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Combating Methicillin-Resistant *Staphylococcus aureus* at Equine Clinics: Recommendations for a Future Control Program at the Department and Clinic of Equine Medicine - Literature Review

A Meticillin Rezisztens *Staphylococcus aureus* elleni küzdelem a Lóklinikákon: Ajánlások egy Jövőbeli Kontrollprogramhoz a Lógyógyászati Tanszék és Klinikán - Irodalmi Áttekintés

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2023

Abstract

Nosocomial infections, meaning those contracted while hospitalized or receiving medical attention, are an important area of research not only in human medicine, but in veterinary medicine as well. Equine Methicillin-Resistant *Staphylococcus aureus* (MRSA), and specifically ST398 in Europe, is able to affect both horses and humans in many ways, ranging from subclinical nasal carriage in both horses and those who work with them, to fatal infections in affected horses. As *Staphylococcus aureus* (*S. aureus)* is naturally part of environmental and animal flora, it easily survives in the hospital setting. This combined with its genetic ability to adapt to various antimicrobial drugs used in attempts to treat these infections, poses a great risk for equine health. There are also potential zoonotic considerations with this strain of bacteria. Given this, creating and implementing effective biosecurity protocols into equine hospitals is of great importance to eradicate MRSA from the equine hospital setting and prevent future MRSA introduction.

Összefoglaló

A nozokomiális fertőzések, azaz a kórházi kezelés vagy orvosi ellátás során elkapott fertőzések nemcsak a humán-, hanem az állatgyógyászatban is fontos kutatási területet jelentenek. A lovak meticillin rezisztens *Staphylococcus aureus* (MRSA), és különösen az 398-as szekvenciatípusa Európában, sokféle módon érinti a lovakat és az embereket egyaránt, kezdve a lovak és a velük dolgozók tünetmentes hordozásától egészen az érintett lovak halálos kimenetelű fertőzéséig. Mivel a *Staphylococcus aureus* (*S. aureus*) természetes módon része a környezeti és állati flórának, könnyen túlél a kórházi környezetben. Ez, valamint az a genetikai képessége, hogy alkalmazkodik a különböző antimikrobiális szerekhez, amelyeket e fertőzések kezelésére használnak, nagy kockázatot jelent a lovak egészségére. E baktériumtörzsnek potenciális zoonotikus vonatkozásai is vannak. Mindezek alapján a hatékony biológiai biztonsági protokollok létrehozása és végrehajtása a lókórházakban nagy jelentőséggel bír az MRSA lókórházi környezetből való kiirtása és az MRSA jövőbeni behurcolása szempontjából.

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Frequently Used Abbreviations

CA-MRSA	Community-Acquired MRSA
CC	Clonal Complex
CFU	Colony Forming Units
DCEM	Department and Clinic of Equine Medicine
HA-MRSA	Hospital-Acquired MRSA
LA-MRSA	Livestock-Associated MRSA
MRSA	Methicillin-Resistant *Staphylococcus aureus*
SSI	Surgical Site Infection
Spa	*Staphylococcus aureus*-specific protein A
ST	Sequence Type

1. Introduction

Nosocomial, or hospital acquired, infections have been a large concern for years in human medicine, and are of increasing importance in the veterinary profession as well. Aside from the risk to veterinary patients, there are also zoonotic concerns regarding pathogens like Extended Spectrum β-lactamase (ESBL) *E. coli*, as well as *Salmonella* species and Methicillin-Resistant *Staphylococcus aureus* (MRSA). If the bacterial load of these pathogens can be reduced or eradicated from veterinary hospitals, and specifically within the scope of this literature review, equine hospitals, there will be a decreased risk for zoonotic disease and patient harm. The objective of this review is to develop general recommendations which can be used as a guide for future development of specific biosecurity protocols in equine hospitals like the Department and Clinic of Equine Medicine (DCEM) for MRSA eradication and prevention.

2. Characterization of MRSA of Equine Origin

2.1 Overview of the Pathogen

Staphylococcus aureus (*S. aureus)* is a gram-positive cocci bacteria that has good environmental resistance (able to remain active in a dry environment for up to one month), naturally occurs in the environment as well as part of normal mammalian flora, present on skin and mucous membranes. It is a catalase and coagulase positive, oxidase negative, fermentative, non-spore forming, non-capsule producing bacterium that grows in irregular clusters. *S. aureus* is non-fastidious, and is easily culturable on simple nutrient agar in both aerobic and anaerobic conditions, and typically produces white or yellow pigments. Other culturing techniques can be used for its selection from samples and various other needs, and animal-infecting strains of *S. aureus* will cause either incomplete α- or complete β-haemolysis[1]. As with any facultative pathogen, once an opportunity presents itself an infection can occur, and *S. aureus'* ability to replicate both in the presence and absence of oxygen enables good wound colonization. As the development and use of antimicrobial drugs, which started with the β-lactam antibiotic penicillin, became more widely available, bacteria including *S. aureus* were selectively pressured for genetic mutations that lead to its

[1] Dr. L. Makrai, Personal Communication, September 28, 2020

β-lactam antimicrobial resistance, and the eventual development of Methicillin-Resistant *Staphylococcus aureus*, or MRSA, which is still continually mutating and becoming resistant to an alarmingly high number of antimicrobial agents. With this fast development of resistance, there is a significant need to counter it with the creation and implementation of pathogen specific biosecurity protocols to identify transmission risks and prevent the spread of this bacteria.

P.C. Appelbaum [1] describes the first strain of *S. aureus* penicillin resistance as appearing in humans in 1942, just two years after the antibiotic was introduced, and over the next two decades this resistance was found in approximately 80% of both hospital and community acquired isolates due to the bacterium's production of a specialized enzyme - penicillinase - which could disrupt the antibiotic. To combat this resistance, the penicillinase-stable methicillin was developed and used to treat these resistant infections starting in 1961. However, soon after its introduction there were reports of methicillin resistance in infections caused by *S. aureus* as well. These strains of staphylococci were then termed MRSA. This chain of events leading up to the evolution of MRSA are corroborated by a review published in 2017, which also followed MRSA development to the first reports in veterinary medicine, where it was discovered for the first time in livestock in the early 1970's in Belgium, and from there it too began to spread [2]. Since the discovery of MRSA, it has been characterized into three main types: Hospital-Acquired (HA-MRSA), Community-Acquired (CA-MRSA), and Livestock-Associated (LA-MRSA). HA-MRSA and CA-MRSA are used to describe mostly human-related infections, whereas LA-MRSA has veterinary importance. LA-MRSA has been isolated from many livestock species, including horses, and is currently a cause of infections for the patients of the Department and Clinic of Equine Medicine (DCEM), and thus will be the focus of this literature review.

Classification and typing of MRSA species in a basic sense is based on the allelic variation of seven carefully chosen and standardized housekeeping gene loci. Those with five or more identical alleles are called Clonal Complexes (CC), and within this, strains with the same seven identical alleles are placed into the same Sequence Type (ST) [3]. The genetic makeup of MRSA strains has been widely studied, and it has been found that the Staphylococcal Chromosome Cassette *mec* (SCC*mec*) is a mobile genetic element that

plays a major role in the development of resistance. The SCC*mec* carries a variant of the *mec* gene that is responsible for the development of an altered penicillin binding protein, PBP2, which is the cause of the decreased ability for β-lactam antibiotics to bind to MRSA [1]. Within LA-MRSA, the main strain of concern in Europe is CC398, and more specifically, the strain afflicting the DCEM is ST398, *spa*-type t011, SCC*mec type* IV [4].

2.2 Clinical Manifestation in Equines

MRSA infections in horses have a wide range of outcomes, from asymptomatic nasal carriage, to fatal infections. The main clinical manifestations include infections of skin and other soft tissues, metritis, osteomyelitis and implant infections, thrombophlebitis, pneumonia, and omphalitis in young foals [3]. Pneumonia, bacteraemia and septic arthritis have also been reported [5]. Throughout the reviewed literature, surgical sites seem to be the predominant site of MRSA colonization and infection. In a retrospective study from the University of Veterinary Medicine Vienna, it was found that the majority (48.5%) of reported equine MRSA infections were classified as wound infections [6], and the Equine Clinic of Bern described presentations of MRSA infections in their 2011 study as having 34 horses develop post-surgical infections and 6 that developed thrombophlebitis following catheterization [7]

2.3 Epidemiology

Albert et al. [4] found that once MRSA has been introduced to an equine clinic, it can establish a population in the environment for years, causing outbreaks of disease in patients that are susceptible. The transmission from horse to horse mainly occurs via fomites, as well as clinic workers and personnel when objects and hands are not disinfected properly between patients. The strains of MRSA found in the hospital setting are very infrequently found in horses and their handlers outside equine hospitals, suggesting that the intense selection pressures from antibiotic use in clinical practice is driving the continual mutation and adaptation to these medications.

