METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS*: ECOLOGY AND MOLECULAR EPIDEMIOLOGY OF ENVIRONMENTAL CONTAMINATION IN VETERINARY AND HUMAN HEALTHCARE SETTINGS DURING NON-OUTBREAK PERIODS

Dissertation

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ABSTRACT

Within healthcare settings, patients, healthcare workers (HCWs) and the environment interact in the transmission cycle of MRSA. In particular, the role that inanimate surfaces might play in this cycle is unclear, even though a plausible involvement of the environment has been described during outbreaks. Therefore, the main goal of this dissertation was to establish the ecology (presence, distribution and maintenance) and molecular characteristics of MRSA strains present in human and animal hospitals during non-outbreak periods. Yearlong active MRSA surveillances were established in two veterinary hospitals where contact surfaces and incoming patients were sampled every month. Similarly, monthly surveillance was performed for one year in two human general medicine wards, in addition to the collection of aggregated data of MRSA patient infection burden within these wards. The first two studies (Chapter 2 & 3) were performed in a small animal veterinary hospital. Overall, 13.5% of surfaces and 5.7% of incoming dogs were positive for MRSA. The majority of the isolates (≥80%) were SCCmec typeII/USA100. Gurneys, doors, and examination tables/floors were the most frequently contaminated surfaces. The third study (Chapter 4), performed in an equine hospital, found that 8.6% of the surfaces and 5.8% of the horses sampled were positive for MRSA. The majority of the isolates (≥60%) were classified as SCCmec typeIV/USA500. The most common contaminated surfaces were: computers, feed/water buckets, and surgery tables/mats. Molecular analysis performed in both veterinary hospitals showed that new pulsotypes were constantly introduced into the hospital and circulated throughout several areas and surfaces. Furthermore, maintenance of strains in
the environment was also observed when unique clones were detected for 2-3 consecutive months on the same surfaces. The fourth study (Chapter 5) performed at the human hospital demonstrated that 23.7% of the surfaces were positive for MRSA. The majority of the isolates (≥46%) were SCCmec typeII/USA100. Chart holders, medicine carts, elevators and doors were the most common contaminated surfaces. Similarly to the veterinary settings, analysis of contamination patterns showed the introduction and/or reintroduction of clones into the environment. Conversely, the frequent contamination of surfaces for 2-3 consecutive months did occur but with different pulsotypes. Over 85% of the strains recovered from the human and animal hospitals were resistant to three or more classes of antimicrobials, which represent a clinical and public health concern. Finally, the original results presented in this dissertation demonstrated that MRSA is frequently found in the environments of human and animal hospitals in the absence of an outbreak. It was shown that MRSA is constantly introduced and/or reintroduced into the hospital likely by patients, visitors and hospital personnel. Such influx of MRSA strains, along with lack or inadequate cleaning and disinfection protocols, promoted the persistence over time of this bacterium within the hospital environment. Most importantly, strains were moved across different areas of the hospitals (by HCWs and/or patients) allowing the spread of MRSA throughout each facility. Therefore, it was concluded that the hospital environment plays a key role as a reservoir and as a plausible source of MRSA within human and veterinary hospitals.
DEDICATION

To all the people that helped me and encouraged me
when I needed it during this long journey

Family, friends, and mentors…

You have my humble gratitude
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CHAPTER 1

METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS IN HUMAN AND ANIMAL HEALTHCARE SETTINGS: REVIEW OF CURRENT KNOWLEDGE

1.1 INTRODUCTION

Methicillin-resistant Staphylococcus aureus (MRSA) is a well recognized opportunistic pathogen frequently associated with hospital-acquired infections [1-3]. Every year, MRSA is responsible for causing severe infections in more than 80,000 people, and roughly, over 13% of such infections become fatal [4]. Furthermore, MRSA is also considered a zoonotic pathogen affecting a variety of animal species, and it has been found to be the 2nd most common occupational pathogen affecting veterinary personnel, in addition to being the 2nd most common infectious agent responsible for severe nosocomial infections in animal patients [5]. Although MRSA awareness within the veterinary community has heightened in recent years, there is much uncertainty regarding the establishment, spread, and public health implications associated with this pathogen in veterinary settings. Particularly, the role that environmental contact surfaces might play in the transmission of MRSA in both human and veterinary healthcare settings remains undefined. Without such knowledge, our ability to establish effective prevention and control measures against this nosocomial and zoonotic pathogen is severely limited.
Therefore, the main objective of this dissertation was to evaluate the epidemiology, contamination patterns and plausible sources of MRSA in the environment of three healthcare facilities (2 veterinary and 1 human) during non-outbreak periods.

Briefly, this dissertation begins with a literature review of MRSA as a nosocomial and zoonotic pathogen, transmission cycle within healthcare settings and current knowledge regarding MRSA environmental contamination in human and veterinary hospitals (chapter 1). The following chapters (2 through 5) describe the research performed in three different healthcare settings. Chapter 2 describes the presence of MRSA on environmental surfaces within a small animal teaching hospital, and chapter 3 evaluates the epidemiological profile of MRSA carrier dogs arriving at the same facility as well as their role (if any) in the contamination of the hospital environment. Similarly, chapter 4 reveals the results obtained from incoming patients and the environment of an equine veterinary center. Conversely, chapter 5 depicts the research performed in a large tertiary human healthcare facility regarding MRSA environmental contamination. Lastly, a summary of all the results obtained is described in the conclusion section (chapter 6), in addition to a brief description of future projects planned to continue studying the significance of MRSA environmental contamination in healthcare settings.

1.2 MRSA IN HUMAN AND ANIMAL POPULATIONS: IMPLICATIONS AND SIGNIFICANCE

When a single human body accommodates over 100 trillion bacteria, one can only be curious as to what and who these life companions are. One of the most commonly known bacteria present in the normal flora of humans is *Staphylococcus aureus*. 

1
According to the Center for Diseases Control and Prevention (CDC), roughly 25-30% of the United States human population is colonized with *S. aureus* [6]. In humans and in different animal species (e.g. cows, pigs, horses, among others) this bacterium commonly colonizes the skin, mucous membranes, urogenital tract and in some cases the gastrointestinal tract [7, 8]. However, this microorganism is not only capable of harmlessly colonize humans and animals, but it can also be the cause of life threatening infections and, therefore, it is considered an opportunistic pathogen. *S. aureus*, which is a facultative anaerobic Gram-positive cocci, can be armed with several virulent factors, from which some of the most important ones are the production of the coagulase enzyme, the Panton-Valentin leukocidin (PVL) toxin and the Toxic Shock Syndrome Toxin (TSST) [9]. Some of the consequences associated to the presence and expression of these factors are the alteration of the host’s immune response and cellular damage. As a result, some *S. aureus* strains (including MRSA) are more invasive, destructive and lethal than others, depending on the number and combination of virulence factors present in their repertoire.

Since the discovery of penicillin and methicillin, the treatment of choice for staphylococcal infections has been the use antimicrobials from the beta-lactam class, such as penicillin, ampicillin, amoxicillin, cefadroxil and cephalxin. However, some *S. aureus* strains developed a resistance against these drugs, and they are known as methicillin-resistant *S. aureus* (MRSA). This resistance is the result of the acquisition of the *mecA* gene present in a mobile genetic element, the staphylococcal cassette chromosome *mec* (SCCmec) [9, 10]. Under normal circumstances, an enzyme known as penicillin-binding protein (PBP) is essential to synthesized peptidoglycan in
staphylococci bacteria, which is one of the most important components of the bacterial cell wall. When staph infections are treated with beta-lactams, these drugs will actively bind to the PBPs active site due to their similar chemical structure to peptidoglycan, permanently blocking the activity of the enzyme. Consequently, this interaction causes a disruption in the formation of the cell wall, leading to the destruction of the bacteria [11]. With the acquisition of the mecA gene, S. aureus strains can express an alternate PBP (known as PBP2a). Both enzymes (PBP and PBP2a) have similar functions but PBP2a has a different active site, to which beta-lactam antimicrobials cannot bind and as a result the enzyme is not inactivated. Therefore, the bacterium proliferates even under antimicrobial pressure, and for this reason MRSA infections are difficult and expensive to treat. Moreover, in the last 40 to 50 years the number of MRSA isolates that are resistant to other classes of antimicrobials (including but not exclusively macrolides, lincosamides, aminoglycosides and quinolones) has been increasing, and they are usually referred to as multidrug resistant strains (MDR) [9, 12].

The first MRSA clinical case was reported in 1961 in the United Kingdom [13], and for the next thirty years this pathogen was solely related to hospital acquired (nosocomial) infections. Since then, hospitals around the world started reporting the occurrence of cases and outbreaks of MRSA infections in individuals with healthcare associated risk factors [12, 14, 15], and it became one of the most important pathogens present in hospitals. However, during the 1990s, MRSA infections were observed among healthy individuals living in the community or patients who had no recent contact with healthcare facilities [9, 12, 16, 17]. Moreover, it was observed that these new MRSA clones were genetically different from those previously observed in hospital settings [18].
For this reason, MRSA infections were classified as hospital acquired / hospital-associated / health care-associated (HA-MRSA) and community acquired / community-associated (CA-MRSA) based on the patient’s clinical epidemiology, as well as phenotypic and genotypic characteristics of the MRSA isolate. (see Table 1.1).

With every passing year, the case definitions for HA and CA-MRSA appear to be less accurate. Within the last decade, several reports have given examples of CA-MRSA strains causing healthcare associated infections, suggesting that traditional nosocomial MRSA strains are being overcome and/or replace by community strains in healthcare settings [18-25]. Similarly, HA-MRSA strains have been described to circulate within the community, in most cases associated with prior healthcare contact [1]. Consequently, a sub definition has been included in the hospital-associated category to better differentiate MRSA strains, known as health care-associated community onset (HACO). MRSA infections are classified as HACO when patients are diagnosed in 48h or less after hospital admission with at least one identified health care-associated risk factor [9, 26].

The time it will take to establish a new case definition due to the constant evolution of this pathogen remains to be determined.

1.2.1 Public Health Perspective

MRSA has become a well establish pathogen in healthcare settings and the general community, especially since it is so easily transmitted by direct (person to person) and indirect (fomites) contact [27-31]. Worldwide, the number of nosocomial MRSA infections vary between geographical regions and populations, but it has been reported to range from 0.6% in The Netherlands to 66.8% in Japan [18]. Currently,
MRSA colonizes over 4 million people in the U.S. (approximately 1.5-2% of the population) [32]. Of those, over 80,000 people develop severe infections every year and at least 11,000 of them succumb to such infections [4].

MRSA has been associated to skin and soft tissue lesions, bloodstream infections (BSI), pneumonia, endocarditis, osteomyelitis, among others [33, 34]. In many hospitals this pathogen has been reported to be endemic and infections are usually associated with patients that have undergone major surgery or that are in intensive care units (ICU) [35, 36]. Between 1992 and 2003, the prevalence of MRSA among the S. aureus cultures isolated from patients in ICUs went from 35.9% to 64.4% in the U.S. [37]. Another study that evaluated eight acute care hospitals found that MRSA was the pathogen most commonly associated with BSI (23%) followed by methicillin-susceptible S. aureus (MSSA, 10.6%) [38]. Moreover, MRSA was considered one of the leading causes of death by an infectious agent in the U.S. in 2005, with a 20% mortality rate in patients with severe invasive infections, similarly or exceeding other diseases like AIDS, tuberculosis and viral hepatitis [1, 35].

Since MRSA has become an important and frequently increasing problem in U.S. hospitals, several guidelines and strategies have been implemented to prevent and control the transmission of MRSA in and out of healthcare settings. As a result, the number of MRSA clinical cases in the U.S. has declined in the last few years [4, 39]. Nonetheless, MRSA is still considered one of the most important nosocomial pathogens present in hospitals, which represents a critical public health problem that requires further research to better understand and prevent nosocomial transmission within healthcare settings [6, 18, 35, 39-41].
In addition to the high morbidity, patient distress and mortality, it is estimated that the cost to treat hospitalized patients with MRSA infections is between $3.2 – $4.2 billions annually including the extra costs that patients are required to pay and the costs to hospitals (counting infection control and/or legal expenses for litigious cases). It has been reported that patients with MRSA infections have longer stays in the hospital and higher average costs than cases with MSSA infections [42-44]. An example of this was described by Ben-David et. al. where patients with MRSA BSI had a longer hospital stay compared to patients with MSSA BSI (20.5 days vs 10.5 days respectively) and the median total hospital cost was $113,852 vs $42,137 (MRSA vs MSSA) [43]. On the other hand, MRSA infections also represent an extra cost to healthcare settings which include the costs for infection control and (in some cases) the costs for litigious cases [45]. An example of this was described by Goetghebeur et.al. where the use of additional barrier precautions, antimicrobial therapy and laboratory investigations represented 19% (average of $2,321 per positive patient) of the costs of a MRSA infection. The other 81% (average of $9895) of the costs was represented by the extended hospitalization [44].

In the community, MRSA prevalences range from <0.5% in Europe to 11.1% in Australia [46, 47]. However, some authors suggest that such numbers appear to be increasing [46, 48]. In the U.S., it is estimated that 1.5-2% of the population carry MRSA in their nose [6, 32], but the exact number of people with MRSA skin infection in the community is unknown [6]. Nonetheless, based on the information obtained in 9 states of the U.S., the CDC Active Bacterial Core Surveillance estimated that 15,560 cases of invasive skin infection occurred in the general community in 2011 [49]. In addition, MRSA (especially CA strains) remains a common etiological agent associated with skin
and soft tissue infections in the U.S. [50]. Since colonized and/or infected people can directly or indirectly transmit MRSA, it is assumed that the high transmission rate among the community is associated with poor hygiene practices, activities that involve close skin contact (e.g. sports), crowded living conditions and sharing contaminated items [51-53]. Therefore, is it not surprising to observe outbreaks in athletes, prisoners, military recruits, children, drug users, among others, which are also considered as high risk populations in the community [20, 34, 53-55]. More importantly, changes have been observed in CA-MRSA clones (e.g. USA300, the most prevalent strain is the U.S.) which are becoming more virulent and resistant to more classes of antimicrobials while they migrate and slowly replace HA-MRSA clones in hospitals [18, 21, 56-58].

