from equine clinics has revealed a distinct set of this characteristics, with spa type t011 frequently observed as the predominant strain in the Austrian veterinary teaching hospital [45, 61]. The first reported isolate from infections in a veterinary hospital for horses in Ireland, and other countries such as Australia and Canada, were the MRSA ST8, t064, IV strain. It is believed that this strain was introduced into the hospital and quickly spread to other facilities [45, 62]. During the mid-2000s, outbreaks of nosocomial MRSA infections were observed, with the most common strain being MRSA ST254, t009/ t036 in Central Europe [43]. Interestingly, strains differ in their resistance profiles to antibiotics and adapted over time [50]. Since then, these successfully adapted lineages have become increasingly prevalent and are now emerging as the dominant MRSA strains, often replacing others. This hypothesis is supported by resistance profiles from isolates tested at the Department and Clinic of Equine Medicine (DCEM) in Üllö, Hungary. The analysis revealed very broad resistance pattern that has widened over time [50].

5.5. Antibiotic usage

Like all domesticated animal species, horses have also benefited from the introduction of antibiotics for the treatment of bacterial infections [2, 63], but CC398-MRSA infections exhibit widespread antimicrobial resistance to antibiotics commonly used in equine medicine [8]. After the discovery of penicillin, *S. aureus* could initially be effectively controlled. However, due to increased use, a series of resistances occurred [11].

Methicillin-resistant *S. aureus* is generally resistant to almost all ß-lactam antibiotics and often presents resistance to other antibiotic classes [43]. Within the CC398 clonal complex, the equine hospital-associated subtypes frequently show gentamicin resistance, while fluoroquinolone and streptomycin resistance are less frequently reported [50]. Studies have examined susceptibility to chloramphenicol and rifamycin, but information available on the occurrence of rifampicin resistance is limited [50], and further investigations will be necessary.

The DCEM at the University of Veterinary Medicine in Budapest, Hungary, conducted monitoring and surveillance to assess the emergence and prevalence of antimicrobial resistance in clinical isolates of MRSA of equine origin. To better understand the dynamics of antimicrobial resistance in MRSA, record-keeping has been made over time, including the acquisition of resistance to different classes of antibiotics and the appearance of new resistance mechanisms. The collected data was analysed to compare and evaluate the observations [50]. The key discoveries will be summarized below.

Over time, MRSA strains sampled from patients of the DCEM have developed resistance to many antibiotics. The first MRSA strains investigated in 2011 were found to be resistant to multiple antibiotics, including penicillin, cefoxitin, trimethoprim, tetracycline, streptomycin, gentamicin, kanamycin, and ciprofloxacin. During the first MRSA outbreak in the hospital, one isolate showed additional resistance to chloramphenicol, while the last isolate was susceptible to streptomycin by the end of July 2014.

In mid-2015, during the second MRSA outbreak, several isolates exhibited resistance to chloramphenicol. The inclusion of rifampicin in MRSA therapy resulted in an increase in its prevalence, and by mid-2016, the second isolate also showed resistance to it. Besides, the study observed the emergence the resistance of phenicol in the hospital's gram-positive flora due to use of chloramphenicol. The study also documents the simultaneous development of

resistance to both antibiotics within a short period of time, likely due to significant selective pressure from antibiotic use and incorrect dosing. The occurrence is traced back to combination therapy of chloramphenicol and rifampicin being used as the last option for the treatment of MRSA infections at DCEM.

In conclusion, the limited therapeutic options emphasize the significance of cautious antibiotic use and necessitate the implementation of alternative preventive and therapeutic approaches in the clinic.

6. Risk factors and clinical manifestation in horses

6.1. risk factors

Equine infections caused by MRSA can affect multiple body sites and present an unspecific variation of symptoms. As these colonisations are primarily opportunistic, horses with MRSA infections generally have a good prognosis and tend to survive until discharge [53]. All in all, equine MRSA infections are curable diseases that need to be handled responsibly and urgently to ensure their cure.

Several variables have been identified in equine medicine as significantly associated with the non-survival of horses infected with MRSA, and these are generally known as risk factors that manifest an infection. They include the use of intra-venous (IV) catheters and infections on surgical sites (SSI) from routine procedures, among others [53]. Many of these infections may have originated from iatrogenic contamination during joint injections and investigations requiring arthrocentesis [53]. There have also been reports of MRSA infections occurring outside the clinical environment, spreading to other regions of the body, which also serve as risk factors.