While it is known that in the equine hospital setting, the environment, patients and clinic staff can become colonized, it can be difficult to determine the direction of transmission. An editorial by Gerrit Koop [8] summarizes a study out of Japan looking into transmission

between racehorses and veterinarians at thoroughbred training centers [9]. Each facility had separate equine hospitals and separate staff. Following nine cases of MRSA-related diseases between these two hospitals, this study was conducted, and it was found that the veterinary colonization rate was slightly over 30%, and in healthy horses at the training facilities, it was 0%. As the healthy horses were not the source of infection, they looked deeper into where the MRSA came from - with the high prevalence of veterinary colonization, were they the source, or did it originate from contact with MRSA positive horses? Unfortunately, this study did not include sampling of the clinical environment or other hospitalized patients outside the specified 9 horses. Therefore, while it was determined that both the equine and veterinary MRSA isolates were indistinguishable, the original source was unable to be determined, but the high veterinary colonization rate may suggest infections stemming from MRSA introduction via the vets working with susceptible horses.

Transmission of different MRSA clones can also occur through staff that transfer from clinic to clinic. This was proven during a study from 2011 in which, during screening, it was discovered that a new hire was colonized with a strain of MRSA that had not before been detected at their clinic but was present at this employee's previous clinic in Germany [7]. This strain was then later detected for the first time in equine patients, proving transmission from human to horse.

At the DCEM, MRSA ST398, *spa*-type t011, SCC*mec type* IV has been isolated in 36 equine patients with a total of 40 strains over a five year span, from 2011 to 2016, in two outbreaks according to an investigation by Albert et al [4]. On top of this, the investigation found that 10 strains were isolated from samples of 36 clinic personnel in 2017. All isolates in the first outbreak followed the same genotyping and with similar antibiotic resistance profiles, with the exception of the second last isolate, which showed additional resistance to chloramphenicol. In the second outbreak, the first isolated strain also showed chloramphenicol resistance, and the second isolate showed resistance to rifampicin as well. Clones of the rifampicin resistant strain were isolated another 18 times in this second outbreak. MRSA isolates from the clinic workers showed the same genotype as those from the equine patients, with mild variations in antibiotic resistance profiles. The authors also reported that this was the first time that MRSA from CC398 was isolated in Hungary from

horses, and the genetic and resistance pattern of the isolates from patients and staff show that all infections are from the same clonal lineage. They have also developed increased resistance as specific antibiotic treatments have been introduced. The authors concluded that these infections are nosocomial in nature, and hygiene regulations were implemented to try and reduce or prevent the continued introduction of MRSA to patients.

An important consideration in screening for and controlling the possible introduction of MRSA into clinics via carriers, is to take into consideration the general prevalence of carriers at the population level in the area around the clinic. Unfortunately there is little research into screening the general equine population, as much of the concentration is on human MRSA screening. One study had a detection rate of 10.9% in horses screened for nasal carriage of LA-MRSA ST398 (with either SCC*mec* type IVa or V, and *spa* type 011 or t1451) upon admission to an equine clinic in Flanders, Belgium, compared to a similar equine facility in Canada where 2.5% of the horses screened on admission tested positive for MRSA carriage [10]. The authors postulated that it may be suggestive of an endemic status of MRSA ST398 within the equine population of Western Europe. It should be noted that the equine clinics in this study are tertiary referral hospitals, and therefore may provide a more biased patient screening base than the regular equine population, but does provide a good model for clinics such as the DCEM. The stress of travel may have been a contributing factor. Moreover, Van den Eede et al. [11] reported a prevalence of 0-4.7%, varying greatly in region and specific strain. The authors state that at the time of the study, up to 55% of hospitalized horses have tested positive for MRSA in clinics that do regular testing. Due to the limited information available, it could not be concluded whether this is representative of the general population, or if this is higher due to a bias in the patients entering the clinic. With this information, Van den Eede et al. [11] began a study in Belgium to screen horse farms for the presence of LA-MRSA CC398. Out of the 373 samples taken from 189 horses on 10 farms, one sample was positively cultured for MRSA CC398, further classified as *spa* type 011, with the SCC*mec*V cassette. All farms included in the study were in the area of the previously mentioned equine clinic, with an arrival screening isolation rate of 10.9% [10]. Therefore the authors concluded that the overall general population carriage is low. Contrasting this, a study by J.F. Busscher et. al. [12] indicates no population carriage in clinically healthy horses on the European mainland.

According to the referenced literature herein, the resistance to clindamycin, ciprofloxacin, erythromycin, gentamicin, kanamycin, tetracycline, trimethoprim-sulphonamide, and oxacillin is frequent among isolates.

2.4 Special Emphasis on the Role of Equine Clinics

Equine veterinary clinics are potentially very significant amplification sites for MRSA, in part due to the nature of equine interaction. Unlike in small animal clinics, where patients are generally kept in stainless steel cages that are smooth surfaced, and more easily cleaned and disinfected, equine patients are kept in stalls, generally constructed from concrete, wood and metal, with various types of bedding from straw to rubber matting. These materials are naturally more difficult to disinfect, as they are rough surfaced, and commonly have small cracks and areas that are very challenging to reach with cleaners and disinfectants. On top of this, the surface area is much larger, and therefore more time consuming and labor intensive to clean properly between patients. This leaves room for environmental contamination that is easily passed to the next patient, potentially resulting in clinical infection.

A study from the Equine Clinic of Bern, Switzerland found that the number of MRSA-related infections increased from less than 1% to almost 16% in their patients with postoperative infection complications over a four year period [7], and with the previous mention of confirmed transmission from personnel to horse, this shows that it is important to prevent colonization and spread via hygiene and surveillance.

There have been a few reports of human clinical infections caused by equine MRSA, such as one of transmission between a previously hospitalized foal, which caused a severe infection in its owner [13], and one report out of Canada where a veterinarian developed a tattoo infection caused by the same strain of MRSA that was affecting two of his patients [14]. However the vast majority of studies show little to no risk of equine MRSA causing infections in humans [5], and a study out of Germany found that only 0.14% of human MRSA infections were attributed to CC398 associated with equine clinics [10]. This indicates that while this is a significant pathogen within equine hospitals, it is not a very significant zoonotic agent.

3. Options of MRSA-Infection Prevention and Control In Equine Clinics

3.1 Frequency of Carriers

A suspected case of MRSA ST398 transmission from a foal to a young Dutch girl resulting in a wound infection was reported in the Netherlands [13]. While this girl had been hospitalized various times due to various medical conditions, the strain isolated from her wound was not one that was present in her place of hospitalization, however, the exact strain was isolated from the nares of her clinically healthy foal, which, due to its own wound infection, had been hospitalized about two months before. The girl was successfully decolonized with the use of mupirocin ointment applied to her nares and perineum three times a day for five days, seven days of oral fusidic acid and rifampin, as well as washing with chlorhexidine shampoo three times a day for five days. The foal was repeatedly monitored with culturing, and was confirmed MRSA negative within three months of detection, without any decolonization therapy. The most likely cause of this human infection is transmission of the bacteria from the foal, which was likely initially colonized while hospitalized. Therefore, while being a rare occurrence - only 2 other reports with similar etiology were known to the authors at the time of this case study - this indicates the potential for zoonotic transmission to the owners of hospitalized equine patients if they become colonized or contaminated while in an equine hospital.

In a study by Weese and Rousseau [16], samples were taken from horses on two farms where outbreaks of endemic MRSA were discovered. The results showed that 17% of horses were carriers for MRSA on the farm evaluated in Canada, and the New York farm showed initial MRSA colonization in 43% of the horses. The specifics of this study and success of decolonization are detailed in the following subsection.

As previously mentioned, it is important to take into consideration the carrier state of the horses being admitted to the hospital for treatment, as well as the general equine population in the areas surrounding an equine veterinary clinic. Admission rates of MRSA carriage can be as high as 55% with a surrounding population carriage rate of 0-4.7% [11]. Other clinics have found carriage rates upon admission to be 10.9% and 2.5% [10].

3.2 Characteristics of Natural Decolonization

In animals acting as carriers, who do not have an active MRSA infection, we are able to see spontaneous clearance of the bacteria without any treatment or intervention, as mentioned previously, where a foal was confirmed negative for nasal carriage after three months, while not receiving any decolonization treatments [13].