1.2.2 Zoonotic Perspective

MRSA is typically described as a human pathogen but it is a highly adaptable microorganism [59]. It has been found in several animal species, primarily dogs/cats (0-9%), horses (0-12%) and pigs (0-80%) [3, 7]. Colonization in animals appears to be transient and in most cases the pathogen is eliminated within weeks [3, 60]. However, as an opportunistic pathogen, MRSA is responsible for causing severe infections mostly associated with skin, ears and surgical wounds [7, 34, 61-63]. More concerning is the fact that MRSA is also considered a zoonotic pathogen, which is why colonized animals (especially pets) could act as a reservoir and as a source of MRSA infections or reinfections in humans [7, 12, 34]. Based on the high exposure (contact and time) that pet owners, farmers and veterinary personnel have with animals, they are considered as high risk populations for MRSA exposure and transmission [7, 64, 65].
When dealing with MRSA in animals, it seems that each species represents a different scenario. For example, in the case of household pets, the transmission of this bacteria is highly likely to occur due to the direct contact observed in owner-pet interactions (petting, bathing, licking, sharing couch/bed space, among others) or through indirect contact with contaminated household surfaces [66]. In the U.S., the most prevalent MRSA clone present in dogs and cats is known as USA100, which is also the most important HA strain found in humans in the same country [32, 67, 68]. In essence, the MRSA clones found in pets reflect those strains that are carried by colonized/infected people in close contact with them [3, 69, 70]. An example of this was described in a study performed in 30 households across the U.S and Canada [69]. The first part (n=22) of the study included homes where a pet (dog or cat) was diagnosed with a MRSA infection in the past 30 days. The second part (n=8) involved houses where one or more family members have had ≥2 MRSA infections within the past year. MRSA was found concurrently in humans and pets in 23.3% (7/30) of the households (from both parts of the study); however, isolates were only obtained from only 5 households. After molecular characterization (by PGFE and spa typing) the authors discovered that the MRSA isolates obtained from humans and pets within the same house were indistinguishable. In addition, 3/5 households were colonized/infected with the USA100 MRSA strain (most prevalent HA clone in the U.S). The other 2/5 homes were colonized/infected with the USA300 strain (which is the most prevalent CA clone in the U.S population) [69]. A similar study was performed in 122 households in Canada. However, in this case, the only inclusion criteria was for houses to have ≥ 1 dog and/or cat regardless of the previous history of MRSA infections in owners or pets [70]. The authors found
concurrent human and animal *S. aureus* colonization in 8.2% (10/122) of the households (8 houses with dogs and 2 with cats). After molecular characterization, 50.0% (5/10, 4 with dogs and 1 with a cat) of those households had indistinguishable MRSA strains present in owners and pets. Unfortunately, no concurrent MRSA colonization was found [70]. Nonetheless, these findings are further proof of the MRSA interspecies transmission, and the similarity of MRSA strains colonizing/infecting humans and their pets.

Similarly to what has been observed in pet populations, the transmission between horses and humans has been reported [62, 71-73]. However, USA500 is the most prevalent MRSA clone in horses and it is not very prevalent in the general human population. Nevertheless, USA500 is commonly isolated from people in close contact with horses, like equine veterinary personnel and horse owners [3, 65, 74-76], highlighting again the possibility of animals acquiring the bacterium by contact with colonized and/or infected humans and in turn animals serve as a source of re-colonization or re-infection for humans [61, 71].

A slightly different scenario occurs in food animals, specifically in the swine population. In the last decade, a new MRSA strain named ST398 emerged in swine and it is known as livestock-associated MRSA (LA-MRSA). The first cases were described in The Netherlands but currently it has been found in several European countries, Canada and some states of the U.S. [12, 77-79]. ST398 is unique among all the MRSA clones because it originated in swine populations instead of humans [79]. In addition, ST398 is capable of producing long term colonization in pigs, which differs from what is has been described with other MRSA strain in other animal species [80, 81]. Nonetheless, ST398
can be zoonotically transmitted and cases of colonization and/or infection have been reported in pig farmers and veterinarians [3, 12].

Regardless of the scenario or animal species involved, there is no doubt that zoonotic transmission (directly or indirectly) of MRSA between humans and animals occurs and it is bidirectional [82]. In most cases, with the exception of ST398, it is very difficult to establish the origin of the bacterium (human or animal). Nonetheless, since the clones observed in animal populations are usually related or indistinguishable to those found in the humans in close contact to them, it seems that animals serve mostly as a reservoir and a source of MRSA only after they acquire the bacteria from their human caregivers [71].

1.3 MRSA AS A NOSOCOMIAL PATHOGEN IN HUMAN AND ANIMAL HOSPITALS

1.3.1 Nosocomial Scenario and Plausible Sources of MRSA in Human Healthcare settings

Nosocomial or healthcare-associated infections (HAI) are defined as those infections that are associated with clinical treatments and hospital environments. The transmission of the microorganism responsible for the infection is acquired in the hospital, most likely as a result of invasive medical procedures (e.g. measures involving central lines, catheters and/or ventilators) and procedures that occur in the institution. According to a study performed in U.S. acute hospitals, approximately 721,800 HAI occurred in 2011. Among the 481 microorganisms reported in the study, *S. aureus* was the second most common pathogen associated with these HAI [83]. In particular, the
authors estimated that at least 9,700 hospital-onset cases of MRSA bacteremia occurred during the same year [83], which is only one of the many complications (e.g. pneumonia, osteomyelitis, cellulitis, endocarditis, wounds or surgical incisions, among others) this pathogen is associated with in healthcare settings [49].

The occurrence of isolated cases of HAIs is not the only concern. Every year numerous reports of nosocomial outbreaks caused by MRSA are described (see Table 1.2) [19, 28, 84-117]. Ultimately, after longer hospital stays, treatments or decolonization procedures, outbreak investigations, establishment of preventive measures and the cleaning/disinfection of hospital areas result in substantial extra costs for the patients, hospitals and insurance companies, all these in addition to an increase in the physical and mental stress that most affected patients undertake [118-120].

Generally, outbreaks start with an index case (usually a patient) that is colonized or infected with MRSA. Then, this patient becomes the source of this pathogen from where the outbreak strain is transmitted to other patients and/or healthcare workers (HCWs) [19, 95, 104]. The spread and severity of the outbreak will depend on several factors that mainly involve other patients (e.g. density or physical closeness between one another), healthcare workers (e.g. colonization/contamination/infection rates) and fomites (e.g. contamination of hospital contact surfaces). In most cases, analysis of epidemiological data and molecular characterization of isolates allows researchers to determine the index case. However, establishing the route and directionality of transmission is a more difficult and complex task. An example of this was observed during the first MRSA outbreak in a Danish neonatal intensive care unit (NICU) [106]. During the outbreak period 32% (32/99) of the neonates present in the Glostrup NICU
were colonized with the same MRSA strain spa-type t127. The index case was a triplet that was transferred from another hospital located in Copenhagen (along with the other 2 siblings) 34 days prior to be identified as MRSA t127 positive in a pharyngeal swab. The authors assumed that the triplets were exposed to the MRSA strain at the Copenhagen hospital, where they were delivered and located in a room adjacent to an isolation room occupied by a neonate positive for MRSA t127. There was no other suspicion of another source introducing the outbreak strain into the Glostrup NICU, even though family members from positive babies (20%, 13/68), HCWs (1.2%, 2/161) and the hospital environment (2 locations, total number of samples was not specified) were found positive for the outbreak strain during the same period. Cesarean section and nasal continuous positive airway pressure treatment were identified as risk factors for MRSA acquisition in neonates. Since both of these procedures may require extra handling and closer contact between HCWs and the babies, some of the measures established to control the outbreak involved barrier precautions and isolation procedures, along with intensified cleaning and disinfection of the environment. The outbreak was successfully contained through infection control procedures and, even though the index case was identified, the authors refrained from discussing how this pathogen was spread throughout the unit [106].

Similarly, the index case could be represented by a colonized HCW instead of a patient [27, 28, 99]. Such was the case described by Ali et. al. where three neonatal patients in England were found positive for a MRSA clone that has never been seen before in the region (South West Pacific clone) [99]. It was later discovered that one staff member (who was in contact with all 3 babies) was an asymptomatic carrier of the same clone. Furthermore, it was assumed that the colonized HCW acquired this particular
strain during a trip to the Philippines (where the South West Pacific MRSA clone is prevalent [40, 121]) only months before the outbreak started [99].

Alternatively, contaminated objects or surfaces (fomites) can also serve a role in the propagation of MRSA throughout the hospital areas and to patients, in the presence or absence of outbreaks [64, 122-124]. These fomites are usually represented by inanimate surfaces present in the environment of healthcare settings. An example of this was observed in a prospective study conducted in an intensive care unit (ICU) for a 14 months period [29]. In each of the 24 sampling events, samples were collected from patients and four areas adjacent to their bed space (underneath the bed, the workstation, the control buttons on the monitors, and a ledge positioned behind the bed). Throughout the study, no changes were made in cleaning and disinfection protocols routinely performed in the unit, which follows the National Health Services Standards. When evaluating environmental contamination, overall MRSA was found in 21.8% (188/864) of the surfaces sampled and it was present even in areas next to non-colonized patients. The area underneath the bed was the most prevalent one (37.5%, 81/216) followed by the monitors (19.9%, 43/216), the workstations (17.1%, 37/216) and the ledges behind the bed (12.5%, 27/216). When environmental contamination was compared to patient colonization, in 35.7% (20/56) of the occasions indistinguishable MRSA isolates were obtained from patients and their nearby environment concurrently. In addition, the authors reported that during the times of environmental screening 61 patients were colonized with MRSA, and 26 of them acquired this pathogen during their stay in the ICU. Twelve of these 26 patients acquired a MRSA strain that was also colonizing at least one other patient during their stay, highlighting the possibility of direct or indirect
transmission between patients. However, the other 14/26 cases acquired MRSA strains that were distinct from the ones found in other patients. In particular, 3 of those 14 cases were colonized with MRSA strains that were detected in the ICU environment within 3-10 days before the patients were found positive. The hypothesis that the hospital environment was the source of the MRSA strains in these 3 cases is supported by the fact that no other patients were found positive 7 days (2/3) and 54 days (1/3) before these cases were diagnosed with MRSA. The authors did not describe which environmental locations were positive with the same clone, and neither did they specify if those surfaces were adjacent to the patient’s bed. Unfortunately, HCWs were not screened during the study period. Therefore, their contribution (if any) in the transmission of the MRSA strains found in the environment to susceptible patients was not established and should be considered as a possibility when interpreting the results obtained. Nonetheless, these findings demonstrate that even if environmental surfaces were not the main (direct) source of MRSA for patients, they still played a big role in the transmission of this pathogen within the ICU unit. In addition, the authors also emphasized the need for intensive and more effective cleaning and disinfection protocols that should include areas with and without colonized patients to minimized environmental contamination [29].

1.3.2 Nosocomial Scenario and Plausible Sources of MRSA in Veterinary Healthcare Settings

In veterinary hospitals, MRSA transmission occurs similarly to human settings, with two major differences. First, veterinary personnel usually have a much closer and longer contact with their patients than human HCWs. Second, animals (as patients) have
a much closer contact with their surrounding (e.g. sniffing, licking or lying on the floor), in this case the hospital environment. Even with this higher exposure for patients and personnel, the number of veterinary outbreaks reported in the literature is much less compared to humans (Table 1.3) [72, 73, 125-130]. Several scenarios could be related to this phenomenon: outbreaks occur but are not reported, outbreaks occur but are not detected (since active surveillances are less common in veterinary settings), or in reality less outbreaks take place in veterinary settings. Regardless, nosocomial infections and zoonotic transmission do happen as described on an NICU of an equine veterinary hospital [72]. At the time of admission, a foal and its dam were found positive for MRSA. Only days after this event, 3 students that have been in close contact with the patients developed skin infections that were later confirmed as the same MRSA strain. Screening of the hospital personnel found 10 other staff members (including students, clinicians and technicians) colonized with the outbreak strain. The authors explained that protective barriers (e.g. gloves and clean coveralls) were mandatory at all times while handling the patients. However, “contact with horse bodily secretions is inevitable because of the duration of the contact and the sometimes fractious or excitable nature of foals” [72]. When the outbreak investigation was completed, the authors concluded that the foal and dam were the most probable source of the MRSA strain that was then transmitted by direct contact to the hospital personnel [72].

Another unrelated outbreak was described on the same institution two years later, this time in their small animal ICU. After the first MRSA case was identified, all the ICU personnel was notified about the patient, hand hygiene was emphasized and infection control practices were implemented (including minimizing the contact with the patient,
and the use of gloves and disposable gowns) to prevent the spread of the bacterium. Regardless, within two weeks, 23% (6/26) of the patients (dogs and cats) that were treated at the ICU became MRSA positive with the same strain as the index case. Since the animals had very little contact between them, the authors assumed that the most plausible route of transmission was by indirect contact and/or personnel-born transmission [126]. Both of these reports highlight the importance of the close contact/exposure of veterinary personnel and the contamination of their hands, gloves and/or gowns when treating positive patients in the plausible nosocomial and zoonotic transmission of MRSA within veterinary settings.