In general, animals that have previously been hospitalized may be more susceptible to acquire MRSA outside the hospital environment. This could be due to a compromised immune system resulting from a medical condition or as they are recovering from a chronic illness [53]. In case of companion animals, the use of surgical implants has also been identified as a risk for an infection [62]. On top of that, these animals might be undergoing antimicrobial therapy or have recently been transported, which can be stressful for the patient

[53]. To prevent and treat MRSA infections in their patients, veterinarians must consider these factors and implement the necessary precautions [53].

Magalhaes [62] has conducted a case-control study focused on the risk factors for MRSA infection in dogs and cats. This research explores the factors contributing to MRSA infections, shedding light on their implications for both animal and human health. The key findings highlight several important aspects.

Firstly, pets having contact with humans who have been ill and hospitalized are at higher risk of being infected with MRSA. Secondly, challenges like overcrowding and insufficient staff in human hospitals can compromise the effectiveness of MRSA control programs, potentially leading to spread of infection within the community [64, 65]. In addition, close social interaction between companion animals and humans, whether at home or in veterinary practices, is a significant factor in MRSA transmission [64, 66]. Documented cases show MRSA strains transferring between different species within households [64, 67].

Graffunder and Venezia [66] also consider the length of the stay in clinical conditions as a factor that increases the chance of contracting MRSA in human medicine. Conversely, the infection might be the cause for a delayed discharge. Their investigations, based on a study with 121 patients, more ward changes, especially among patients from the intensive care and the rehabilitation units, are associated with a higher risk [68, 69]. The research also revealed that enteral feeding and urinary catheterization might be reasons, as well as the prolonged use of specific devices [66]. Diseases affecting the cardiovascular system, kidney diseases and diabetes appear to be foremost cofactors just as chronic illness are. As we know, the performance of surgical interventions pose potential risks in both veterinary and human medicine [66]. When the patient's immune systems function is decreased, it may lead to a compromised host defence and, therefore, creates a portal for microorganisms. In this context, surgical techniques and post-operative care are noteworthy [66].

The majority of literature agrees that using antimicrobial medications wisely is one of the most important strategies in minimizing the growing problem of antibiotic resistance, along with appropriate infection control procedures [66].

6.2. clinical manifestation

A retrospective study was performed by Anderson et al. [53] between 2000 and 2006 to evaluate the characteristics and outcomes of the disease in 115 patients across six equine hospitals in the USA and Canada; the main aspects will be discussed as follows.

Among the 115 infected horses evaluated, the age range varied from new-born to thirty-one years, with a median age of four years. Surgical incisions were the most frequently reported site of infection, with horses used for breeding being the most commonly infected, followed by non-racing performance horses and racehorses. The most common symptoms for admission and treatment of the patients were colic, wounds, and incision infections. Only one case of MRSA bloodstream infection was documented. Overall, 83.8% of the cases survived to discharge, indicating a high survival rate. Farther, the study discovered that higher infection risk was associated with horses with comorbidities, a finding confirmed in human medicine as well [55, 68]. The factors that were linked to CA-infections included having an incision infection, previous gentamicin treatment thirty days prior admission, and having been hospitalised within thirty days before admission [53].

The study found that 50.9% and 49.1% of the cases were attributed to infections that were either healthcare- or community-associated, respectively. It was discovered that hospital-associated infections could manifest anywhere from 48 hours to 170 days following admission, with a median duration of six days. It is worth noting that there was no significant difference in the survival rate between hospitals- or community-associated infections. In summary, the study demonstrates that both HA- and CA-infections are prevalent, but survival rates do not appear to be significantly affected the type of infection or the length of the hospital stay [53].

Almost always, the infections caused by MRSA exhibit the same symptoms as infections caused by methicillin-sensitive *Staphylococcus aureus* (MSSA) infection [68]. The spectrum ranges from minor skin and soft tissue infections to bone and joint infections, and even acute bacterial endocarditis [68]. In both human and animal patients, a common clinical sign is surgical wound infection, which affects the stratum basale of the dermis. The spread of the pathogen via bloodstream may lead to primary or secondary bacteraemia [68]. The formation of abscesses within the pelvic and abdominal cavity, as well as inflammatory responses in joints and bones resulting in osteomyelitis, are considered possible complications but are less common compared to surgical wound infections [70, 71]. The respiratory system can

also be affected, but nosocomial pneumonias, lung abscesses and infections of the chest wall have minor significance [68].

By analysing the data from these cases, we hope to improve the understanding of the epidemiology, risk factors, and clinical manifestations of MRSA infection in horses. This information may lead to the development of more effective strategies for the prevention and treatment of this influential equine disease.