Weese and Rousseau [16] conducted studies on two separate horse farms, one in Ontario, Canada, one in New York State, USA, in order to describe an eradication program to rid them of their MRSA endemicity. MRSA was first introduced to the Canadian farm in 2002 after a mare returned from the Ontario Veterinary College Veterinary Teaching Hospital, where she had developed a postoperative infection following a surgical procedure. MRSA-5 (the dominant equine MRSA type in Canada) was isolated from both the infected incision as well as the nares of this mare. The following month, three out of 63 horses on the premises also tested positive for the identical strain of MRSA, collected from the nares. In 2003, two horses from this farm were also admitted to the same university hospital on two separate occasions, and both tested positive on routine admission screening for asymptomatic nasal carriage with the same MRSA strain as the original. With the addition of the sixth horse testing positive over this relatively short period, there was a question of endemicity of the bacteria on the farm, therefore screening of horses and personnel was implemented.

On the New York farm, the veterinarian noticed an increase in postoperative infections following routine interventions during the summer of 2003. Preceding this, a foal from this farm was admitted to a veterinary teaching hospital, where MRSA was isolated from this young horse. No control measures were recommended at that time, and the foal returned to the farm once discharged. Due to this history, it was suspected that MRSA was the cause of the increase in incision infections on the farm. The protocol for this study was not on a strict schedule, but nasal swabs were initially taken from all horses, and were continued until there were two consecutive negative samples, and the horse had no continued contact with another infected horse. All new horses were sampled upon arrival. Samples were taken from the farm personnel as well. The results from the Canadian farm showed that overall, 17% of horses and 10% of personnel were carriers for MRSA, and only one of the horses was negative in the initial screening sample at the beginning of this surveillance

program but was found positive on the second sample. All isolates were the same as the original strain detected. After the initial screening, a control program was implemented, which successfully decreased the number of positive samples to only two horses after just over a month. At 84 days, both horses were negative, however were again positive on the following sample. They both remained carriers by day 100, and due to concerns of long term sustainability of the control program, they were prescribed amikacin treatment with a nebulizer. One horse remained positive after this treatment, therefore further testing and treatment was performed, eventually resulting in MRSA-negative status, the details of which are described in the following section. Following this, all collected samples from horses and personnel remained negative. The farm was considered free from MRSA, and they instituted periodic screening of all horses on the farm, as well as screening of new arrivals to the property.

The New York farm showed initial MRSA colonization in 43% of the horses and 7% in personnel, and as with the Canadian farm, the numbers of carriers decreased following the control program implementation, to a total of only one horse on day 120, however the farm failed to take further samples so it is unclear of eradication was achieved.

The infection control measures described by Weese and Rousseau [16] are as follows:

1. After initial sampling, all horses with carrier status were moved to a separate area on the farm, and all in-contact items were not used for non-carrier horses
2. Carrier horses were prevented from co-mingling with non-carrier horses, or allowed to graze the same pastures
3. Carrier horses were restricted, where possible, from contacting other carrier horses
4. Personnel working with carrier horses were prevented from contacting non-carrier horses as much as possible
5. Gloves were required for any horse handling/contact, and there was strong encouragement for the use of alcohol based hand sanitizers, and good hand hygiene
6. When negative cultures were obtained from a previously MRSA positive horse, it was moved to an intermediate area while awaiting a second

negative result. When a second negative result was confirmed, the horse was moved to the area with the non-carrier horses

Forced decolonization therapy was not initially selected, as, based on other literature and observation, the authors of this study had knowledge that MRSA colonization in horses is generally transient and spontaneously clears with no antimicrobial intervention, which was proved with this study, with the exception of three of the total 120 horses remaining long term carriers, and only one of them requiring multiple antimicrobial treatments for eradication of the bacteria. The results of this study were very encouraging, with the speed at which MRSA was cleared from carriers using only the implementation of a strict infection control program, and support the past findings that equine MRSA colonization is, in most cases at least, transient. Minimal risk and simplicity of testing and isolation measures make the practical application more desirable as well. Further evidence of the effectiveness of pathogen control programs is the fact that in 2003 on the Canadian farm, a similar level of MRSA was detected, but the management did not feel the need to introduce measures to eradicate MRSA this time. Within the next six months, 11 horses tested positive on entry screening to the same university hospital, which included one foal that eventually was euthanized due to MRSA sepsis and septic arthritis.

3.3 Role and Options of Forced Equine Decolonization (Decolonization Therapy)

As far as the author is aware, there are no true studies on the efficacy of decolonizing equine asymptomatic carriers - infection control centers around isolation of positive MRSA carriers, human decolonization, and increased hygiene. This is due to the general findings that asymptomatic MRSA carriage is generally temporary and will clear without antimicrobial treatment, as in Weese and Rousseau [16]. This is also supported by the spontaneous clearance of MRSA from a foal within three months of detection [13]. Although both these cases did not take place in a veterinary hospital setting, their applicability is supported by similar protocols in a hospital setting where a decolonization and infection control program was put in place, and while the patients were not decolonized, only clinic personnel, there were very promising results. During this protocol implementation, there were no new MRSA infections [7], however there was an increase in MRSA clinical infections in hospitalized patients when these protocols were relaxed. This

seems to suggest that isolation of positive equine carriers, personnel decolonization and increased hygiene may be enough to control and eventually eradicate MRSA from a hospital setting.

However, as mentioned in the previous section, Weese and Rousseau [16] reported two horses that remained carriers long term, and therefore were forcefully decolonized with amikacin. One horse remained positive after this treatment, so two treatments of oral chloramphenicol were administered, also unsuccessfully, even though there was *in vitro* sensitivity to both antimicrobial agents. Due to this, samples were taken from the rectal area, guttural pouch and nasal cavity on day 153 to determine if there were multiple colonized sites that may be the source of the continued carrier status, and a second course of chloramphenicol was given. All samples were negative.

4. Role of Staff and Personal Hygiene Measures

4.1 Human MRSA Carriage

Walter et al. [17] conducted a long-term study to determine the status of nasal colonization among veterinary clinic workers and their family/household members, which spanned six years, following veterinary conference participants. They initially tested 225 participants, 45 of which initially tested positive for MRSA CC398. These participants, as well as those in their households were tested four times over this six-year period. Of the initial 45 MRSA positive conference participants, 31 were continually tested, and 26% of them were colonized with CC398 at all four testing points, however only 13% of these were of the same *spa* type consistently. Of the initially negative participants, 7% tested positive for MRSA CC398 at least once in the follow up tests. In total, this study followed 185 households, and in 11% of them, there was at least one non-veterinary staff member that tested positive for MRSA CC398 at least once. Veterinarians made up 89% of the initially positive participants, and 2% were veterinary assistants, indicating that veterinarians may play the largest role in personnel transmission of MRSA within the clinic setting. It should be noted that the majority of the initially positive participants also had contact working in swine farms. Although there were other strains of MRSA CC398 detected, CC398 *spa* type 011 was constantly the most common during all four testing periods, making up 55% of all

samples. They determined that households were 12 times more likely to have at least one MRSA positive non-veterinary member if there was a MRSA positive veterinary clinic worker in the house. As well, 77% of the strains found in household members matched that of the initial conference participant positive strain, supporting the idea of transmission from the participants to members of their households. While this study does well in bringing to light the possibility of long-term nasal colonization in clinic workers, and transmission to those outside the clinic setting, the authors also bring up the point that based on the layout of their study, they are not able to differentiate colonization versus temporary nasal contamination without colonization. They conclude that while they found the colonization of the same MRSA clones rare, there is a significant increase in the positive colonization status of those in the same household as people colonized with MRSA CC398. A Swiss study was also undertaken to screen veterinary clinic workers for MRSA and Methicillin-Resistant *Staphylococcus pseudintermedius* (MRSP) carriage, and found that 3.8% of the 340 participants tested positive for MRSA carriage, and all those that tested positive and worked with large animals were carriers of LA-MRSA ST398, including ST398-t011-IV [18].