A good example of plausible nosocomial transmission involving a colonized veterinary surgeon as the source of MRSA, was described in an orthopaedic referral veterinary hospital [131]. During the study, 10 veterinarians, 12 nurses and 4 reception/domestic staff were screened for MRSA for 18 months. Of those, 2 orthopaedic surgeons and one nurse were found positive for MRSA. Surgeon A was colonized with MRSA for at least a year without developing any related infections, surgeon C was colonized during two sampling periods (3 weeks apart) and the nurse was positive only once. Decolonization was not performed in surgeon A, however, under his/her general practitioner’s direction surgeon C and the nurse were successfully decolonized. The authors reviewed the medical records of all patients that underwent orthopedic or spinal surgery during the same period. Of the 180 surgeries performed by the colonized surgeon A, 4 cases (2.2%) developed wound complications caused by MRSA. Conversely, none of the 141 surgery cases performed by surgeon C developed MRSA infections. These results highlight the possibility of MRSA transmission from the long-term colonized
surgeon to the patients. Antimicrobial resistance patterns were indistinguishable between the MRSA isolates, but molecular characterization was not performed [131].

In conclusion, there is strong evidence that nosocomial transmission of MRSA occurs within human and veterinary healthcare settings involving patients, hospital personnel and/or the hospital environment. Establishing the transmission directionality and source(s) of the outbreak strain can be a cumbersome task. Some of the tools use for such purposes includes the analysis of epidemiological data and the molecular characterization of all MRSA isolates obtained during the outbreak. However, more often than not, this information is very difficult to ascertain and the final answer is usually a combination of assumptions and possibility. Nonetheless, regardless of the source, the implementation of control and preventive measures is key to minimize the plausibility of zoonotic and nosocomial transmission within veterinary settings. In the case of positive patients, these measures should include the appropriate use of personal protective equipment (PPE, e.g. gloves, gowns, coveralls) to reduce and/or avoid direct skin-to-skin contact between the staff and patients. Additionally, the isolation of MRSA positive patients will reduce their contact with other patients and the hospital environment, minimizing the risk of spreading the bacterium within the facility. In the case of colonized/infected personnel, compliance with hand washing as well as the proper use of PPE could decrease the risk of nosocomial MRSA transmission to susceptible patients. Lastly, fittingly routine cleaning and disinfection protocols must be establish to control environmental contamination and prevent the maintenance of MRSA on hospital contact surfaces.
1.4 TRANSMISSION CYCLE OF MRSA IN HEALTHCARE SETTINGS

As it was briefly described in the previous section, three components (patients, HCWs and environment) are usually involved in the transmission of MRSA within human and veterinary hospitals. However, a more detailed analysis of available epidemiological information is necessary to first, establish a plausible transmission cycle of MRSA in healthcare settings and second, to better understand how these sources interact in such cycle.

Within healthcare settings, the sources of MRSA for a susceptible patient could be classified as endogenous and exogenous (see Figure 1.1). An endogenous source involves the self inoculation of an already colonized patient that undergoes an invasive procedure (e.g. surgery) and infects itself with the MRSA strain that is already present in him/her body. This scenario has already been proven in human surgical patients, were those that were nasally colonized had a 9-fold greater odds of developing a surgical site infection compared to patients that were not colonized [132]. On the contrary, an exogenous source consists in the participation of an external component (another colonized/infected patient, a colonized/infected HCW or a contaminated environment), and how transmission occurs in these cases will be further discussed below.

**Patients.** There are 3 scenarios where colonized or infected patients can be involved in the nosocomial transmission cycle of MRSA. First, they are capable of shedding MRSA and contaminate the hospital environment, especially the areas in close proximity to them (surfaces and air) [29, 30, 133]. The amount of bacteria shed could vary mainly depending on which anatomical sites are colonized or infected [133]. Transmission occurs by direct contact of the environment with body secretions, skin
lesions, or simply hand touch. Several studies have already determine the presence of MRSA strains in the environment (contact surfaces and air) that are indistinguishable (by molecular typing) to those present in the patient [29, 30, 134-137]. Second, colonized/infected patients can directly transmit the bacterium to HCWs most likely when the personnel are very close to the patient during examinations or administration of medicaments [27]. In this case, the HCW could develop clinical symptoms of an infection (as it was observed in a dermatology ward [95]), could become colonized [138], or could simply be temporarily contaminated and act as a vehicle to spread the bacterium to other patients or environments [139]. This vector/vehicle role has been described and proven by experimental studies, and it is highly associated to the contamination of the personnel’s hands or gloves when they are working in close proximity and interacting with the patients [139]. Third, colonized/infected patients could be capable of directly transmitting the bacterium to other patients in the hospital [138, 140]. However, since direct patient-to-patient interaction in hospital settings is extremely limited (almost non) this scenario is only an assumption of a situation that could potentially happen.

**Medical Personnel.** There are 3 scenarios where HCWs participate in the transmission of MRSA, and they depend on the carrier status of the personnel. First, when a HCW is colonized/infected, they could act as a reservoir for the bacterium and are capable of contaminating the hospital environment by direct contact, fomite that could later be in contact with a patient. Second, it is well know that a colonized/infected HCW can also transmit the bacterium to any susceptible patient that is under their care, or to other HCWs, by direct contact through their contaminated hands and/or clothes [27, 28]. The third scenario involves HCWs that simply act as a vehicle. As described in the
previous section, these HCWs are simply contaminated (most likely their hands and/or clothes) with MRSA and under the right conditions they could potentially transfer the bacteria to susceptible patients and/or the environment [30, 139].

**Hospital Environment.** There are 2 scenarios where contaminated contact surfaces participate in the transmission of MRSA. First, HCWs in close or direct contact with positive surfaces could simply contaminate their hands/clothes and serve as a vehicle for transmission to patients and other HCWs [30, 31]. Alternatively, they could acquire the bacterium and become colonized or develop an infection and serve as a reservoir of the bacteria for patients and other HCWs [141], as described before. Second, patients could acquire the bacterium by direct or close contact with a contaminated surface and become colonized/infected; in turn they could also transmit the bacteria to susceptible patients that are in contact with them [29]. In fact, it has been suggested that when a new patient is admitted to the hospital and placed in a room that was previously occupied by a MRSA case, the new patient has 1.4 times the odds of developing a MRSA infection compared to patients staying in rooms previously occupied by negative patients (mostly likely related to contaminated fomites present in the room) [142]. This scenario is of major concern in the veterinary field due to the closer contact and interaction that animal patients have with the environment compared to human patients. In both scenarios, the evidence that has been found in the literature is only circumstantial, reflecting the plausibility of transmission as well as the necessity to develop research projects to have clearer answers.

Lastly, in the particular case of animals, colonized/infected owners should also be considered as a plausible source of MRSA for animals since they can act as reservoirs in
their households/farms [143]. It is important to highlight that usually there is a very close interactions between owners and their animals. In the case of household pets (dogs and/or cats), these interactions occur daily, including but not exclusively to feeding and washing bowls, spending time on the couch, playing and/or exercising, grooming (e.g. bathe, brushing and trimming nails), training and sharing bed space during the night hours. In the case of larger species (e.g. horses) the interaction might not be as frequent but the exposure is almost the same since the majority of the activities described before also apply. The biggest differences in regards to animals might be observed in the case of farm animals (e.g. cows and pigs), but farmers still check their animals daily and perform a lot of hands on activities that guarantee direct contact between them (e.g. treating sick animals and vaccinations). Essentially, the close contact between owners and animals may facilitate the transmission of MRSA between them, and therefore, it could increase the chances of developing infections in susceptible individuals.

1.5 MRSA IN THE ENVIRONMENT

1.5.1 MRSA Prevalence and Commonly Contaminated Surfaces

Every day, humans and animals live their life surrounded by diverse environments, including but not exclusively households, work space and public areas (e.g. schools, parks, grocery stores, hospitals and farms). Each of these locations is unique in the number and type of surfaces they have, number of people and/or animals that are in contact with them, how often they are cleaned and how much exposure they have to the elements.
Healthcare settings are often visited by sick individuals (human or animal patients) with active MRSA infections seeking treatment. In addition, hospitals are also frequently visited by a great number of otherwise healthy people (visitors and hospital staff), and it is highly likely that some of them will be colonized with MRSA (prevalence vary between regions). Both of these populations (sick and healthy) bring this bacterium into the hospital and are capable of transfer MRSA directly and/or indirectly to susceptible individuals. In addition, considering that only a single touch is required to contaminate any given surface, and that hospitals are “enclosed” spaces that harbors a large number of sick and healthy individuals carrying microorganisms, it is highly likely that environmental surfaces around these settings will be at some point contaminated by pathogens like MRSA.

The level of MRSA environmental contamination found on a given hospital, and accurate detection of such contamination, will depend on several factors. Some of these include how much of the bacterium is shed by colonized/infected individuals, sampling methodology, type of surface (e.g. type of material, texture and location), cleaning/disinfection products and technique applied, and sampling period (outbreak or non-outbreak) [144, 145].

During the last 15 years, studies have been performed to establish MRSA environmental contamination in healthcare settings (human and veterinary) (Tables 1.4 and 1.5) [29, 30, 36, 64, 94, 123, 124, 131, 134-136, 146-158]. However, the lack of a standard sampling methodology causes major differences between studies in regards to surfaces sampled, sample collection method, culture methods and cleaning/disinfection protocols; therefore, results are hardly comparable. In addition, a greater part of them
have been performed during outbreak investigations, to evaluate a new cleaning/disinfection protocol or product, or for very short periods of time (with the exception of some long term studies showing data from interrupted samplings). There are only few studies that have been performed for long periods and with consecutive samplings of the same contact surfaces [36]. Such studies are necessary to better understand the role that the hospital environment might play (as a source and as a reservoir) in the transmission cycle of MRSA within healthcare settings.

Regardless of the differences between study designs and the methodology applied, there are two major aspects to consider when evaluating MRSA environmental contamination, which surfaces are most commonly contaminated and for how long such surfaces can be contaminated. First, it is necessary to establish which areas or surfaces are most commonly contaminated that could potentially become “hot spots” of MRSA contamination. In the case of human healthcare settings surfaces could be classified as personnel contact, patient contact and general public contact depending on who touches the surface most frequently (or exclusively in the case of personnel contact) and their locations. Patient contact surfaces tend to be heavily contaminated [159], and big differences have been described in regards of environmental contamination present in rooms of MRSA positive cases and rooms of negative patients (58.8% vs 23.3% of environmental contamination respectively) [149]. Examples of these surfaces are bed linen/mattresses/pillows, bed rails/frames, and tables next to the beds, with prevalence ranging from 6 - 60% [30, 36, 134-136, 149]. Other highly prevalent surfaces are floors (in most cases the area around and below the beds) with a prevalence as low as 9% to up to 60% [29, 30, 123, 135, 136]. Humans in general (patients and HCWs) hardly have any
direct contact with floors and therefore, exposure to this contaminated surface is very limited. However, one study suggested that floors could play a passive role in the transmission cycle of MRSA within hospitals, specifically by transferring the bacterium to high contact surfaces via the movement of contaminated dust in air currents [29]. Such hypothesis is supported by another study describing that MRSA-containing particles were present in the nearby air during the process of bed-making in rooms of MRSA positive patients [160]. Another important surface with prevalence ranges from 4-59% [30, 123, 135, 148] is door handles, even though they are considered high-touch surfaces and it is recommended that they should be cleaned more frequently than minimal-touch surfaces [161]. Contamination of medical equipment and workstations with prevalences from 13% to 33% [29, 30, 123] does come as a surprise, especially since in most cases the medical staff are the only ones regularly touching such tools. Moreover, these pieces of equipment (if contaminated) could represent a higher risk of MRSA transmission to patients every time the equipment is used on them. Lastly, with the advance of technology, electronic equipments were slowly introduced into healthcare settings to facilitate and improve the management of data. Electronic devices like desktop computers, laptops and monitor touch screens are some of the examples of the new equipment that assists HCWs 24/7. Unfortunately, this equipment also represents a new challenge since they require more sophisticated and unique cleaning techniques. Therefore, it is not surprising to find these personnel contact surfaces as some of the most contaminated around hospitals in more recent studies (up to 20% of surface contamination) [29].
In the case of veterinary settings, the number of studies performed in the last
decade to establish MRSA environmental contamination are less numerous than those in
human healthcare settings (Table 1.5). In any case, the overall MRSA prevalence ranges
from 0.0% to 12.0% [64, 124, 131, 153-158], significantly lower when compared to
studies in human hospitals, which could be attributed to the fact that *S. aureus* is not part
of the normal flora in animals. In addition, prevalences between human and animal
contact surfaces vary particularly during non-outbreak investigations. Conversely, during
outbreak periods, animal contact surfaces become more prevalent probably due to the fact
that animals with active MRSA infections are being treated and shedding the bacterium
in their surrounding areas [153]. Examination/lying floors, cages and stalls are some of
the most commonly contaminated animal contact surfaces, with a prevalence as high as
62.0% during active MRSA outbreaks [153], which is expected especially due to the
close contact that animals have with the floor of their stalls/cages. On the subject of
human contact surfaces, very similar results are observed and compared to human
hospitals, where doors, computer keyboards, telephones and stethoscopes are among the
most prevalent MRSA contaminated surfaces [124, 154, 155, 158].