7. Prevention

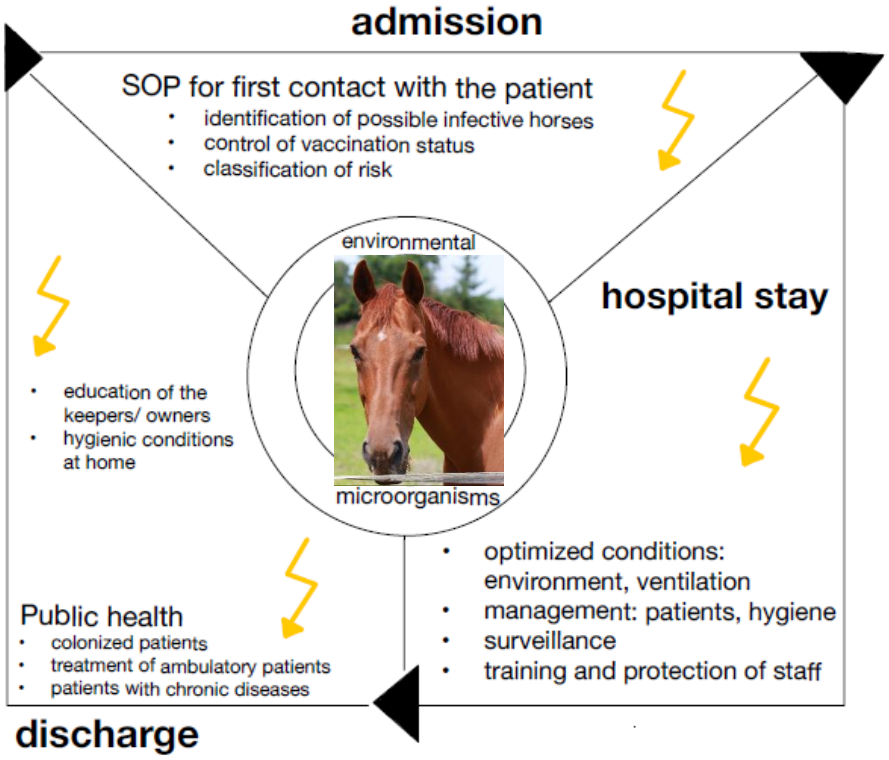

Figure 3: Infection prevention in horse clinics: Measures and influencing factors

The German 'Infection Protection Act' (IfSG, 2011), aims to implement such measures [2]. The application of multimodal infection prevention concepts, as well as continuous active surveillance, is required. The veterinarian is responsible for realizing biosecurity procedures during treatment and the entire hospital stay, to minimize the threat posed by multidrug-resistant pathogens to humans and animals [70]. Thus, better preventive hygiene management is essential. This is intended to avoid nosocomial infections and the spread of infectious agents to the patients. To achieve long-term reductions in hospital-acquired infections in the practice or clinic, different approaches and perspectives must be considered.

Explaining the relevance to veterinarians and their assistances' is the aim to raise awareness and sensitivity to the importance of MRSA and its transmission. Sensitizing non-veterinary staff to this issue is also necessary; spreading of pathogens through the hands of veterinarians and staff is a critical point [72, 73]. Since not only hands act as vectors for transmitting pathogens, but also the veterinary equipment used, such as stethoscopes, nasogastric tubes, endoscopes, clippers and their heads, twitches, endoscopy instruments, thermometers and more [72]. Therefore, it is essential to adhere to the standardized guidelines of the "Gesellschaft für Pferdemedizin" (GPM) [72], the latest of which were issued in 2019. The problems listed above can be improved by regularly washing and disinfecting hands, wearing non-sterile disposable gloves [72, 75] and protective aprons, as well as continuously cleaning and disinfecting surfaces, counters, and equipment. For inpatient clinic patients, each horse should have its own equipment, including halter and lead rope, noseband, muzzle, and grooming tools. After discharge, this equipment should be cleaned and disinfected by soaking it in an instrument disinfection solution [72].

It is noteworthy, that specific hygiene and infection prevention measures for equine clinics is not yet satisfactory due to species-specific requirements: patient size, stable ventilation, bedding, and feeding [70]. Compliance with basic hygiene using "Standard Operating Procedures" (SOPs) [74] should be a matter of course. Hence, it is recommended, especially in large clinical settings with a high risk of infection, that a qualified infection control manager should be assigned to establish, monitor, and continuously improve infection management, hygiene, as well as conduct regular training sessions [72, 77]. To ensure compliance with appropriate hygiene standards, this training should be conducted at least annually with all employees [70].

Moreover, collecting infection data can effectively reduce infection rates, especially those involving MRE. This may help to reduce the often incorrect use of antibiotics, which leads to antibiotic resistances. Data collection, i.e. recording postoperative wound infections with MRE, such as MRSA. Besides, catheter-associated infectious thrombophlebitis can be used as indicators or measurable parameters for outcomes [70].