4.2 Hand Hygiene (Gloves, Sanitization)

From the time hand hygiene was determined to be an important factor in the control of infection transmission, attributed to Ignaz Semmelweis in 1847 [19], there have been efforts made to improve on the techniques and substances used to ensure hands are properly disinfected. A randomized controlled crossover study by Espadale et al. [20] was performed in a veterinary hospital with the goal of comparing the efficacy of lactic acid and alcohol based disinfection methods, the former having residual activity, which is a desirable trait for antimicrobial agents. Due to the known importance of the role of hands for transmission of bacteria and other pathogens, the use of alcohol-based hand rubs and sanitizers have long been introduced into the healthcare field, as well as to the general public, and as noted by the authors of this study it has also "...been shown to be more effective, for decontamination of healthcare workers' hands, than handwashing with an unmedicated soap." The alcohol is able to very rapidly lyse microorganism cells via denaturation and coagulation of cell membrane proteins. However, there are some notable

disadvantages to the use of alcohol-based disinfectants, namely flammability, skin irritation, especially on broken skin, toxicity when ingested, lack of residual action, and effectiveness in the presence of organic matter. As well, in *in vitro* experiments, use of alcohol increases the production of biofilm formation in some *Staphylococcus aureus* strains, while having a positive correlation between increased alcohol concentration and increased production of these biofilms, while maintaining bacterial viability within [21]. Furthermore, alcohol also has no impact on protozoan oocysts, nor bacterial spores, although there is some efficacy on inactivating nonenveloped viruses. More detail on hand hygiene compliance in healthcare settings will be discussed later in this paper, but the general consensus is that it is, on average, poor due to many factors. Due to this, products with residual antimicrobial activity, such as lactic acid, are desirable as the impact of disinfection lasts longer post-application to hands, lessening pathogen transmission between patients and the environment. The authors performed a two week-long study in a small animal veterinary clinic setting with the primary goal of evaluating the hand Colony Forming Units (CFU) after hand hygiene protocols, as well as 6-8 hours afterward, right before they left work at the end of their shift. The results of this study showed that the alcohol rub was more effective at immediate CFU reduction after application in comparison to the lactic acid solution. However, in the pilot study for this experiment, the lactic acid solution only showed residual activity for an hour post-application, and neither had any significant residual effects at the end of a routine work shift. The authors interpreted this as demonstrating the importance of disinfectant reapplication. One possible explanation for these results is the evaporation time - as alcohol evaporates faster than the lactic acid, there is a possibility that the hand cultures were inoculated when hands were still wet with the lactic acid, leading bacteria to be more easily transferred. The authors therefore interpreted this to indicate a need for quick-drying disinfectants to achieve the best results in a healthcare setting. Another finding was that risk factors for increased hand contamination were touching patients compared to the environment, as well as being a veterinarian compared to other veterinary staff roles.

While transmission of pathogens in an everyday clinical setting, such as from patient to patient, via contaminated equipment, or the general environment, is an important step in infection control, this concept becomes even more important during surgical procedures.

This requires creating and maintaining a sterile working environment, and ensuring the asepsis of everything introduced into it. One of the most important aspects of aseptic surgery is the surgical scrubbing of the surgeons hands. Even though it is now routine to wear one or two pairs of sterile surgical gloves depending on the type of surgery being performed, it is still very important that the surgeon's hands also be sterile in order to prevent contamination in the case of glove perforation. Currently, the most common soaps used in both human and veterinary medicine for surgical hand scrubbing are 4% chlorhexidine gluconate, and povidone-iodine, both of which are medicated water-based soaps [22, 23]. Increasing research has been conducted into the effectiveness of alcohol based aseptic techniques for surgical hand preparation as well, and has shown promising results. The importance of this research includes decreased application time, therefore showing increased compliance from surgeons, decreased water usage, and overall improved skin health and decreased irritation. This in turn decreased the colonization of staphylococci and other bacteria in the damaged skin [22]. In 2016, a study was published by da Silveira et al. [22] comparing the use of traditional 4% chlorhexidine gluconate infused sponges and water, and a neutral soap wash paired with an alcohol-based hand rub on pre-surgical hand preparation before and after elective surgeries in an equine hospital. While there are many studies showing the efficacy of alcohol hand preparations as equally or more effective on decreasing CFUs on surgeons hands, in veterinary medicine, particularly equine medicine, is it common that the surgeon examines the horse before the surgery takes place. As organic matter such as dirt and feces decreases the antimicrobial effects of alcohol, the authors implemented a hand wash protocol with a neutral soap before the application of the alcohol in order to maximize the effects. All hand preparation techniques were done in a standardized fashion, and samples were taken from the surgeons' hands before washing, after the neutral soap wash, after the traditional five minute scrub technique or 90 second alcohol rub technique, and at the end of the elective surgical procedure. Glove puncture tests were also performed to evaluate the level of potential surgical site contamination during surgery. The results of this study showed that the alcohol hand preparation technique was of equal efficacy for hand asepsis compared to the traditional hand scrubbing method used at all sampling time points, as there was no significant difference in CFUs across the sampling points, and the pattern of decrease

through steps of hand preparation and post-surgery also had no significant difference. While one horse developed a superficial SSI after traditional pre-surgical hand preparation, it was uncomplicated and resolved with medical treatment, therefore the authors did not consider this a significant impact to the results of the experiment. As well, while the WHO recommends a contact time of 3 minutes for alcohol application, this study confirms previous findings that 90 seconds is adequate to, at minimum, match the efficacy of traditional chlorhexidine pre-surgical hand scrubbing methods. This conclusion is also supported by a comparative study published in the *New Zealand Veterinary Journal* by Edwards et al. [24], as well as a 2011 experimental study out of Belgium [23].

Rocktäschel et al. [25] conducted a study to compare individual, everyday pre-surgical handwashing techniques to a standardized method, in order to determine the efficacy of surgical hand asepsis in a veterinary university equine teaching hospital. The standardized protocol used for this experiment was taken from "VAH method 12", from the German Association for Applied Hygiene (VAH) Disinfectants Commission. The focus was on time spent on both hand washing and disinfection afterward, brush use during handwashing, and the amount of disinfectant used. Samples were taken before and after hand washing, after disinfection, and after surgery was completed. A glove perforation test was also performed on the surgical gloves worn by the participants. The authors noted significant differences in the individual routines of participants; some participants took up to eight minutes for their pre-surgical hand washing and some used up to 48 ml of disinfectant. The largest variation in time and disinfectant use was found among veterinary students, whereas veterinarians and veterinary assistants had less variation in these parameters. Over all, the time spent during the pre-surgical hand washing did not impact the bacterial counts between individual and standardized methods. The results of the standardized hand disinfection showed an overall more significant decrease in the bacterial contamination of hands than the non-standardized methods. From the 42 study participants, initially eight were found to be MRSA positive. Genotyping revealed that there were 14 different MRSA strains involved, and later in the study, these 14 MRSA strains were found on an additional three people, now totaling 11 MRSA positive participants, including nine students, one staff member, and one surgeon. All MRSA samples were typed as ST398 *spa* type t011 and t6575, and all with gentamicin resistance. Glove perforations were also determined to be

more prevalent in those worn by the surgeons, as well in more invasive surgical procedures.

An extensive review by Allegranzi and Pittet [26] found that there are many reasons for poor hand hygiene compliance in a human healthcare setting, which similarity occurs in developed and developing countries. Interventions that were implemented in these studies included increased promotion of hand washing, as well as increased utilization of alcohol-based hand rubs. Factors influencing compliance included professional category (i.e. doctor vs. physiotherapist), working in a specific area or care unit (i.e. intensive care vs. surgery), overcrowding, understaffing, and the use of gloves and gowns, as well as a simple lack of availability of handwashing or sanitizing areas. Other factors such as personal knowledge of pathogen transmission risk, social expectation and personal conviction to maintain hand hygiene were also found to be important factors. The authors classified hand hygiene practice into two types: inherent (occurring when hands are noticeably soiled) and elective (where hands are disinfected or washed in the absence of visible or obvious physical contamination). The highest hygiene compliance rate of all the papers included in this review was 81% after intervention, with another paper reporting the highest long term compliance rate of 66% after a follow up period of eight years. Based on this review, the authors concluded that, with short term awareness training and an implemented protocol, compliance shortly drops back to around where it was before the interventions. However, long term compliance can be marginally maintained at a higher level when the interventions involve continuous education and monitoring. In Finland, researchers Verkola et al. [27] sent out a questionnaire to 129 ambulatory large animal veterinarians in order to assess self-reported hand hygiene practices while vets are working with livestock and in equine stables. Adequate hand washing areas were reported on livestock farms by 66.9% of respondents, compared to 21.4% in equine stables. There was also a higher number of participants reporting cleaning hands, either via washing or with a hand sanitizer, with those working with livestock vs. equines, at 75% and 42.5% respectively. A similar trend was also reported in regard to wearing protective clothing or cover-alls while working with the animals, with 91.6% of livestock veterinarians reporting that they do, and only 27.7% of equine veterinarians doing the same. As well, 30% of respondents reported cleaning their stethoscopes less than once a week.