Of concern is the fact that the majority of the surfaces described above are
touched by numerous people in the hospital. HCWs in particular have been under the
microscope after studies demonstrated how successful is the transmission of pathogens
like MRSA from contaminated surfaces into the hands and gowns of hospital personnel
[30, 31]. For example, Bhalla et. al. evaluated the frequency of acquisition of pathogens
on the hands of HCWs after they have been in contact with environmental surfaces in
rooms occupied with patients vs empty cleaned rooms (after patient discharge). They
reported that roughly 30% of the HCWs hands that were in contact with surfaces in occupied rooms were positive for *S. aureus*, compared to less than 10% of hand contamination in HCWs that touched surfaces in cleaned unoccupied rooms [31]. Additionally, another study performed in rooms occupied by patients colonized or infected with MRSA reported that 58% of the gloves and 65% of the nurses’ gowns were contaminated with MRSA following routine nursing-care activities on the patients or after being in contact with contaminated surfaces near the patient. Therefore, contaminated hands of HCWs could serve as a very efficient vehicle for transmitting MRSA to patients, other HCWs and/or other contact surfaces [30, 31].

In addition to establishing which contact surfaces are most commonly contaminated, the second aspect to consider when evaluating environmental contamination is the length of time that surfaces remain contaminated with MRSA. One explanation for this persistence is the possibility of having the same clone or strain continuously contaminating the same surface for weeks or months. Such survivability could be even longer if there is a lack of appropriate cleaning and disinfection. However, this scenario of survival over time has only been described during in vitro experiments [122, 144, 162, 163]. Alternatively, another explanation for the continuous persistence could be due to a continuous re-contamination of the surface with different clones instead of the same strain surviving over time. For example, MRSA clone A could be contaminating surface A at one sampling point, but in the next sampling date the surface could be contaminated with MRSA clone B (after the removal or perish of clone A). To be able to prove that these scenarios could normally occur in healthcare settings, the hospital environment (same surfaces) must be continuously sampled for long periods of
time, and all the isolates recovered must be genotypically characterized (e.g. using PFGE) to establish their clonality. Since the majority of the studies evaluating environmental contamination have been performed for short periods, and mostly during outbreak investigations, the current available data only suggests that these scenarios of survival over time and re-contamination plausibly occur.

Based on the information obtained from epidemiological data of each hospital in particular and the molecular characterization of the isolates obtained from their environments, control and prevention measures (including cleaning and disinfection protocols) should be adapted to minimize the presence of “hot spot” surfaces and reservoirs in the hospital environment. Some of the strategies that could be used to eliminate environmental reservoirs of MRSA contamination include: verifying that cleaning and disinfection protocols are properly followed, confirm that the cleaning products are being prepared and used at the correct doses/dilutions, and/or changing the cleaning product altogether for something more effective. For controlling hot spot surfaces that are constantly contaminated with different bacterial clones, strategies could include: increase the number of times the surface is cleaned/disinfected per day, assess the HCWs compliance with hand washing, and verify that PPE (e.g. gloves, gowns) are appropriately used especially when caring for infected patients.

Some of these measurements have already proven to be helpful, as it was described by Dancer et. al. in a study performed to evaluate the impact of one additional cleaner in the rate of environmental contamination in two surgical wards [159]. During a yearlong study, hand-touch sites (including bedside areas and clinical equipment) were screened weekly and patients were monitored for the development of MRSA infections.
As expected, after the enhanced cleaning was implemented in one of the wards the authors found a 32.5% reduction in the level of environmental contamination compared to the ward that continued their normal cleaning routine. More importantly, a 26.6% reduction in new MRSA infections was observed on the ward receiving the improved cleaning, even though MRSA positive patients stayed in this ward for more days compared to the control ward. The authors concluded that reducing the level of environmental contamination apparently has an effect in the number of patients that develop further MRSA infections during their stay in the hospital. Additionally, the authors estimated that this reduction in the number of new MRSA infections saved the hospital £30,000 to £70,000 (roughly $44,000 to $104,000) during the study period [159].

Similar results were reported in another study that described a MRSA outbreak that lasted over 2 years in a male general surgical ward and affected 69 patients total [123]. Standard infection control and outbreak measures (including closure, emptying and cleaning of ward bays) were instituted on 4 different occasions and failed to eradicate the outbreak. Therefore, a year and a half after the outbreak started, new measures were initiated and the whole ward was closed for deep cleaning and disinfection. In addition, from that point forward, the number of hours spent in the routine domestic cleaning was doubled (from 66.5 to 123.5 hours per week), emphasizing on dust control over surfaces, furniture and floors. All other areas from the ward continued to be cleaned following the routine hospital policy. Before the intervention, 30 patients acquired the MRSA outbreak strain during their stay on the ward. Contrary, only 3 patients acquired the outbreak strain in the 6 following months after the enhanced cleaning and disinfection was implemented, demonstrating that the outbreak could not be controlled until the pathogen was eliminated.
from the ward environment [123]. Both of these studies highlight the role that environmental contamination has in the maintenance of MRSA within healthcare settings, therefore increasing the probability of susceptible patients to be exposed and develop nosocomial infections.

1.5.2 MRSA Survival in the Environment

In regards to survival outside a suitable host (human or animal), MRSA (as any other staphylococci bacteria) has the ability to survive in a wide range of temperatures, humidity, desiccation and sunlight exposure. These abilities give the bacteria all the necessary tools to survive on different contact surfaces. In vitro, studies have found that *S. aureus* (including MRSA) persists on inanimate surfaces between 7 days for up to 7 months [122, 144, 162] with some variations between strains [163]. An example of this was described by Wagenvoort et.al. where the influence of desiccation on bacterial survival between outbreak and sporadic MRSA isolates was evaluated [163]. The tested isolates survived longer than 6 months, but the two outbreak strains survived 1-3 months longer compared to the sporadic isolates [163]. Another study evaluated survival times of MRSA and MSSA isolates on 5 different materials commonly found in hospitals (cotton, terry, cotton-synthetic blend, polyester and polyethylene) [162]. Even though variations were observed between materials, both MRSA and MSSA survived longer (between 40-51 days and 22-90 days respectively) on polyethylene [162], highlighting the possibility of MRSA clones to survive in vivo on hospital contact surfaces for long periods of time. The differences denoted between these two studies in regards to the time that a strain is capable of surviving on a surface, could be attributed to the inoculums size, the types of
materials evaluated and laboratory conditions. Nonetheless, there are other factors (not evaluated in the studies described above) that could be associated to the survivability of a particular clone (e.g. the ability to produce biofilms) [164]. However, as it was described in the previous section, to provide proof of this scenario occurring in vivo, environmental samplings must be performed continuously for long periods of time and isolates detected must be genotypically characterized. Currently, only few studies have reported results from long-term environmental surveillances with enough positive surfaces to follow over time. For example, a prospective study performed in a 9-bed ICU collected environmental samples from each of the nine bed spaces present in the unit [29]. Samplings were performed monthly for up to 6 consecutive months on 3 different occasions. After sampling 216 spaces in 24 sampling dates, MRSA was found in 21.8% of the surfaces. PFGE characterization of the isolates allowed the authors to detect certain pulsotypes contaminating the ICU environment for consecutive samplings dates. However, the authors did not specify if either consecutive contamination occurred on the same surface/bed-space nor for how many consecutive months the same pulsotype was found in the environment [29]. Another study performed in 3 hospitals for 11 uninterrupted months found that 1.3% of the surfaces sampled were positive for MRSA, mostly cause by 1 to 3 dominant clones [36]. However, then again, the authors did not specify either if the contamination occurred for consecutive months or if it happened on the same surface [36]. Based on this information, it is not possible to establish if MRSA long-term survival actually occurs beyond the walls of a laboratory where in vitro experiments take place.
Environmental survival of MRSA strains over time can be affected by extrinsic factors related to outer conditions, as well as intrinsic factors associated to the bacteria [162, 163, 165-168]. In the case of extrinsic factors, the characteristics of the surface (including type of material and texture) [165-167], as well as environmental conditions [163], could have an effect in the persistence of MRSA contamination over time. In vitro studies have described an increase probability of survival in materials like polyester and polyethylene plastic compared to cotton, terry and blends (60% cotton – 40% polyester) [162, 165]. The most feasible explanation for these findings is related to the presence of crevices or niches on a particular surface where the bacteria could proliferate, especially since these narrow spaces are harder to access through regular cleaning and disinfection [165]. Fortunately, *S. aureus* strains that have acquired the *mecA* gene and became MRSA only gained the capacity to be resistant to beta-lactams antimicrobials. Therefore, as any other staphylococci bacteria, MRSA is still susceptible to a wide range of cleaning and disinfectant products [6]. However, these products must be use appropriately (including accurately diluting the product and applying it for the recommended time) to be able to efficiently eliminate environmental contamination. Unfortunately, every now and then even appropriate routine cleaning and disinfection protocols (and sometimes more aggressive measure like the use of hydrogen peroxide vapor) are simply not enough to eliminate all bacterial particles from contact surfaces [148, 169], suggesting the potential involvement of factors inherent to the bacteria in the microorganism persistence.

The most notorious intrinsic factor associated with staphylococci survival on over time is their capability to produce biofilms [168]. These structures consist on the aggregation of a bacterial community that constructs a polymeric conglomeration of
extracellular DNA, proteins and polysaccharides around them to create a “mesh or film” structure on a given surface [164]. As a result, the film works as a protective layer for the interior community of microorganisms against antimicrobials, cleaning and disinfectant products, and adverse environmental conditions; therefore, biofilms are categorized as a non-specific mechanism of resistance [168]. An example demonstrating how well these biofilms work was presented in a recent study evaluating the efficacy of 3 different biocides against MRSA biofilms. The authors found that when the bacteria produces biofilms, the biocides were ineffective against the microorganism at the normal label concentrations recommended for their use [170]. As a result, it is reasonable to assume that biofilm producer MRSA strains could be more adapted to survive for longer periods of time on surfaces, increasing the chances of creating reservoirs of bacteria in hospital environments.

1.6 CURRENT PROTOCOLS FOR SCREENING AND SURVEILLANCE OF ENVIRONMENTAL MRSA CONTAMINATION IN HOSPITAL ENVIRONMENTS

Since MRSA became a problem in healthcare settings, opinions on how to approach the possible problem that the environment represents in the transmission of nosocomial MRSA has varied over time. In the late 1970s and early 1980s, studies determined that the role of the environment was insignificant [171, 172]. As a result, there was much uncertainty regarding the implementation of surveillance programs, and the number of studies performed including environmental samplings declined drastically [144]. Despite the rise of CA-MRSA in the early 1990s [17, 25] and the new discoveries
that provided support for the role that the environment plays in MRSA transmission [29-31], today only minimal changes in regards to surveillance and monitoring programs have been observed. In fact, in the CDC guidelines for environmental samplings screening of contact surfaces is only recommended to be performed during outbreak investigations [161]. Nonetheless, with the rise of new technologies and discoveries, the role of the environment is little by little returning to the debate table.

One of the biggest problems that the scientific community is facing at this time is the fact that there is no standardized protocol or methodology for the detection and isolation of MRSA from the environment in healthcare settings. As a matter of fact, depending on the personal experiences and preferences of the principal investigator (PI) in charge of the project, many different methods have been employed to screen for MRSA. Some of the observed differences are the use of electrostatic cloths or swabs for sample collection [147, 148, 155-157], which could be pre-moistened or not in sterile solutions [29, 36, 124, 155, 156], the use of pre-enrichment media and further incubation or simply direct plating [30, 64, 124, 127, 155], the addition of salt or not to pre-enrichment media (if used) [64, 124, 127, 134, 155], and selection of mediums for bacterial isolation which vary from mannitol to ChomAgar plates [64, 124, 155]. This large number of variations and combinations on how to screen surfaces will have an effect in the MRSA prevalence found on each particular setting that is sampled.

Another challenge that investigators usually face when performing environmental screenings is the fact that each hospital is different from the other; therefore the selection of contact surfaces to sample is particularly unique to each location and circumstances. Presence and spatial locations of certain hospital areas will vary based the level of
healthcare delivery (primary, secondary or tertiary). Since primary care institutions involve the widest and more general scope of healthcare, these facilities generally consist of a given number of exam rooms plus some general areas (including the administrative department and waiting area). In addition, surfaces available will be the most basic (e.g. computers, counter tops, phones, chart holders and exam tables) and the patient population visiting will include healthy and sick members from the general community. In contrast, tertiary institutions are specialized healthcare settings with a large number of units or specialties (e.g. neurosurgery, cardiac surgery, and advance neonatology services). Contact surfaces will range from basic (e.g. computers, phones and chart holders) to very complex medical equipment (e.g. x-rays and computed tomography scanners and surgical equipment). Moreover, the patient population will include mostly referral patients from primary and/or secondary institutions, in many cases severely ill. Based on all these differences, selecting a standard list of surfaces to sample in hospital is a challenging process. Generally, surfaces are selected based on how frequently they are touched by multiple individuals and/or based on the level of exposure that patients could potentially have to them.

Considering the discrepancy in sampling techniques and the surfaces present in healthcare settings, it is not surprising that comparisons between studies are extremely difficult to make and extrapolation of findings and results to other institutions is nearly unfeasible. Consequently, answering the question of how much involvement the hospital environment has in the transmission cycle of MRSA in hospitals has become a difficult challenge to overcome.
1.7 DISSERTATION’S OBJECTIVES AND JUSTIFICATION

Even though the problem that MRSA environmental contamination represents in healthcare settings has been studied worldwide for the past 30-40 years, there are still many questions that remain unanswered. First, the role that the environment plays in the transmission cycle of MRSA within the hospital during non-outbreak periods is still unclear. As described throughout this review, multiple studies have highlighted the possibility of contaminated contact surfaces being the source for nosocomial infections during outbreaks [133, 144]. Therefore, it is important to establish if the environment can be a reservoir of MRSA clones that are either transient or endemic in the institution, which could ultimately increase the probability of nosocomial infections and/or outbreaks to occur. Second, only few long term studies with continuous sampling have been performed, which are necessary to generate enough data to establish patterns of environmental contamination and persistence over time. Third, there is a lack of standardize environmental sampling protocols (including surfaces selection and bacterial recovery), which are necessary to obtain harmonized data that could be compared and extrapolated among institutions. Fourth, little research has been performed regarding environmental contamination in veterinary settings, even though animal patients are more likely to have a closer interaction with their surroundings during the hospital visit.