Summarized, the key points of basic hygiene are education and training of clinic staff, owners and visitors to ensure a low-bacteria environment. Examples from human medicine prove that the efforts can lead to a reduction in the number of preventable nosocomial infections and ease their potential consequences [70].

8. Outlook

Recent publications on infection control in equine clinics have demonstrated that creating an individualized intervention program can significantly reduce the incidence of MRSA in these clinics. However, current data also suggest that up to 3.5% of horses admitted to veterinary clinics may carry MRSA in their nasal passages [72, 78].

Targeted hygiene management is based on the classic principles of risk management outlined on the websites of the Robert Koch Institute (www.rki.de), the Federal Institute for Risk Assessment (www.bfr.bund.de), and the European Food Safety Authority (www.efsa.europa.eu) [2]. As part of the interdisciplinary network "MedBVe-Staph", funded by the German Federal Ministry of Education and Research, hygiene concepts for equine clinics will be developed over the next two years by Subproject 8 within the Department of Veterinary Medicine at the University of Berlin. This development will follow an evaluation of practical measures to enhance biosafety, infection prevention, and antibiotic stewardship [2].

Certain concepts encompass these aspects with through a "search-and-destroy" strategy [11]. In this method, the pathogen is initially searched for and then managed by eradication. This involves the identification of risk groups, the implementation of isolation procedures, regular monitoring of carriers, and the decolonization of positive tested individuals. The measures put in place have shown notable success in reducing the MRSA burden in the environment, all achieved with relatively manageable efforts. In the Netherlands and some countries in Scandinavia, this approach is the norm in human healthcare and has led to a low incidence of MRSA, as well as reduced antibiotic use [11]. As it stands today, equine clinics have also effectively adopted screening programs [11].

9. Conclusion

To conclude, colonization rates of multi-resistant organisms are notably higher among veterinarians and their staff compared to the general population. The adaptability of MRSA to human-altered environments and its capacity to move between different host species emphasizes the complexity of controlling its transmission, highlighting the necessity for comprehensive strategies to mitigate its impact. It is crucial to note that prudent antibiotic use plays a critical role and should be a targeted objective to stop MRE proliferation effectively. Implementing strict preventive measures such as thorough hand disinfection prior each patient interaction, consistent use of disposable gloves, and the isolation of MRSA-positive horses are essential strategies. Nevertheless, despite the known preventive measures, their optimal execution remains a challenge in practice. Factors like high workload, time constraints, and various other reasons often hinder their effective application. Future efforts should concentrate on overcoming these obstacles and improving compliance with preventive protocols to manage MRE colonization rates. While the introduction of MRSA through incoming horses cannot be entirely prevented, protective measures should be implemented to hinder its spread.

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Thesis progress report for veterinary students

Name of student: ……LINKE JULIA……..…….

Neptun code of the student: ……JKHCOJ.....

Name and title of the supervisor: ……Dr. Ervin Albert, junior lecturer……

Department:……Department of Parthology……

Thesis title: …Characteristics of hospital-associated infections in horses caused by methicillin-resistant *Staphylococcus aureus*…….....

…….....

Consultation – 1st semester

Timing				Topic / Remarks of the supervisor	Signature of the supervisor
	year	month	day		
1.	2023	03	07	Regular consultation on the structure and content of thesis	
2.	2023	03	18	Online consultation on details regarding literature review	
3.	2023	04	04	Email correspondence	
4.	2023	05	06	Email correspondence	
5.	2023	06	21	Email correspondence	

Grade achieved at the end of the first semester: 5 (excellent)

Consultation – 2nd semester

Timing				Topic / Remarks of the supervisor	Signature of the supervisor
	year	month	day		
1.	2023	09	30	Consultation	
2.	2023	10	10	Consultation via email on final edits	
3.	2023	10	28	Final checks and edits	
4.					

5.					

Grade achieved at the end of the second semester: 5 (excellent)

The thesis meets the requirements of the Study and Examination Rules of the University and the Guide to Thesis Writing.

I accept the thesis and found suitable to defence,

……...
signature of the supervisor

Signature of the student: ……

Signature of the secretary of the department: ……..……

Date of handing the thesis in…… ……..…...

I here by confirm that I am familiar with the content of the thesis entitled

„Characteristics of hospital-associated infections in horses caused by methicillin-resistatant *Staphylococcus aureus*"

written by Julia Linke (JKHCOJ) which I deem suitable for submission and defence.

Date: Budapest, 13th November 2023

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Dr. Ervin Albert
Department of Pathology