Another reason for lack of handwashing compliance in healthcare settings is the dryness and irritation that occurs in the skin when repeatedly washed long term. This may be reduced when frequent handwashing is replaced with use of an alcohol based hand rub when hands are not grossly contaminated. A comparative experimental study was done by Boyce et al. [28] to compare the dryness and irritation in the hands of nurses in a human healthcare setting, during a two week period. The findings were that skin irritation and dryness significantly increased, and epidermal water content significantly decreased when using an unmedicated soap and water for frequent handwashing. These criteria showed either almost no change or much less significant changes with alcohol based disinfection. The conclusion of the study was that hand asepsis with an alcohol based gel was very well tolerated by all participants, and did not significantly alter the parameters of the skin from before the experiment was conducted.

Another possible area of hand contamination is the hand drying process after washing. The efficacy of proper hand drying includes factors like the speed and degree of drying, bacterial removal, and recontamination prevention. A systematic review by Huang et al. [19] set out to identify the mode of hand drying that resulted in the least recontamination of hands after hand washing, though there is limited research into this specific area as more studies focus on the role of handwashing. The authors note that multiple studies have investigated and found increased moisture on the hands can result in more bacterial transfer than from hands that are dry, which is why it is important to dry hands properly. The available research used included studies on cloth and paper towels, hot air and jet air dryers, and evaporation. In the conclusion of this review, it was determined that while there is more environmental impact and an increased cost, paper towels are a more hygienic method of drying. The research indicates that when housed in a closed holder to prevent cross contamination in settings such as washrooms, paper towels consistently show a decreased level of hand contamination after washing than other methods, and are therefore recommended by the authors when hand hygiene is of importance, such as in a veterinary hospital.

The use of gloves in the reduction of hand contamination and transmission of pathogens has been well documented, preventing contamination of the hands 77% of the time

according to Muto et al. [29] in a human healthcare setting. This study also found that use of gloves and gowns together significantly reduced the transmission of pathogens when compared to gloves alone. The recommendations made in this paper were:

1. The use of active surveillance cultures, including hospital wide initial sampling of the environment and patients, upon patient admission, weekly screening of patients remaining in the hospital, and with a goal to identify every patient carrying or infected with MRSA
2. Proper hand hygiene implementation and compliance monitoring
3. Isolation and barrier protection for patients found to be carrying or infected with MRSA, including gloves, gowns, and masks to prevent staff nasal colonization.
4. Prudent use of antibiotic agents
5. Decolonization of patients
6. Ensuring proper environmental decontamination before patients are reintroduced to infected areas

4.3 Face Covers and Masks

There is conflicting information to be found in regard to mask use to prevent contraction of nasal MRSA carriage in the human healthcare setting, and even less so in the veterinary healthcare setting. However, Muto et al. [29] recommends mask use in a human healthcare setting as a precaution when in contact with MRSA positive patients, as well as for the patient to wear one in order to decrease nasal shedding. While patient use is not applicable to the equine hospital environment, staff may benefit from the use of facial masks to prevent colonization from aerosolized MRSA when handling horses with known positive MRSA status, and during an outbreak when there is a higher potential chance of MRSA to be airborne [18]. This could also decrease the potential risk of staff shedding the bacteria colonizing their nasal cavity into the environment via breathing, sneezing, blowing or picking one's nose etc.

4.4 Smartphones

It is important to remember that inanimate objects are also important factors in pathogen transmission, and as mentioned previously, fomites such as stethoscopes often are forgotten about in routine cleaning and disinfection. However, while a veterinarian is rarely without a stethoscope, even more universally carried is a mobile smartphone, especially with the increase in applications that are utilized by veterinarians and other medical workers, such as ultrasonography, radiographic evaluation, for reference material and reading textbooks, and drug and other calculations. Unlike a stethoscope, mobile smartphones are of course used outside the professional setting as well, increasing the possibility of pathogen contamination and transfer. An experimental study by Lieberman et al. [30] conducted a series of experiments to test the efficacy of 6 mobile smartphone sanitization methods on decreasing aerobic bacteria colony formation. The methods tested were a 70% ethanol spray, 2 different 254-nm UVC sterilization lights, a quaternary ammonium disinfectant solution, delicate-task wipes, and wipes impregnated with sodium hypochlorite. All mobile phones used in this experiment were left in their cases, if applicable, to mimic realistic conditions and sampling was done before and after sanitization, from both the screen surface and the inner and outer surfaces of the phone case, as well as the junction of the phone and its case. The results of this experiment showed that all methods significantly reduced bacterial growth from all sampling sites, however the most effective methods were the UVC devices, as they were the only ones where aerobic colony numbers were reduced to zero after sanitization. The authors also suggest that UVC lights might be the most beneficial for long term use, as there is a potential for smartphone damage if liquid disinfectants are routinely used for cleaning and sanitization.

4.5 Human Decolonization Therapy

Human MRSA decolonization has been extensively studied over the years with overall positive results to various decolonization therapies, and in general seems to have a higher success rate than that of horses. In a 2015 retrospective cohort study, Sai et al. [31] evaluated two different decolonization methods in hospitalized patients, and their effects

on MRSA infection rates. The two protocols each lasted a total of five consecutive days. The first involved the application of 2% mupirocin intranasally, paired with washing the body with a chlorhexidine soap. The second protocol also involved an intranasal treatment and body wash, but both were done with a povidone-iodine solution. Including both protocols, 34% of the patients involved were successfully decolonized on the first attempt, with 20% of the remaining patients decolonized after a second round of treatment. The research indicated that there was no further success in decolonizing patients after a third or fourth attempt at decolonization. In total, the overall success rate was unimpressive at 39%. However, when evaluated separately the rates of decolonization were much more successful with the mupirocin and chlorhexidine protocol than the povidone-iodine protocol, at 56% and 23% respectively.

In 2012, a study published out of a Swiss hospital evaluated the success of forced decolonization on patients, as well as aimed to set a determination on how long of a follow up period should be established in order to accurately determine recolonization after a patient has been confirmed negative for MRSA carriage [32]. For the decolonization therapy, the authors used a five day treatment of mupirocin ointment administered nasally twice a day, a mouth rinse of chlorhexidine also twice a day, and a didecyldimonium chloride body wash once per day. Systemic antibiotics were not used, and this decolonization treatment was compared to a control group of patients that did not receive any decolonization therapy. The results showed an overall tripling of decolonization success of those in the treatment group (65% forced clearance vs. 22% spontaneous clearance). Over 42% of the treatment group were successfully decolonized on the first round of treatment, 11% needed two rounds of decolonization therapy, 3% needed three rounds, and 5% showed spontaneous decolonization of MRSA in between decolonization treatments. Of those not treated for decolonization, only 22% of the patients were able to be followed throughout the study, but all of these 22% showed spontaneous clearance of MRSA carriage. According to the authors, the main reasons for decolonization failure identified were respiratory/throat colonization compared to nasal carriage at the beginning of decolonization treatment, age (those successfully decolonized had a mean age of 49.7 compared to 68.6 years of age), and colonization with MRSA *spa*-type 002 which was endemic to this hospital and therefore assumed to be more resistant to the antimicrobials

used for therapeutic decolonization. These factors correlated to a longer time and more decolonization treatments to reach a MRSA-free state. The authors identified a mean recurrence time of 95 days (with a range of 59-205 days), a 75% detection rate of MRSA recolonization within 180 days, and 90% of recurrences detected within 270 days (approximately 9 months) from confirming negative colonization following successful decolonization. Those remaining free of MRSA recolonization were followed up with for a median time of 339 days (ranging from 216-553 days). Based on this, the authors suggested a follow-up period of 1 year after a negative confirmation swab is obtained. It should be noted that all those involved in this study were patients, and had other comorbidities that may have contributed to the colonization status and response to decolonization therapy or spontaneous clearance of MRSA.

Eed et al. [33] set out to determine whether MRSA decolonization treatment with chlorhexidine and mupirocin lead to the development of resistance against these agents. At the beginning of the study, baseline resistance to both agents were determined to be "moderate" at 13.9% for mupirocin and "rare" at 3.5% for chlorhexidine. This study consisted of analyzing 115 MRSA isolates, separated into 81 isolates from facilities that then introduced a targeted decolonization protocol, and 76 isolates from facilities that implemented a universal decolonization protocol. The results of the study concluded that neither decolonization protocol resulted in any significant changes in the development of resistance to either antimicrobial agent used. According to the authors, a 2% ointment of mupirocin is commonly applied 2-3 times daily to the anterior nares of the patient, and there is approximately a 90% success rate for clearance of MRSA within 5-7 days, but formulations can also be useful for addressing skin and soft tissue infections. Chlorhexidine is most commonly used as a skin antiseptic agent, as well as oral rinse. The concentration and exact formulation of chlorhexidine was not specified in this paper. Both these agents may be combined for addressing MRSA infection or carriage. The results of this study found that there was no significant change in resistance development for either mupirocin or chlorhexidine, from either a targeted or universal decolonization protocol, therefore showing favorable results. However, the authors do acknowledge that the sample size was relatively small, and the study length was fairly short, being only 14 months. The

authors recommended periodic surveillance in order to monitor possible increases in resistance to decolonization agents.