Therefore, the main goal of this dissertation is to establish the ecology (presence, distribution and maintenance) and molecular characteristics of MRSA strains present in human and animal healthcare settings during non-outbreak periods. To accomplish this goal, the following secondary objectives were evaluated:
• To establish a year long active environmental surveillance in a small animal hospital with continuous samplings performed monthly (Chapter 2)

• To determine an epidemiological profile of MRSA positive incoming patients in the same small animal hospital, as well as to evaluate their role in the introduction of MRSA into the hospital environment (Chapter 3)

• To establish a year long active surveillance in an equine medical center, including the environment and incoming patients, with continuous samplings performed monthly (Chapter 4)

• To establish a year long active environmental surveillance in a human tertiary healthcare facility with continuous samplings performed monthly (Chapter 5)

The results obtained from this dissertation will allow establishment of environmental MRSA contamination patterns and potential routes/points of transmission in which contact surfaces could be involved. In turn, this information could allow the design of precise intervention strategies to minimize the persistence and spread of the bacterium across the hospital. In addition, surfaces that are persistently contaminated and acting as “hot spots” of MRSA contamination are a potential source for nosocomial infections. Therefore, it is imperative that such surfaces are identified and targeted in cleaning and disinfections protocols. Ultimately, the final goal is to be able to generate information that will help minimize nosocomial transmission of MRSA, as well as diminish occupational exposure and zoonotic transmission within human and veterinary healthcare settings.
1.8 LIST OF REFERENCES


<table>
<thead>
<tr>
<th>CHARACTERISTIC</th>
<th>Hospital Acquired or Health Care-Associated (HA)</th>
<th>Community Acquired or Community-Associated (CA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time frame</td>
<td>Patient diagnosed 48h after hospital admission</td>
<td>Patient diagnosed in an outpatient or within 48h of hospital admission</td>
</tr>
<tr>
<td>Clinical</td>
<td>Surgical site and wound infections, nosocomial bacteremia</td>
<td>Skin and soft tissue infections</td>
</tr>
<tr>
<td>Epidemiology</td>
<td>Primarily elderly, healthcare</td>
<td>Primarily children or young adults, athletes, drug users, prisoners and military</td>
</tr>
<tr>
<td>Risk factors</td>
<td>1) Receipt of hemodialysis, surgery, hospitalization or residence in long-term care facility in the previous year</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>2) Presence of indwelling percutaneous devices or catheter</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3) Previous MRSA isolation</td>
<td></td>
</tr>
<tr>
<td>Antibiotic Resistance</td>
<td>Frequently multidrug resistant</td>
<td>Frequently susceptible to non-B-lactams antimicrobials</td>
</tr>
<tr>
<td></td>
<td>Frequently Clindamycin resistant</td>
<td>Frequently Clindamycin Susceptible</td>
</tr>
<tr>
<td>SCCmec</td>
<td>Types I, II, III</td>
<td>Types IV, V</td>
</tr>
<tr>
<td>Virulence genes</td>
<td>Usually PVL negative</td>
<td>Usually PVL positive</td>
</tr>
<tr>
<td>PFGE Type (^1)</td>
<td>USA100, USA200, USA500, USA600, USA800</td>
<td>USA300, USA400, USA1000, USA1100</td>
</tr>
<tr>
<td>spa Type (^1)</td>
<td>t001, t002, t004, t008, t018, t032, t037, t051</td>
<td>t008, t012, t044, t127, t216</td>
</tr>
<tr>
<td>MLST (sequence type) (^1)</td>
<td>ST5, ST36, ST45</td>
<td>ST1, ST8, ST30, ST59, ST80</td>
</tr>
</tbody>
</table>

\(^1\) Types most frequently associated to HA or CA MRSA strains

Abreviations. MLST: Multilocus Sequence Typing, MRSA: Methicillin-resistant Staphylococcus aureus, PFGE: Pulsed-field Gel Electrophoresis, SCCmec: Staphylococcal Cassette Chromosome mec, spa: Staphylococcal Protein A, ST: Sequence Type

**Table 1.1:** Epidemiological and molecular characteristics used to classified methicillin-resistant *Staphylococcus aureus* strains into Hospital Acquired (HA) and Community Acquired (CA)
<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Pub</th>
<th>Time Frame</th>
<th>Country</th>
<th>Location</th>
<th>Total Patients</th>
<th>Components Evaluated</th>
<th>Contaminated Surfaces</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mongkolrattanothai</td>
<td>2010</td>
<td>84</td>
<td>Feb 2009 to Mar 2009</td>
<td>USA</td>
<td>NICU</td>
<td>5 positive patients</td>
<td>1 positive HCW</td>
<td>N/E</td>
</tr>
<tr>
<td>Murillo</td>
<td>2010</td>
<td>85</td>
<td>Jul 2005 to Nov 2005</td>
<td>USA</td>
<td>NICU</td>
<td>29 positive patients</td>
<td>6 positive HCWs</td>
<td>N/E</td>
</tr>
<tr>
<td>Schlebusch</td>
<td>2010</td>
<td>86</td>
<td>Oct 2008 to Nov 2008</td>
<td>Australia</td>
<td>NICU</td>
<td>5 positive patients</td>
<td>N/E</td>
<td>N/E</td>
</tr>
<tr>
<td>Song</td>
<td>2010</td>
<td>87</td>
<td>Sep 2004 to Mar 2009</td>
<td>USA</td>
<td>NICU</td>
<td>218 positive patients</td>
<td>Not described</td>
<td>N/E</td>
</tr>
<tr>
<td>Nagao</td>
<td>2010</td>
<td>88</td>
<td>Mar 2009 to Dec 2009</td>
<td>Japan</td>
<td>General Ward</td>
<td>4 positive patients</td>
<td>6 positive HCWs</td>
<td>N/E</td>
</tr>
<tr>
<td>Comejo-Juarez</td>
<td>2010</td>
<td>89</td>
<td>Jan 2006 to Dec 2007</td>
<td>Mexico</td>
<td>Oncology Center</td>
<td>55 positive patients</td>
<td>2 positive HCWs</td>
<td>N/E</td>
</tr>
<tr>
<td>Teare</td>
<td>2010</td>
<td>90</td>
<td>Aug 2007 to Dec 2008</td>
<td>England</td>
<td>Plastic Surgery and Burns Center</td>
<td>18 positive patients</td>
<td>10 positive HCWs</td>
<td>All negative</td>
</tr>
<tr>
<td>Sanchez Garcia</td>
<td>2010</td>
<td>91</td>
<td>Apr 2008 to Jun 2008</td>
<td>Spain</td>
<td>ICU</td>
<td>12 positive patients</td>
<td>None (all negative)</td>
<td>15 (16.5%) positive surfaces</td>
</tr>
<tr>
<td>Romanelli</td>
<td>2010</td>
<td>92</td>
<td>Sep 2004 to May 2005</td>
<td>Brazil</td>
<td>Transplant Unit</td>
<td>11 positive patients</td>
<td>None (all negative)</td>
<td>N/E</td>
</tr>
</tbody>
</table>

**Table 1.2:** Summary of MRSA Nosocomial Outbreaks Reported between 2010-2014 in Human Healthcare Settings
<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Time Frame</th>
<th>Total Time*</th>
<th>Country</th>
<th>Location</th>
<th>Components Evaluated</th>
<th>Patients</th>
<th>Personnel</th>
<th>Environment</th>
<th>Contaminated Surfaces</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ben Ayed</td>
<td>2010</td>
<td>Apr 2002 to Jun 2002</td>
<td>2M</td>
<td>Tunisia</td>
<td>Dermatological Ward</td>
<td>6 positive patients</td>
<td>1 positive HCW</td>
<td>N/E</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Heinrich</td>
<td>2011</td>
<td>Feb 2005 to Jun 2006</td>
<td>11M</td>
<td>Germany</td>
<td>NICU</td>
<td>27 positive patients</td>
<td>9 positive HCWs</td>
<td>2 (1.3%) positive surfaces</td>
<td>Stethoscope (2x)</td>
<td></td>
</tr>
<tr>
<td>Mine</td>
<td>2011</td>
<td>Feb-08</td>
<td>1M</td>
<td>Japan</td>
<td>Dermatology Unit</td>
<td>1 positive patients</td>
<td>3 positive HCWs</td>
<td>N/E</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Lee</td>
<td>2011</td>
<td>Mar 2006 to Jun 2006</td>
<td>3M</td>
<td>Taiwan</td>
<td>Respiratory Care Ward</td>
<td>8 positive patients</td>
<td>11 positive HCWs</td>
<td>All negative</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Kassis</td>
<td>2011</td>
<td>Nov 2003 to Jan 2004</td>
<td>2M</td>
<td>USA</td>
<td>Cancer Center</td>
<td>2 positive patients</td>
<td>7 positive HCWs</td>
<td>N/E</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Gilomen</td>
<td>2011</td>
<td>Summer 2003 to Dec 2008</td>
<td>5.5Y</td>
<td>Switzerland</td>
<td>Dermatology Unit</td>
<td>19 positive patients</td>
<td>2 positive HCWs</td>
<td>N/E</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Ali</td>
<td>2012</td>
<td>Jun 2010 to Dec 2010</td>
<td>6M</td>
<td>England</td>
<td>NICU</td>
<td>4 positive patients</td>
<td>4 positive HCWs</td>
<td>N/E</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Giuffre</td>
<td>2012</td>
<td>Apr 2011 to Aug 2011</td>
<td>4M</td>
<td>Italy</td>
<td>NICU</td>
<td>14 positive patients</td>
<td>N/E</td>
<td>N/E</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Koser</td>
<td>2012</td>
<td>2009</td>
<td>1M</td>
<td>England</td>
<td>NICU</td>
<td>12 positive patients</td>
<td>3 positive HCWs</td>
<td>N/E</td>
<td>N/A</td>
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</table>
Table 1.2: Continued

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Time Frame</th>
<th>Total Time</th>
<th>Country</th>
<th>Location</th>
<th>Components Evaluated</th>
<th>Patients</th>
<th>Personnel</th>
<th>Environment</th>
<th>Contaminated Surfaces</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alsubaie</td>
<td>2012</td>
<td>Oct 2009 to Nov 2009</td>
<td>1M</td>
<td>Saudi Arabia</td>
<td>Infant Nursery</td>
<td>13 positive patients</td>
<td>All negative</td>
<td>All negative</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Manoharan</td>
<td>2012</td>
<td>Jun 2009 to Jan 2010</td>
<td>7M</td>
<td>India</td>
<td>OBGYN Ward</td>
<td>20 positive patients</td>
<td>N/E</td>
<td>Not described</td>
<td>Not described</td>
<td></td>
</tr>
<tr>
<td>Nubel</td>
<td>2013</td>
<td>Feb 2010 to Aug 2010</td>
<td>6M</td>
<td>Germany</td>
<td>NICU</td>
<td>32 positive patients</td>
<td>2 positive HCWs</td>
<td>N/E</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Pinto</td>
<td>2013</td>
<td>May 2011 to Jan 2012</td>
<td>8M</td>
<td>Australia</td>
<td>NICU</td>
<td>20 positive patients</td>
<td>6 positive HCWs</td>
<td>1 positive surface</td>
<td>Not described</td>
<td></td>
</tr>
<tr>
<td>Ramsing</td>
<td>2013</td>
<td>Jul 2008 to Sep 2008</td>
<td>2M</td>
<td>Denmark</td>
<td>NICU and SCBU</td>
<td>32 positive patients</td>
<td>2 positive HCWs</td>
<td>5 positive surfaces</td>
<td>N/A</td>
<td>NICU: Alarm button and floor. SCBU: Chair seat, laundry cupboard handle and floor</td>
</tr>
<tr>
<td>Patel</td>
<td>2013</td>
<td>Jan 2010 to Feb 2010</td>
<td>2M</td>
<td>England</td>
<td>Paediatric Burns Center</td>
<td>4 positive patients</td>
<td>2 positive HCWs</td>
<td>1% (1/100)</td>
<td>Toy in ward playroom</td>
<td></td>
</tr>
<tr>
<td>Hidaka</td>
<td>2013</td>
<td>Apr 2010 to Dec 2011</td>
<td>1.5Y</td>
<td>Japan</td>
<td>ICU</td>
<td>24 positive patients</td>
<td>N/E</td>
<td>2 positive surfaces</td>
<td>Suction tube and ventilator panel</td>
<td></td>
</tr>
<tr>
<td>Hail</td>
<td>2013</td>
<td>May 2011 to Mar 2012</td>
<td>14M</td>
<td>England</td>
<td>Cardiac Surgery Unit</td>
<td>13 positive patients</td>
<td>1 positive HCW</td>
<td>N/E</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Harris</td>
<td>2013</td>
<td>2011</td>
<td>6M</td>
<td>England</td>
<td>SCBU</td>
<td>26 positive patients</td>
<td>N/E</td>
<td>N/E</td>
<td>N/A</td>
<td></td>
</tr>
</tbody>
</table>
Table 1.2: Continued

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Time Frame</th>
<th>Total Time*</th>
<th>Country</th>
<th>Location</th>
<th>Components Evaluated</th>
<th>Patients</th>
<th>Personnel</th>
<th>Environment</th>
<th>Contaminated Surfaces</th>
</tr>
</thead>
<tbody>
<tr>
<td>van der Zee</td>
<td>2013</td>
<td>Nov 2001 to Dec 2009</td>
<td>8Y</td>
<td>The Netherlands</td>
<td>Teaching Medical Center</td>
<td>1230 positive patients</td>
<td>153 positive HCWs</td>
<td>N/E</td>
<td>N/A</td>
<td></td>
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<tr>
<td>Agea</td>
<td>2014</td>
<td>Not described</td>
<td>Not described</td>
<td>Turkey</td>
<td>NICU</td>
<td>10 positive patients</td>
<td>Not described</td>
<td>N/E</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Scheithauer</td>
<td>2014</td>
<td>Oct 2012 to Nov 2012</td>
<td>1M</td>
<td>Germany</td>
<td>NICU</td>
<td>8 positive patients</td>
<td>9 positive HCWs</td>
<td>0.7% (1/135)</td>
<td>Backpack</td>
<td></td>
</tr>
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<td>Carey</td>
<td>2014</td>
<td>Jan 2000 to Dec 2008</td>
<td>8Y</td>
<td>USA</td>
<td>NICU</td>
<td>260 positive patients</td>
<td>14 positive HCWs</td>
<td>N/E</td>
<td>N/A</td>
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<tr>
<td>Williams</td>
<td>2014</td>
<td>Jan 2010 to Feb 2011</td>
<td>13M</td>
<td>England</td>
<td>Neonatal Units</td>
<td>Not described</td>
<td>Not described</td>
<td>Not described</td>
<td>Not described</td>
<td></td>
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<tr>
<td>Lee</td>
<td>2014</td>
<td>Jun 2012 to Jul 2012</td>
<td>1M</td>
<td>Korea</td>
<td>Nursery</td>
<td>6 positive patients</td>
<td>24 positive HCWs</td>
<td>All negative</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Faccone</td>
<td>2014</td>
<td>Jan 2006 to May 2006</td>
<td>4M</td>
<td>Argentina</td>
<td>Neonatal Unit</td>
<td>10 positive patients</td>
<td>6 positive HCWs</td>
<td>N/E</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Hart</td>
<td>2014</td>
<td>Jan 2000 to Dec 2009</td>
<td>9Y</td>
<td>Australia</td>
<td>Teaching Hospital</td>
<td>1.1 - 20.6% positive patients</td>
<td>0.0 - 5.52% positive HCWs</td>
<td>N/E</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Brust</td>
<td>2014</td>
<td>April 2007</td>
<td>1M</td>
<td>Brazil</td>
<td>General Hospital</td>
<td>13 positive patients</td>
<td>N/E</td>
<td>N/E</td>
<td>N/A</td>
<td></td>
</tr>
</tbody>
</table>

* Time described in years (Y), months (M) and days (D)

Abbreviations. HCW: Health Care Worker, ICU: Intensive Care Unit, MRSA: Methicillin-resistant *Staphylococcus aureus*, N/A: Not Applicable, N/E: Not Evaluated, NICU: Neonatal Intensive Care Unit, OBGYN: Obstetric and Gynecology Ward, SCBU: Special Care Baby Unit
<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Pub</th>
<th>Time Frame</th>
<th>Total Time</th>
<th>Country</th>
<th>Location</th>
<th>Components Evaluated</th>
<th>Contaminated Surfaces</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seguin[1]</td>
<td>1999</td>
<td>73</td>
<td>Sep 1993 to Oct 1994</td>
<td>13M</td>
<td>USA</td>
<td>Veterinary Teaching Hospital</td>
<td>11 positive horses</td>
<td>62.0% (18/29) Stalls MRSA positive horses</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3 positive HCWs</td>
<td>25.0% (1/4) Muzzles</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>N/E</td>
<td>10.5% (2/19) Twitches</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6.9% (2/29) Stalls MRSA negative horses</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5.6% (1/18) Medical equipment</td>
</tr>
<tr>
<td>Weese[3,13]</td>
<td>2006</td>
<td>72</td>
<td>May 2002 to Aug 2002</td>
<td>3M</td>
<td>Canada</td>
<td>Large Animal Clinic</td>
<td>27 positive horses</td>
<td>4/7 Stable units</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>17 positive HCWs</td>
<td>3/6 Isolation units</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>25 positive surfaces</td>
<td>2/2 Preparation rooms</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2/2 Examination rooms</td>
</tr>
<tr>
<td>Weese[3]</td>
<td>2007</td>
<td>126</td>
<td>Not described</td>
<td>13D</td>
<td>Canada</td>
<td>ICU</td>
<td>4 positive dogs and 2 positive cats</td>
<td>2/2 HCWs rooms</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>13 positive HCWs</td>
<td>1/2 Students’ room</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>N/E</td>
<td>1/1 Farriery</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>N/E</td>
<td>1/1 Anaesthesia unit</td>
</tr>
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<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>1/1 Intensive care supply room</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1/1 Animal attendants’ canteen</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1/1 Students’ canteen</td>
</tr>
<tr>
<td>Weese[10]</td>
<td>2007</td>
<td>125</td>
<td>Not described</td>
<td>13M</td>
<td>Canada</td>
<td>NICU</td>
<td>2 positive horses</td>
<td>4/7 Stable units</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>13 positive HCWs</td>
<td>3/6 Isolation units</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>2/2 Preparation rooms</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2/2 Examination rooms</td>
</tr>
<tr>
<td>Van Duijkeren[17]</td>
<td>2010</td>
<td>127</td>
<td>2 outbreaks: Dec 2006 to Early 2007 and Jan 2008 to Oct 2008</td>
<td>12M</td>
<td>The Netherlands</td>
<td>Veterinary Teaching Hospital</td>
<td>24 positive horses</td>
<td>2/2 HCWs rooms</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>20 positive HCWs</td>
<td>1/2 Students’ room</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>19 positive surfaces</td>
<td>1/1 Farriery</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1/1 Anaesthesia unit</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1/1 Intensive care supply room</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1/1 Animal attendants’ canteen</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1/1 Students’ canteen</td>
</tr>
</tbody>
</table>

Table 1.3: Summary of Significant MRSA Nosocomial Outbreaks Reported in Veterinary Healthcare Settings
Table 1.3: Continued

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Time Frame</th>
<th>Total Time*</th>
<th>Country</th>
<th>Location</th>
<th>Components Evaluated</th>
<th>Contaminated Surfaces</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Personnel: N/E</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Environment: N/E</td>
<td>Not described</td>
</tr>
<tr>
<td>Schwaber</td>
<td>2013</td>
<td>Jan 2010 to Apr 2010</td>
<td>4M</td>
<td>Israel</td>
<td>Veterinary Teaching Hospital</td>
<td>Patient: 12 positive horses</td>
<td>16 positive HCWs</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Personnel: N/E</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Environment: N/E</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Personnel: All negative</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Abbreviations. HCW: Health Care Worker, ICU: Intensive Care Unit, MRSA: Methicillin-resistant *Staphylococcus aureus*, N/A: Not Applicable, N/E: Not Evaluated, NICU: Neonatal Intensive Care Unit
<table>
<thead>
<tr>
<th>Author (Year Pub)</th>
<th>Total (Year Pub)</th>
<th>Time*</th>
<th>Outbreak</th>
<th>Components Evaluated</th>
<th>Sample Collection</th>
<th>Environmental Sampling</th>
<th>Surfaces Sampled</th>
<th>Top Contaminated Surfaces (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boyce (1997)³⁹</td>
<td>N/A</td>
<td>No</td>
<td>N/E</td>
<td>N/E</td>
<td>27%</td>
<td>Rayon-tipped swab, premoistened</td>
<td>Floor, bed linen, bedside rail, bedside commode, overbed table, patient's gown, intravenous pump button, blood pressure cuff, stethoscope, door handle (room and bathroom), window sill</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1Y 8M</td>
<td>Yes</td>
<td>69 positive patients</td>
<td>All negative</td>
<td>10.7% (72/673)</td>
<td>Cotton swab, premoistened</td>
<td>Furniture – Locker, wardrobe, chair, television, telephone</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Floor – Edge by door, edge by window, under one bed</td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Medical equipment – Urine catheter bag stand, commode, O2/suction, sphygmanonometer, note carrier, note trolley, medicine trolley, pressure relieving mattress/motor</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Bed – Wheels, frame, cot sides, bed light, curtain rail</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Flat surfaces – Overbed table, window sill, shelf for holding medical equipment</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Door handle</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ventilator duct/grill</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Radiator</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Nurse call button</td>
<td></td>
</tr>
<tr>
<td>Devine (2001)⁴⁰</td>
<td>1D</td>
<td>No</td>
<td>Not described</td>
<td>N/E</td>
<td>24%</td>
<td>Swab, premoistened</td>
<td>Computers (keyboard and mouse)</td>
<td>N/A</td>
</tr>
</tbody>
</table>

**Table 1.4:** Summary of studies evaluating MRSA environmental contamination in human healthcare settings
<table>
<thead>
<tr>
<th>Author (Year Pub)</th>
<th>Total Time*</th>
<th>Outbreak</th>
<th>Components Evaluated</th>
<th>Sample Collection</th>
<th>Surfaces Sampled</th>
<th>Top Contaminated Surfaces (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oie (2002)61</td>
<td>3M</td>
<td>No</td>
<td>N/E</td>
<td>Sterile gauze, premoistened in sterile physiological</td>
<td>Room door handles</td>
<td>N/A</td>
</tr>
<tr>
<td>Lemmen (2004)89</td>
<td>1Y 10M</td>
<td>No</td>
<td>N/E</td>
<td>Polyester fibre-tipped swab, premoistened in sterile saline and Rodac plates</td>
<td>Bed cover, night-dress, bed sheet, urinal, infusion pump, shower fitting, bedside table, chair, respirator, basin fitting, bathroom seat, shower</td>
<td>~36.0% Bed Cover -34.0% Night-dress -34.0% Bed sheets -33.0% Urinal -31.0% Infusion pump</td>
</tr>
<tr>
<td>French (2004)38</td>
<td>N/A</td>
<td>No</td>
<td>N/E</td>
<td>Sterile cotton swab, premoistened in brain heart infusion broth</td>
<td>Floor corners and floor areas beside the bed, over-bed tables, bed frames and bed-raising panels, bedside chairs and lockers, door handles, light switches, sink taps, televisions and remote controls</td>
<td>43.0% Bed frames</td>
</tr>
<tr>
<td>Hardy (2006)29</td>
<td>1Y 10M</td>
<td>No</td>
<td>38 positive patients</td>
<td>Sterile cotton swab, premoistened in phosphate-buffered saline</td>
<td>Underneath the bed, workstation, control buttons on monitors, ledge positioned behind the bed</td>
<td>37.5% (81/216) Underneath the beds 19.9% (43/216) Monitors 17.1% (37/216) Workstations 12.5% (27/216) Ledges behind the beds</td>
</tr>
<tr>
<td>Sexton (2006)49</td>
<td>N/A</td>
<td>No</td>
<td>N/E</td>
<td>Mannitol agar plates (sweeping motion)</td>
<td>Bed frame, mattress, bed linen, bedside table, chair and window ledge</td>
<td>66.7% (56/84) Table 58.3% (49/84) Bed 55.4% (46/83) Chair 53.6% (45/84) Mattress 44.6% (37/83) Linen 42.9% (36/84) Window Ledge</td>
</tr>
<tr>
<td>Boyce (2007)69</td>
<td>8M</td>
<td>No</td>
<td>N/E</td>
<td>Swab, premoistened in nutrient broth</td>
<td>Bedside rails, blood pressure cuff, TV remote, overbed table, toilet, toilet rails, dresser, door knob, IV pump, call button</td>
<td>~100.0% Bedside rail ~90.0% Blood pressure cuff ~75.0% TV remote ~60.0% Table ~60.0% Toilet seat ~50.0% Dresser ~50.0% Toilet rail ~40.0% Door</td>
</tr>
</tbody>
</table>

Continued
Table 1.4: Continued

<table>
<thead>
<tr>
<th>Author (Year Pub)</th>
<th>Total (Year Pub)</th>
<th>Time*</th>
<th>Outbreak</th>
<th>Patient</th>
<th>Personnel</th>
<th>Environment</th>
<th>Sample Collection</th>
<th>Surfaces Sampled</th>
<th>Top Contaminated Surfaces (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mahamat (2007)(^1)</td>
<td>4Y</td>
<td>No</td>
<td>Not described</td>
<td>N/E</td>
<td>1.5% (57/3784)</td>
<td>Charcoal swab, premoistened in sterile water</td>
<td>Areas that were frequently touched (e.g. telephones, door handles)</td>
<td>Medical telephones (2)</td>
<td>Public telephones (2)</td>
</tr>
<tr>
<td>Oie (2007)(^2)</td>
<td>1Y</td>
<td>No</td>
<td>N/E</td>
<td>N/E</td>
<td>19.7% (83/421)</td>
<td>Swab, premoistened in sterile physiological saline</td>
<td>Items of patient care equipment</td>
<td>Commode (1)</td>
<td>Commode (1)</td>
</tr>
<tr>
<td>Wang (2011)(^3)</td>
<td>11M</td>
<td>No</td>
<td>N/E</td>
<td>N/E</td>
<td>34.1% (858/2520)</td>
<td>Cotton swab, premoistened in normal saline solution and Contact plates</td>
<td>Areas where dust may accumulate</td>
<td>Drug trolley (1)</td>
<td>Drug trolley (1)</td>
</tr>
<tr>
<td>Heinrich (2011)(^4)</td>
<td>11M</td>
<td>Yes</td>
<td>27 positive patients</td>
<td>9 positive HCWs</td>
<td>1.3% (2/160)</td>
<td>Contact plates</td>
<td>14 sites from bed linens near the patient's body</td>
<td>Oxygen mask (6)</td>
<td>Oxygen mask (6)</td>
</tr>
<tr>
<td>Javidnia (2013)(^5)</td>
<td>9M</td>
<td>No</td>
<td>44 positive patients</td>
<td>N/E</td>
<td>1.3% (16/1181)</td>
<td>Swab</td>
<td>4 sites from bedside rails</td>
<td>Ventilators (3)</td>
<td>Ventilators (3)</td>
</tr>
<tr>
<td>Creamer (2014)(^6)</td>
<td>8M</td>
<td>No</td>
<td>12 positive patients</td>
<td>N/E</td>
<td>3.3% (21/664)</td>
<td>Neutralizing buffer swab and Chromogenic agar plate</td>
<td>Mattress, pillows, bed rail or bed frame and patient lockers</td>
<td>Mattress, pillows, bed rail or bed frame and patient lockers</td>
<td>Mattress, pillows, bed rail or bed frame and patient lockers</td>
</tr>
</tbody>
</table>