At the Equine Clinic of Bern, a study was performed to determine the cause of the increase of MRSA-related postoperative infections, and both patients and clinic personnel, including students, were screened [7]. Human samples were taken over a six-year period, consisting of five samples per person, via nasal swab. After a prescribed decolonization therapy for all MRSA-positive personnel in the study, follow up samples were taken from the nasal cavity, throat and inguinal area. For the decolonization therapy, mupirocin was administered into the nares three times a day, 0.1% chlorhexidine solution was gargled two times a day, and a 4% chlorhexidine solution was also used to wash the body and hair. This protocol was continued for a five day period, and all clothes and bedding were changed after each shower. Forced decolonization was not performed on the horses, but hygiene measures were put in place which included mandatory hand gloving and sanitization in between all patients, as well as stabling horses that have tested positive with MRSA carriage or infection in isolation areas. Decolonization of the clinic personnel was started three months after the implementation of the new hygienic measures, and during the three-month period following decolonization there were no new infections found in patients due to MRSA, even though there were two staff members that could not be decolonized and remained carriers. Within the last year of the study, only seven cases of MRSA-related infections were detected, even though one-fifth of the staff were determined to be carriers during this time. Later in 2010 when the study was ending, there was laxity in maintaining the infection control protocols, and likely as a consequence there was an increase of infections caused by *S. aureus*. Rosenkranz et al. [18] also noted similar findings after an investigation into many MRSA outbreaks in an equine clinic in the Netherlands**.**

5. Role of the Environment and its Hygiene

5.1 Survival of *S. aureus* Under Different Circumstances, Environmental Hot Spots

As *Staphylococcus aureus* bacteria are a normal part of the environment and animal flora, they remain viable in a large range of conditions. *S. aureus* is also able to travel great

distances via the wind. An American study was conducted to detect both aerosol travel and bacterial viability of MRSA isolates from both inside and downwind of a swine facility. The publication by Ferguson et al. [34] found that there was an association between aerosolized MRSA bacteria and particles larger than 5 μm from samples taken inside the facility, but with particles smaller than 5 μm downwind of the swine farm. There were also samples taken from the feed used for the pigs at the facility, and MRSA was isolated from samples of the feed both before it was brought into the facility and from the samples of feed already inside. There were no viable MRSA isolates from air samples found after powerwashing with a biocide inside the facility. Air samples containing MRSA were able to be detected at the surprising distance of 215 meters downwind of the swine farm, which to the authors knowledge at the time of publishing, was the first time downwind detection in these circumstances had exceeded 150 meters. This indicates that MRSA may be able to travel a far greater distance than originally thought in an aerosolized form, and therefore poses an increased risk of spreading to surrounding areas of these MRSA "hot spots", which may also be applicable to equine hospitals with high rates of MRSA infection, although more research will need to be done in this specific area, especially given the difference in animal husbandry between intensive swine farming facilities, and equine hospitals.

More relevant to the clinical setting, it has become apparent in recent years that the clinical environment can be a significant source of hospital-acquired infections, in both human and veterinary medicine, and MRSA is no exception to this. The main reason for this is the fact that these bacteria are able to survive on dry surfaces for months while remaining viable, and interestingly, contrary to the findings for other bacteria, *S. aureus* has been found to prevail longer in low humidity and moisture conditions, as opposed to higher, and temperatures from four to six degrees celsius also improve survival time in the environment [35]. Hoet et al. [36] conducted an investigation into the baseline environmental contamination with MRSA in a veterinary university teaching hospital, during a time where there was no current outbreak of MRSA infections in the patients hospitalized there. The aim was to determine the areas of the hospital environment from which MRSA can be isolated, and therefore create a plan to combat the presence of MRSA, even when there is no active outbreak. While most of the MRSA isolates obtained

were from the small animal area of the hospital, there was one isolate obtained from the equine part of the clinic. Overall, there was no significant difference in type of contact surface (i.e. human or animal contact surfaces), and 12.9% of the total isolates came from those surfaces that had contact with both humans and animals. No MRSA was detected in vacuum equipment or air vents for the facility. The majority of human contact surfaces that contained MRSA were from doors, but these bacteria were also detected on bathroom faucets and computer equipment. The one equine isolate came from a sample of a door. This isolate from the equine part of the hospital had a very broad resistance profile, including all β-lactam antibiotics, gentamycin, trimethoprim-sulfa, and erythromycin. It was also the only isolate from this setting that showed resistance to tetracycline as well. However there was demonstrated susceptibility to doxycycline, vancomycin, amikacin, quinolone antibiotics, clindamycin and chloramphenicol. The results of this investigation prompted a surveillance system to be implemented within the teaching hospital to monitor and combat the presence of MRSA, which involved monthly sampling of the indicated high risk areas, screening of patients upon admission to the clinic, management and handling protocols for MRSA-positive patients, and specific disinfection and cleaning protocols for the areas identified within this study. These findings also suggest that, as a part of a targeted biosecurity protocol, it is important to sample and identify environmental "hot spots" within the clinic setting, in order to efficiently eliminate MRSA from the environment and prevent future transmission to patients and staff.

Environmental contamination with MRSA isolates in an equine hospital setting has also been confirmed by Weese et al. [37] when 25/260 samples taken from various surfaces within an equine veterinary teaching hospital showed the presence of the pathogen. Unsurprisingly, most of the positive samples were from stalls that contained horses with known MRSA infections or carriage, however there were also positive samples from those taken from staff personal items, restraint and medical devices (i.e. a portable ultrasound machine and nose twitches), and other stalls containing MRSA-negative horses. The presence of MRSA in stalls of both MRSA-positive and negative patients is a concern, as these areas are frequently a contact point for both horses, staff, and visitors, and have the potential to be a major source of contact transmission throughout the hospital, especially to other horses. It is important to note that as horses generally investigate their environment

thoroughly with their lips and nose, it is logical that the area they spend most of their time in would be highly contaminated. It is also a potentially important way in which they easily develop nasal carriage if stalls are not properly cleaned before they are admitted. The nasal twitches used at this teaching hospital were constructed of wood and rope, and were not commonly disinfected or cleaned and, due to the materials used, would be very difficult to completely de-contaminate. Research is ongoing to determine better materials to use for horse comfort as well as ease of disinfection. Due to the results of this investigation, increased biosecurity has been introduced, including more strict isolation of MRSA-positive patients, as well as a more thorough stall cleaning after the patient leaves the hospital. This disinfection protocol includes the entire stall being scrubbed three times with quaternary ammonium disinfectant and air dried for a day between each cleaning. All items within the stall are also more intensively cleaned.

5.2 General Hygiene Practices (GHP, the Role of Cleaning, Sanitation, Disinfection)

In 2017, an equine hospital isolated MRSA from environmental and SSI samples, and a deep clean procedure was undertaken to attempt eradication from the clinic [38]. This involved closure of the affected stabling areas, removal of all items and bedding within, removal of all organic matter via scrubbing with an alkaline detergent solution in warm water and steam cleaning or pressure washing the stall, allowing for complete air drying. Walls and floors were then sprayed with Vikron solution and left to air dry for at least 24 hrs. This was also done in all affected drains, walkways and other areas where MRSA had been detected. Horses were only reintroduced into the stalls when the environmental swabs came back negative for MRSA, and monthly samples were taken to monitor for the reintroduction of MRSA to the areas. This protocol showed promising results.

While there is a lot of research into what cleaning agents make effective cleaners, a lesser but still important area of research is the methods of cleaning, including the types of cloth used. Experimentally, ultra-microfiber cloths have been shown to pick up microorganisms more effectively than conventional cloths, across many different surface types, and also on contaminated surfaces to simulate real-life contamination in a clinic setting, according to an experiment by Wren et al. [39]. The authors also found the same is true for microfiber

mops compared to string mops. This is an interesting area of research as the use of many cleaning agents can pose health effects on those exposed to them, as well as contribute to the degradation of surfaces and equipment, not to mention the potential for increasing selection pressures for resistance. Therefore it would be helpful to have cleaning equipment that is able to remove these pathogens from surfaces without or with less need for cleaning agents.