Time described in years (Y), months (M) and days (D)
Abbreviations. MRSA: Methicillin-resistant *Staphylococcus aureus*, N/A: Not Applicable, N/E: Not Evaluated, ICU: Intensive Care Unit, NICU: Neonatal Intensive Care Unit, HCW: Health Care Worker, VRE: Vancomycin-resistant Enterococci
Table 1.5: Summary of studies evaluating MRSA environmental contamination in veterinary healthcare settings

<table>
<thead>
<tr>
<th>Author (Year Pub)</th>
<th>Total Time*</th>
<th>Outbreak</th>
<th>Patient</th>
<th>Personnel</th>
<th>Environment</th>
<th>Sample Collection</th>
<th>Surfaces Sampled</th>
<th>Top Contaminated Surfaces (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>McLean (2008)†*</td>
<td>18M</td>
<td>No</td>
<td>4 positive dogs</td>
<td>14 positive HCWs</td>
<td>0.0% (0/15)</td>
<td>Swab, premoistened in sterile saline</td>
<td>5 random surfaces per sampling (e.g. weighing scales, kennels and examination tables)</td>
<td>N/A</td>
</tr>
<tr>
<td>Heller (2009)†*</td>
<td>14D</td>
<td>No</td>
<td>2 positive HCWs</td>
<td>14 positive HCWs</td>
<td>1.5% (3/200)</td>
<td>Cotton swab, premoistened in</td>
<td>Floors, door handles, work surfaces, taps, kennels and drains (140 surfaces on day 1)</td>
<td>Door handle (2) positive on day 1 and 14 radiology foam wedge (1)</td>
</tr>
<tr>
<td>Murphy (2010)†*</td>
<td>3M</td>
<td>No</td>
<td>All negative</td>
<td>2 positive HCWs</td>
<td>9.0% (9/101)</td>
<td>Electrostatic clothes and Cotton swab</td>
<td>Table surfaces (examination rooms, treatment, surgery, grooming and boarding)</td>
<td>2.0% (2/100) Telephone, keyboards and taps 1.6% (1/61) Otoscope tips 1.9% (1/100) Stethoscopes</td>
</tr>
<tr>
<td>Ishihara (2010)†*</td>
<td>4M</td>
<td>No</td>
<td>All negative</td>
<td>21 positive HCWs</td>
<td>5.3% (8/152)</td>
<td>Cotton swab, premoistened in sterile physiological saline</td>
<td>Waiting area; consulting, procedure, and operation rooms; dispensary; animal wards; administrative office; and a veterinarian’s office</td>
<td>Floors in X-Ray/CT scan room (1) Administration of anesthetics in the cat ward (1) Cat cage in cat ward (1) Top part of Anesthetics stand (1) Floors of rooms in cat ward (1) Meeting room for veterinary technicians (1) Intern/veterinarian’s office (1)</td>
</tr>
<tr>
<td>Weese (2004)††</td>
<td>3M</td>
<td>Yes</td>
<td>26 positive horses</td>
<td>16 positive HCWs</td>
<td>9.6% (25/260)</td>
<td>Cotton swab, premoistened in sterile saline</td>
<td>Stalls (walk, doors, water bowls, feed bowls, and hay nets), examination and treatment rooms, public areas, personal items, medical equipment, and handling equipment</td>
<td>62.0% (18/29) Stalls with MRSA horses 25.0% (1/4) Muzzles 10.5% (2/19) Twitches 6.9% (2/29) Stalls with MRSA negative horses 5.6% (1/18) Medical equipment 4.8% (1/21) HCWs Personal equipment</td>
</tr>
<tr>
<td>Loeffler (2005)†*</td>
<td>1D</td>
<td>No</td>
<td>N/E</td>
<td>4 positive</td>
<td>10.0% (3/30)</td>
<td>Cotton swab, premoistened in</td>
<td>Door handles, desk tops, water bowls) in the waiting area, consulting rooms, procedure</td>
<td>Door handles (2) Board marker pen (1)</td>
</tr>
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</table>

63
**Table 1.5: Continued**

<table>
<thead>
<tr>
<th>Author</th>
<th>Total (Year Pub)</th>
<th>Time*</th>
<th>Outbreak</th>
<th>Components Evaluated</th>
<th>Sample Collection</th>
<th>Surfaces Sampled</th>
<th>Top Contaminated Surfaces (N)</th>
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<tr>
<td>Hoet (2011)</td>
<td>3M</td>
<td>No</td>
<td>N/E</td>
<td>Environmental Sampling</td>
<td>Cotton swab, premoistened in sterile trypticase soy broth and Electrostatic cloth</td>
<td>High-contact surfaces within ICU, surgery suits, examination rooms, dermatology, animal wards and animal pens (full list includes over 30 surfaces)</td>
<td>Cart/gurneys (3)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>Examination tables (1)</td>
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<td>Examination floors (1)</td>
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<td>Water Bowls (1)</td>
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<td></td>
<td></td>
<td></td>
<td>General equipment (1)</td>
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<tr>
<td>Bergstrom (2012)</td>
<td>–6M</td>
<td>No</td>
<td>All negative</td>
<td>Environmental Sampling</td>
<td>Cloth, premoistened in buffered peptone solution with a 10% additive of a neutralising substance</td>
<td>Entrance floor</td>
<td>Floor of Intensive Care (1)</td>
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<td></td>
<td>Waiting Room: carpet, floor ICU: floor, light switches, door handles, lamp handles, taps</td>
<td>Dogs lying area in the call theater (1)</td>
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<td>On call theater: dogs lying area Orthopedic theater: dogs lying area Scale</td>
<td>Lying area in Orthopedic theater (1)</td>
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<td>Corridor: contact areas Dermatology: light switches, door handles, lamp handles, taps</td>
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<td></td>
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<td>Anaesthesia recovery room: cage floor, lower part of grid</td>
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<tr>
<td>Bergstrom (2012)</td>
<td>1Y 9M</td>
<td>Yes</td>
<td>8 positive horses</td>
<td>Environmental Sampling</td>
<td>Cloth, premoistened in a buffered peptone solution with a 10% additive of a neutralising substance</td>
<td>Water feeders and water buckets Muzzles + Twitches Door knobs, light switches Medicine, bandage and dental cart Surgery equipment Tarpaulins + traverse Mobile operating table</td>
<td>28.6% (2/7) Ante-room 20.0% (1/5) Treatment Stall 16.7% (1/6) Walls + Floor inside stall 14.3% (2/14) Door knobs 12.5% (1/8) Surgery Equipment</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>All negative</td>
<td></td>
<td></td>
<td>Walls + Floors</td>
<td></td>
</tr>
</tbody>
</table>

* Time described in years (Y), months (M) and days (D)
Abbreviations. MRSA: Methicillin-resistant *Staphylococcus aureus*, N/A: Not Applicable, N/E: Not Evaluated, ICU: Intensive Care Unit, HCW: Health Care Worker, CT: Computed Tomography
Figure adapted from Hoet, A. MRSA as an Emerging Zoonotic and Nozocomial Pathogen in ACVS Veterinary Symposium Proceedings, 241-247, 2009. Continuous (solid) line: transmission that has been confirmed by several published research articles. Noncontinuous line: transmission that has been reported in a small number of research articles. Dotted line: transmission that plausible occurs but it has not been confirmed by researchers.

**Figure 1.1:** Endogenous and exogenous sources involve in the transmission of MRSA within healthcare setting
CHAPTER 2

PRESENCE, DISTRIBUTION AND MOLECULAR EPIDEMIOLOGY OF MRSA IN A SMALL ANIMAL TEACHING HOSPITAL: A YEARLONG ACTIVE SURVEILLANCE TARGETING DOGS AND THEIR ENVIRONMENT

2.1 SUMMARY

Methicillin Resistant *Staphylococcus aureus* (MRSA) is known to be present in small animal veterinary clinical environments. However, a better understanding of the ecology and dynamics of MRSA in these environments is necessary for the development of effective infectious disease prevention and control programs. To achieve this goal, a year long active MRSA surveillance program was established at the OSU Veterinary Medical Center to describe the spatial and molecular epidemiology of this bacterium in the small animal hospital. Antimicrobial susceptibility testing, SCCmec typing, PFGE typing and dendrogram analysis were used to characterize and analyze the 81 environmental and 37 canine-origin MRSA isolates obtained during monthly sampling events. Overall, 13.5% of surfaces were contaminated with MRSA at one or more sampling times throughout the year. The majority of the environmental and canine isolates were SCCmec type II (93.8% and 86.5%, respectively) and USA100 (90.1% and 86.5%, respectively). By PFGE analysis these isolates were found to be closely related,
which reflects a low diversity of MRSA strains circulating in the hospital. For five consecutive months, one unique pulsotype was the most prevalent across the medical services and was recovered from a variety of surfaces and hospital locations. Carts/gurneys, doors, and examination tables/floors were the most frequently contaminated surfaces. Some surfaces maintained the same pulsotypes for 3 consecutive months. Molecular analysis found that incoming MRSA positive dogs were capable of introducing a new pulsotype into the hospital environment during the surveillance period. Our results suggest that once a MRSA strain is introduced into the hospital environment, it can be maintained and spread for extended periods of time. These findings can aid in the development of biosecurity and biocontainment protocols aimed at reducing environmental contamination and potential exposures to MRSA in veterinary hospital staff, clients, and patients.

2.2 INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is known to be a primary pathogen capable of causing severe health problems in both humans [1], and animals [2-5]. The increasing number of reported cases [1, 2, 6, 7] suggests that MRSA represents a growing animal and public health concern. The risk of zoonotic transmission of MRSA between humans and companion animals has been described in households, the community and healthcare care settings [3, 8-16].

MRSA is capable of contaminating and survive for up to seven months on inanimate objects and contact surfaces in households and healthcare facilities [17, 18]. Several studies have implicated hospital surfaces contaminated with nosocomial
pathogens including MRSA, in the dissemination of hospital acquired infections [17-24]. Paralleling these are reports of the increasing role of MRSA in nosocomial infections in veterinary settings [9, 11, 25-28]. A recent study of veterinary teaching hospitals accredited by the American Veterinary Medicine Association (AVMA) found that MRSA was the second most common pathogen (13/31, 42%) associated with nosocomial outbreaks [25]. However, the interaction between the environment, patients, and hospital personnel in the transmission of MRSA in veterinary hospitals has not been described.

The presence of MRSA in veterinary environments has been reported during outbreaks or limited periods of surveillance [29-33]. The environment in the equine section of a veterinary teaching hospital was sampled after the recognition of a cluster of MRSA infections in horses and humans [29]. MRSA was found on multiple surfaces and on equipment throughout the equine hospital. The authors concluded “that the environment may be an important source of MRSA infection” [29], which could be the result of the close interaction of the animals with their contaminated surroundings. Nevertheless, no longitudinal reports of the ecology and dynamics of MRSA in veterinary environments in the absence of an outbreak have been reported. Therefore, our objectives were to generate baseline data for the presence and distribution of MRSA contamination on surfaces in a small animal teaching hospital and, to describe the temporal dynamics of MRSA in this environment using molecular epidemiological analyses.
2.3 MATERIALS AND METHODS

2.3.1 Active MRSA Surveillance Program

This targeted surveillance was performed over a one year period at the Small Animal Hospital in The Ohio State University Veterinary Medical Center (OSU VMC) between November 2007 and October 2008. The is a tertiary referral veterinary medical hospital that accepts patients from smaller veterinary clinics and/or private practices throughout the Midwestern US, but it also operates as a primary care facility for local companion animals. The active MRSA surveillance program had two major components: regular sampling of the hospital environment and concurrent sampling of incoming dogs prior to their entering the surveillance areas.

2.3.2 Location, Selection and Sample Collection of Environmental Surfaces

Based on results obtained in a 2007 preliminary study [33], specific services of the small animal hospital were targeted for MRSA monitoring. These were: Community Practice (examination room and treatment area), Dermatology (treatment room and wards), Intensive Care Unit, and Surgery (Pre-surgery room, anesthesia room, surgery suits, and wards). These areas were targeted because they previously tested positive to MRSA contamination [33], and because the presence of this pathogen represents an important risk for nosocomial infection of the patients visiting these services.