6. Role and Options of Surveillance - Patients, Staff, Environment

Microbiological surveillance plays an important role in infection control within the hospital environment for many reasons. Isolation and identification of the causative pathogen(s) is one but especially in the case of MRSA, it can also help determine whether the bacteria was introduced such as with a UK study finding multiple different MRSA strains over a 5 year period indicating multiple separate introductions into the equine hospital [38], or if it was acquired from the hospital environment, and therefore endemic. This would require intensive screening and cleaning procedures to eradicate.

A Swedish study by Bergström and Grönlund [39] looked at infection control compliance before and after and intervention to educate staff on the importance of infection control measures, as well as performed an evaluation on reasons leading to deceased compliance with the protocols. The authors found that the overall compliance before intervening was poor in the four equine hospitals they evaluated, mainly due to "...poor or absent [infection control] implementation strategy, lack of active surveillance of compliance with procedures and no monitoring of such as nosocomial infections." This poor compliance with hand hygiene has also been recorded in other veterinary settings in 2014, where compliance only reached 14% [40]. There was a post-intervention increase in gloving compliance in one hospital, but there was no significant increase in glove purchases following the intervention. It was found that dress code was in high compliance at 92-100% across all evaluated facilities, but hand hygiene was generally poor. The reasons given behind the poor hygiene compliance was lack of knowledge of the proper procedures, high workload with not enough time to maintain proper hand hygiene, and lack of supplies and facilities to efficiently follow hand hygiene protocols. Monitoring of

compliance was done through purchase information of the related items (i.e. hand sanitizer and disposable gloves), which were evaluated at a purchase-per-patient and purchase-per-day level, and was evaluated before and after the intervention. While studying the pre-intervention information, it was noted that none of the hospitals had in place a plan for the proper monitoring and implementation of infection control compliance, and at most there were "read and sign" documents outlining proper control protocols. The authors suggested three specific areas with which improvement can be made to ensure proper infection control protocols are being followed; traceable documentation outlining proper hygiene protocols, providing staff with training and information, especially regarding hand hygiene, and staff involvement in dialogue to remove barriers of compliance to these infection control protocols. The authors also note that monitoring compliance with glove and hand sanitizer purchases in a purchases-per-patient manner seems very suitable to the equine clinic setting.

In the development of a surveillance program, there needs to be a specific outline to follow, with specific and attainable goals. In a paper by Paul Morley [41], he outlined four important goals for a good infection control program in human hospitals:

1. "To evaluate the effectiveness of infection control and biosecurity practices. Activities that are used to achieve this goal include estimating rates of nosocomial infection/disease, estimating rates of pathogen shedding, and evaluating environmental contamination with infectious agents. These actions allow estimation of baseline or expected rates, which then allows detection of changes in rates (increases and decreases), which might trigger actions if critical limits are exceeded…"
2. "To evaluate compliance with infection control procedures. This can be achieved through formal or casual monitoring and can relate to routine patient management practices or to procedures that are specifically used as preventive measures for controlling nosocomial infections…"
3. "To provide a basis for logical infection control decisions. Data obtained from the surveillance program can be used in establishing general

overarching policies to answer specific questions or test specific hypotheses and in making management decisions about individual patients…"

4. "To stimulate efficient and economic use of resources. It is too difficult and costly to attempt to function following the most rigorous control procedures at all times and to perform ongoing active surveillance for every type of infectious agent, every type of procedure, and every type of nosocomial disease. Resources are always limited, and it is important to achieve maximal efficiency in surveillance and control efforts. Surveillance data should be used to target prevention and surveillance efforts where they are most needed and to identify areas that require further investigation and corrective action…"

Facilities with intense active surveillance, enforced infection control protocols, a unitized method of feedback to hospital staff on nosocomial infection rates, and well trained staff to implement and supervise protocol compliance have routinely been found to have significantly decreased rates of nosocomial infections [41]. Morley also discusses the use of "critical limits", which trigger action if exceeded. These should be specific to the needs of every facility in which it is implemented, similar to the rest of the goal setting when developing an infection control plan. Providing feedback is discussed as being an important part of infection control, so that staff are aware of the microbial contamination rate of their place of work, and are able to adjust their habits according to the severity of infection or environmental contamination. The author mentions that this correlation has been proven in the human healthcare setting, and should transfer well to the veterinary field as well. It is also mentioned that owners should be made aware of the trends of microbe contamination, even if there is no or low clinical disease present, so that informed decisions can be made regarding their animals' care.

Adequate surveillance of the status of MRSA in a veterinary clinic requires effective sampling techniques and sampling sites. Van den Eede et al. [42] conducted a study and found that the most effective nasal sampling site was the nasal vestibulum, with a relative sensitivity and isolation rate of 81.1% and 44.6% respectively, which were the highest from all sampling sites, including the ventral meatus and diverticulum. Van den Eede et al.

[43] also found nasal samples to be the most accurate in hospitalized horses, however in 30% of the horses tested, they also found MRSA positive samples from various sites on the skin. The highest rates of skin isolation were found to be the carpus, neck, withers and croup of these horses. However, the authors had not found evidence of MRSA being isolated from a skin sample of a horse that was not showing clinical signs of infection, therefore based on current data, it is unlikely that skin carriage poses much risk for asymptomatic transmission in equine hospitals. The authors theorize that this skin positivity might be due to hand transmission from clinic workers, as these areas are ones frequently touched when in contact with horses, and therefore further validates the need for proper hand hygiene. Based on the results of this study, the authors also recommend routinely testing at least one skin site for MRSA when screening horses. The authors also found that a significantly higher number of patients tested positive for MRSA in the surgery and internal medicine sections of the hospital, compared to the obstetrics area.

7. Equine Clinic-Specific Biosecurity Recommendations

Due to the potential severity of MRSA infections in hospitalized horses, its ability to become endemic, the possibility for zoonosis, and the evidence supporting its constant mutation in response to new antimicrobials, there is an ever increasing need to establish strict biosecurity protocols in equine hospitals to mitigate these risks. An editorial published in the *Equine Veterinary Journal* in 1998 highlighted the importance of emphasizing the utilization of pre-antibiotic era techniques, such as hand and environmental asepsis/disinfection to prevent infections, rather than treat them after they are established in patients [44]. These concepts have increased in importance in human medicine and have been effective. Based on this model, it seems a good idea to treat this with the same level of importance in veterinary medicine. Since this editorial was published, there has been an increase in the number of antimicrobial drugs that have become ineffective in fighting MRSA as this pathogen continues to mutate and adapt to new drugs. The concept behind this is that if, by decreasing the selective pressure that drives mutation leading to increasing drug resistance, carefully selecting drugs based on culture and sensitivity screenings and proper antimicrobial administration, over time we

could see an increase in susceptibility to the antimicrobials that currently are ineffective. However, this specific area of study is outside the scope of this paper.

Specific to the practise of equine medicine in the hospital setting, screening all horses arriving for hospitalization and those already admitted by taking samples from both the nasal vestibulum, as well as one frequently contacted skin site is important to identify those that carry MRSA asymptomatically. All MRSA-positive horses should be strictly isolated and allowed to undergo natural decolonization if they are mere carriers for the pathogen, with routine and regular re-testing until two concurrent samples come back negative for MRSA. Exceptions should be made for those patients complicated with an active MRSA infection, requiring antimicrobial treatment based on antimicrobial sensitivity testing, or those that are found to be long-term carriers requiring decolonization treatment to eliminate their carrier status. Isolation should include as minimal staff changes as possible to limit human exposure, and as little contact as possible for staff between MRSA-positive and negative horses. This would decrease the potential transfer risk from staff to other horses. While forced decolonization may not be practical or required for equine patients, it is an option for staff in outbreaks where staff members become asymptomatic carriers for equine MRSA. This can be done with the use of mupirocin and chlorhexidine as described previously in this paper. As well, due to the potential for staff to carry MRSA species when transferring veterinary hospitals, it may be a good idea to screen all new hires that previously worked at another equine facility.

Personal hygiene is another important factor, with an emphasis on proper cleaning protocols for MRSA-positive horse stalls and other hotspots. Hand washing stations should be conveniently located and well stocked with soap and paper towels for use when hands are visibly contaminated, and alcohol-based hand sanitizers should be made readily available and easily accessible for veterinary personnel, with proper education on the importance of its frequent use, especially between patients. Cooperative monitoring and surveillance programs put in place to ensure compliance may be of benefit. Regarding surgical hand scrubbing, the pairing of a neutral soap and a standardized alcohol hand wash has been shown to be at least as effective as more traditional hand scrubbing, however the use of alcohol may be more gentle on the skin when multiple surgeries are to be performed back to back. Either way, standardized hand washing protocols that all

surgical participants follow is needed to ensure consistency of product contact time and efficacy. Personal Protective Equipment (PPE) should also be employed, including disposable gloves, gowns, shoe covers and facial masks to reduce transmission of MRSA from positive patients to other areas of the hospital or other animals, as well as to prevent nasal carriage in staff working with MRSA positive horses. Disinfection of frequently used personal items such as stethoscopes, should be done frequently with appropriate solutions, and UV sanitation of cell phones may also be used to decrease fomite transmission. As seen in this paper, there is a good success rate for human MRSA decolonization with intranasal mupirocin and chlorhexidine solutions for washing the body and mouth. This has been shown to extend into the equine hospital setting, and when combined with proper isolation protocols for MRSA-positive hospitalized horses and cleaning protocols, there is a significant reduction in new MRSA infections.

As *S. aureus* is a normal environmental bacterium, it is able to persist in the environment in a fairly wide range of conditions, including those that make up equine facilities. Therefore proper cleaning and disinfection is crucial to eradicating pathogens like MRSA from the hospital environment. “Hot spots” that are significant in the environmental presence of MRSA are the areas where both MRSA-positive and negative animals are kept (i.e. stalls), equipment used for restraint, and medical equipment, as well as human contact sites like door handles, keyboards, and bathroom taps. These sites may contain MRSA even when there is no active outbreak in the hospital. Dust control and ventilation is also important, as there is evidence that MRSA is able to travel through the air on small particles of dust. On top of screening patients on admission to the hospital, it could also be beneficial to perform monthly sampling of high contact and high risk areas for the presence of MRSA, to ensure that proper cleaning and disinfection is taking place. Quaternary ammonium products used for stalls in multiple rounds of cleaning, allowing for air drying between, may be useful for proper disinfection. Another protocol with effective results is the scrubbing of stalls with alkaline detergent in warm water, steam cleaning or pressure washing, allowing time to air dry, and then spraying Vikron and allowing complete drying. Both these methods require the complete removal of bedding and organic material prior, and the areas should be confirmed MRSA-negative via two consecutive swab samples before using for patient stabling again. For surface cleaning of areas with high staff

contact, microfiber and ultra-microfiber cloths, as well as microfiber mops demonstrate effective pathogen removal, even in the absence of cleaning solutions. Proper education, facilities, monitoring and surveillance is also required to maintain proper hand hygiene, and needs to be implemented in a practical-to-use, and accountable way to ensure staff possess the knowledge on hand hygiene, as well as take the time to clean their hands, and glove appropriately. This can be monitored via tracking purchase materials related to hand hygiene on a per-patient and per-day level, as well as ensuring accountability is maintained for staff who fail to maintain expected standards. However, they're should also be an emphasis on communication from staff for feedback on efficacy and areas for improvement. There is also evidence supporting the effectiveness of transparency with staff over the prevalence of pathogens like MRSA, so that they can make personal behaviour adjustments accordingly. Owners should be made aware of the prevalence of these pathogens, as well as the protocols in place to control the issue, so that they may make informed decisions about their animals' care.

Similar to human protocols, effective biosecurity infection control programs should be able to establish a baseline for pathogen contamination, and with a harmful bacterium such as MRSA, the baseline likely should be zero, where any detection over this threshold provokes immediate response. Compliance for the set protocols for prevention and eradication should be closely monitored. These policies should also be efficient in their use of resources, while being specific and tailored to each scenario, with protocols in place for day- to-day infection prevention, and for various levels of MRSA outbreaks, depending on severity and prevalence.

8. Methods

Research articles for this literature review were found through the use of PubMed, ResearchGate, Web of Science, and Google Scholar, with search terms related to the prevalence of equine MRSA in Europe, more specifically in equine clinics, as well as decolonization methods and success for both humans and horses, hygiene protocols, pathogen transmission and environmental contamination in the clinical setting. Searches were also carried out for research into different aseptic hand washing techniques, and hand

drying techniques. Only English papers were used. General information about *Staphylococcus aureus* was derived from bacteriology lectures taught by Dr. László Makrai, Associate Professor, on September 28th, 2020. Dr. Ervin Albert also provided initial research articles as an introduction to the subject. Research was conducted between June 2022 and September 2023.

9. Review

With the ever-increasing ability for MRSA to adapt to the use of new antimicrobials attempting to treat these infections, and the potential harm that can come from endemic MRSA in equine hospitals, it is crucial to ensure that protocols are in place to identify the presence of this pathogen, as well as screen individuals entering the facilities in order to prevent new introductions. Once MRSA has been detected in an equine clinic such as in the DCEM, the immediate goal should be eradication via proper sampling, isolation, disinfection, and hygiene practices. Once this pathogen is no longer detectable in staff, patients or the environment, strict screening and hygiene measures should continue in order to prevent re-introduction via new staff and patients.

10. Conclusion

Since MRSA ST398 was first detected at the DCEM, it has become endemic. With the consequences of this endemicity having the potential for causing fatal infections in patients, it is important to develop a strict biosecurity protocol to combat it. This should include screening all current and new admissions to the hospital, new staff who previously worked in equine facilities, maintaining proper isolation of MRSA-positive horses and monitoring their natural decolonization. Screening staff during periods of outbreaks and forced decolonization of those positive for MRSA carriage, education and accountability for personal hygiene, and instituting a strict and thorough cleaning and disinfection protocol of all contact areas for equine patients and areas of frequent staff contact, with an emphasis on those areas where MRSA-positive horses have been treated or contained must also be carried out..

11. Summary

Staphylococcus aureus is a facultative pathogen that is able to survive in the environment, and has the ability to cause colonization and infection in those susceptible. Due to a mutation, *S. aureus* evolved the penicillinase enzyme resulting in resistance to penicillin, and was also able to very quickly adapt to the use of methicillin, therefore becoming MRSA. MRSA has continued to evolve resistances to an alarming number of antimicrobial drugs in use today. The main strain of concern in Europe in horses is CC398, and the specific causative agent of concern at the DCEM is ST398, *spa*-type t011, SCC*mec type* IV. Clinical manifestations of MRSA infections in horses can range from mild to severe, with one of the most common presentations being wound or surgical site infections. It has been determined through various studies that equine patients can contract MRSA via the environment, as well as from clinic personnel where MRSA is endemic. Endemicity can cause various outbreaks of disease, and is a factor in the evolution of MRSA resistance to new antimicrobials. Screening for MRSA upon admission is beneficial, and the endemicity of MRSA in the general equine population in the surrounding areas should also be considered. Due to the nature of equine hospitalization, it is much more difficult to properly clean and disinfect stalls between patients, which is a major factor in nosocomial MRSA infections. Equine clinics also serve as an amplification setting for MRSA due to this. And while the zoonotic potential of MRSA CC398 is reportedly very low, there are some cases of equine MRSA strains causing human clinical infections. There is also evidence to support transmission of MRSA from clinic workers to other members of their household, with a high rate of detection from LA-MRSA strains, such as CC398. In the vast majority of cases, MRSA can be eradicated from asymptomatic equine carriers with the implementation of strict infection control and isolation protocols, without the use of antimicrobials.

Hand hygiene is also very important, in both general day to day practice, as well as before surgery. The use of alcohol for frequent hand disinfection between patients is beneficial in reducing transmission, as is the use of neutral soap and a 90 second alcohol wash of surgeons hands. Research has also shown that paper towels are the most sanitary way to dry one's hands after washing. Use of a mask for staff, especially when in contact with MRSA positive horses, may be beneficial to reduce human carriage and transmission.

UVC lights are the most effective long term method of sanitizing cell phones, with very little potential damage to the device with long term use.

In human patients, it is recommended to do follow-up MRSA testing for re-colonization approximately 1 year after negative status is achieved, and the most effective decolonization therapy includes mupirocin and chlorhexidine use together. Due to the ease of environmental persistence by *S. aureus*, it is important to properly disinfect stalls between patients, as well as maintain proper cleaning of the hospital setting with emphasis on high contact areas like door knobs. Another important consideration are dust levels, as MRSA is able to aerosolize and travel on air particles. For sampling of equine patients, it may be most effective to not only do a nasal sample, but also a skin sample from a frequently contacted area on the horse.

Acknowledgements

I would like to thank Dr. Ervin Albert for all his hard work and help in preparing this thesis paper.

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I hereby confirm that I am familiar with the content of the thesis entitled

„Combating Methicillin-Resistant *Staphylococcus aureus* at Equine Clinics: Recommendations for a Future Control Program at the Department and Clinic of Equine Medicine - Literature Review"

written by **Jordan Bagshaw** (ONAMZI) which I deem suitable for submission and defence.

Date: Budapest, 13. November 2023.

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