Once the areas to be regularly monitored were selected, specific high contact surfaces were identified for monthly sampling. The number and type of surfaces to be sampled were determined based on the results from the previous pilot surveillance [33]. An average of 48 samples were collected each month for one year: Community Practice
(5 samples/month), Dermatology (11 samples/month), Intensive Care Unit (8 samples/month), and Surgery (21 samples/month). Samples were also obtained from the hall carts/gurneys that travelled among several services; since they were not limited to one section in particular they were classified as General (3 samples/month). The pre-selected surfaces were divided into human and animal contact surfaces as seen in Table 2.1. Human contact surfaces were defined as those surfaces that are regularly touched by multiple people during routine activities, but are typically out of reach for direct contact with animals, such as computers (keyboard and mouse) and phones. In contrast, animal contact surfaces are those that are mainly touched by multiple animals, such as cages and water bowls.

Dry electrostatic cloths/Swiffer® (for larger surfaces) or sterile pre-moistened cotton swabs (for smaller surfaces) were used to sample the different contact surfaces, either as individual or pooled samples, as previously described [33]. Immediately after sampling, electrostatic cloths were folded and placed into sterile bags, and 90 ml of BPW (BD BBL™ Buffered Peptone Water, Becton, Dickinson and Company, Sparks, USA) was added before incubating them at 35°C for 24 hours. Similarly, the individual swabs were placed in tubes with TSB (BD BBL™ Trypticase Soy Broth, Becton, Dickinson and Company, Sparks, USA) and incubated at 35°C for 24 hours. Negative and positive controls were included in each sampling.

2.3.3 Canine Population and Sample Collection

Parallel to the environmental sampling, incoming dogs admitted to the hospital services being monitored were sampled upon their arrival as previously described [34].
Briefly, only dogs, either referrals or OSU VMC patients that had not been in the hospital in the last 6 months were included in the study. Canines from Community Practice and Intensive Care Unit (n=148), Dermatology Service (n=145) and Surgery Service (n=142) were recruited on a monthly basis, averaging 36 to 37 dogs per month during the surveillance year. Upon arrival, a signed consent form was obtained from the dog’s owner for inclusion into the study and the animal was sampled prior to any clinical examination. To screen for the presence of MRSA, sterile pre-moistened cotton swabs in TSB (BD BBL™ Trypticase Soy Broth, Becton, Dickinson and Company, Sparks, USA) were used. Samples were collected from the nasal cavity, ear canals, external surface of the perianal area and any skin lesions (if present) from each canine. To increase the likelihood of identifying MRSA, these specific anatomical locations were selected based on previous studies demonstrating positive carriage in these sites [35, 36].

2.3.4 MRSA Isolation and Identification

All samples collected (environmental and animal) were processed in the Diagnostic and Research Laboratory for Infectious Diseases (DRLID) at the OSU, College of Veterinary Medicine, for isolation of *S. aureus* and further testing for resistance to Methicillin. As previously described [33], samples were incubated at 35°C in pre-enrichment media for 24 hours and then streaked onto mannitol salt agar (BD BBL™ Mannitol Salt Agar, Dickinson and Company, Sparks, USA) with 2µg/ml of Oxacillin. After 24-48 hours, 1-3 colonies per sample were selected and plated on Trypticase Soy Agar with 5% Sheep Blood agar plates (Remel®, Blood Agar (TSA with 5% Sheep Blood), Lenexa, USA) for further testing. Identification of *S. aureus* was
performed by standard colony morphology (including size, pigmentation and hemolysis pattern), and biochemical tests reactions that included: mannitol fermentation, gram stain, catalase, tube coagulase, anillin fermentation, Polymyxin B susceptibility, acetoin production (Vogues-Proskauer test), and latex agglutination (Sure-Vue® Color Staph ID, Biokit USA, inc, Lexington, Mass) [33]. Phenotypic MRSA confirmation was performed by growth on Oxacillin Screen Agar® (OSA) plates contains 6µg/ml of Oxacillin supplemented with NaCl (BD BBLTM, Becton Dickinson and Company, Maryland, USA) following the Clinical Laboratory Standards Institute protocols [37].

2.3.5 Antimicrobial Susceptibility Testing (Phenotyping)

A total of 161 MRSA isolates (90 from the environment and 71 from dogs) were tested for susceptibility to eighteen antimicrobial drugs important to veterinary and human medicine. Susceptibility to seventeen of these drugs was tested using the Kirby-Bauer Disc Diffusion technique following protocols described by CLSI [37]. Antimicrobials included were: Amikacin 30µg, Ampicillin 10µg, Amoxicillin with clavulanic acid 30µg, Cefovecin 30µg, Cefpodoxime 10µg, Cephalotin 30µg, Chloramphenicol 30µg, Ciprofloxacin 2µg, Clindamycin 2µg, Doxycycline 30µg, Enrofloxacin 5µg, Erythromycin 15µg, Gentamicin 1µg, Oxacillin 1µg, Sulfamethoxazole Trimethoprim 25µg and Tetracycline 30µg. The last antimicrobial, Vancomycin, was tested using Vancomycin Screen Agar plates (6 mg/L) (BD BBL™ Vancomycin Screen Agar, Dickinson and Company, Sparks, USA). For quality control purposes, the following strains were included: S. aureus (ATCC 43300), S. aureus (ATCC 29213), S. aureus (ATCC 25923), Enterococcus faecalis (ATCC 23212),
Escherichia coli (ATCC 25922), and Pseudomonas aeruginosa (ATCC 27853). Isolates resistant to three or more classes of antimicrobials (including beta-lactams after confirmation of the mecA gene) were considered multidrug resistant (MDR). Inducible Clindamycin resistance was tested by placing the Erythromycin disc next to the Clindamycin disc during the phenotyping process, and evaluating the bacterial growth pattern consistent with the D-test [38].

2.3.6 mecA Confirmation and Staphylococcal Chromosome Cassette mec (SCCmec) Typing

Based on the phenotyping results, 118 unique isolates (81 from the environment and 37 from the dogs) were selected for further molecular testing. Since 1 to 3 colonies were selected per plate during the isolation and identification process, selection was performed by ruling out isolates coming from the same sample source with identical morphology and phenotypic results in all the antimicrobials tested. Methicillin resistant status was confirmed by detection of the mecA gene and typing of the staphylococcal chromosomal cassette mec (SCCmec) simultaneously. Briefly, DNA was extracted using a boiling method, where a 100μl bacterial suspension was heated for 7 minutes at 95°C [39]. A multiplex PCR was performed [40] with a few modifications. Each PCR mixture with a final volume of 25μl contained 2μl of DNA template, 12.5μl of 2x Multiplex PCR Master Mix (Qiagen®, Foster City, CA) containing HotStartTaq Polymerase, 3mM MgCl2 and dNTPs. Primer concentrations were doubled compared to what had been previously reported [40]. The PCR products (10μl) were resolved in a 3% Seakem LE (Cambrex, Rockland, ME) agarose gel with 1x Tris-acetate-EDTA buffer (Promega
Corporation, Madison, WI) at 100 Volts for 2 hours, and were visualized with ethidium bromide. All assays were performed in a Gradient Thermocycler (Eppendorf, Hamburg, Germany). The cycling conditions were the following: 95°C for 15 minutes; 35 cycles of 94°C for 30 seconds, 57°C for 90 seconds and 72°C for 90 seconds; and a final extension of 72°C for 10 minutes.

2.3.7 Pulsed Field Gel Electrophoresis (PFGE)

PFGE of SmaI digested chromosomal DNA was performed according to the protocol established by [41]. Restriction fragments were separated using a CHEF mapper system (Bio-Rad Laboratories, Nazareth, Belgium). The resulting band patterns were analyzed by BioNumerics® software (version 6.6, Applied Maths, Ghent, Belgium) to determine the relatedness between strains by using Dice coefficient and Unweighted Pair Group Method using Arithmetic averages (UPGMA) to achieve dendrograms with a 1% band position tolerance [42]. Dendrogram interpretation was performed as follows: each band pattern represented a pulsotype; isolates with ≥98% similarity were considered indistinguishable and characterized as the same pulsotype. These types were grouped in clusters of ≥80% similarity that were considered to be closely related [43]. Designation of USA types was performed comparing our isolates to a CDC database containing 100 S. aureus strains with the most typical band patterns for each USA type, using ≥80% similarity as the cutoff point. Salmonella serotype Branderup strain H9812 was digested with XbaI and used as a molecular size marker. Three different dendrograms were built: environmental isolates alone, canine isolates alone, and a combination of both environmental and canine isolates.
2.3.8 Strain Characterization

Each isolate was characterized and classified as a strain if it possessed a unique combination of phenotypic profile, SCC\textit{mec} type, and pulsotype.

2.3.9 Statistical Analysis

Surface location, date of collection, culture, and molecular strain characterization results were organized and stored. Further analysis was performed using the statistical software STATA (Small Stata 12.0, StataCorp LP, Texas, USA). Chi-square coefficients were calculated to make comparisons between types of contact surfaces (human vs. animal); and also to compare in between hospital services (Community Practice, Dermatology, Surgery and ICU). To be able to establish seasonality, the year was divided into four groups, each with three months (January to March, April to June, July to September, and October to December), and Chi-square coefficients were also used to make comparisons between them. Relationships were considered significant when their p-value was equal to or less than 0.05.

2.4 RESULTS

2.4.1 General Prevalence, Phenotyping, and Molecular Characterization of Environmental Isolates

A total of 569 environmental samples were collected during the active MRSA surveillance; 77/569 (13.5%) were positive for MRSA. The monthly prevalence varied from as low as 2.0% to as high as 29.8% (Figure 2.1). The presence and distribution of MRSA on the human and animal contact surfaces in the different services are
summarized in Table 2.2. From 77 positive environmental samples, 81 MRSA isolates were recovered, indicating that some surfaces (e.g. computer keyboards, doors, and examination tables/floors) were contaminated with two distinctive MRSA strains at the time of sampling.

Genotypically, 93.8% (76/81) of the isolates were SCCmec type II (Table 2.3). A dendrogram of only the environmental samples based on PFGE results revealed two major clusters (C1 and C2) and 30 pulsotypes were identified (P1-P30). Of the 81 isolates, 86.4% (70/81) were grouped in the same cluster.

Ninety percent (73/81) of the isolates were classified as USA100 (Table 2.3). The majority of isolates fell into two pulsotypes; pulsotype 10 (P10) had the highest number of isolates (33 isolates) followed by pulsotype 27 (P27) with 8 isolates. The predominant pulsotype, P10, was observed in the hospital environment from November 2007 until July 2008. A comparison of the prevalence of P10 with the combined prevalence of the other 29 pulsotypes is shown in Figure 2.2. Even though a predominant pulsotype was found during more than half of the year surveillance, other unique pulsotypes were intermittently introduced in the hospital environment. These pulsotypes (other than P10) were sporadically observed while P10 was present, but from June to October the number of isolates representing them gradually increased. By the end of the surveillance (last three months), a complete shift had occurred in the molecular characteristics of the isolates present in the hospital environment circulating mostly new strains not present at the beginning of the surveillance.

Of the 81 environmental isolates characterized in this study, 44 unique MRSA strains were identified based on their combination of phenotypic profile, SCCmec type,
and PFGE pulsotype. Phenotypically, 11 different antimicrobial susceptibility profiles were found. All of the strains (100%) were classified as MDR MRSA. In addition to the expected resistance to beta-lactams, 97.7% (43/44) of the environmental MRSA strains were resistant to Ciprofloxacin, and 95.5% (42/44) were resistant to Erythromycin and Enrofloxacin. Also, inducible Clindamycin resistance was detected in 65.9% of the strains (29/44) obtained from the environment. All of the strains (100%) were susceptible to Doxycycline, Chloramphenicol and Vancomycin.

2.4.2 Human vs. Animal Contact Surfaces

The animal contact surfaces (43/290, 14.8%) had a MRSA prevalence similar to the human contact surfaces (34/279, 12.2%) (Tables 2.2 and 2.4). The distribution of MRSA by type of contact surface over time is presented in Figure 2.1. Table 2.4 summarizes the prevalence of MRSA on each type of human and animal contact surfaces. Doors were the predominant human contact surface with 22.7% (22/97) of samples positive. Of the animal contact surfaces, carts/gurneys were the most prevalent with 44.1% (15/34) MRSA positive.

Genotypically, 17 pulsotypes were found on human contact surfaces and 19 pulsotypes were found on animal contact surfaces. Six (6) of these, including the most prevalent pulsotypes (P10 and P27), were present on both human and animal contact surfaces. Interestingly, P10 was present only on human contact surfaces for the first two months of surveillance (Figure 2.3). Then, between January and March 2008 it was gradually transferred to the animal contact surfaces. By April 2008 it was mainly found
on animal contact surfaces, except in July when only one sample was positive with this pulsotype and it was a human contact surface (Figure 2.3).

It is noteworthy to highlight that some surfaces were contaminated by the same pulsotype for 2 to 3 consecutive months. This was the case for four different surfaces contaminated with the most prevalent pulsotype (P10). These surfaces were a cart/gurney that was contaminated for 3 consecutive months; the access doors in a ward, the water bowls (from the same ward as the doors) and one of the examination floors were contaminated for 2 consecutive months with the same pulsotype. In contrast, four types of surfaces were contaminated with distinct and different pulsotypes each month for 2 to 4 consecutive months, which shows a continuous cycling of strains on those surfaces. The surfaces with such persistent contamination with multiple pulsotypes over time were two of the carts/gurneys, computers, multiple doors, and examination tables and floors.

2.4.3 Hospital Services

MRSA prevalence for each hospital service (overall and by type of contact surface) is shown in Table 2.2. The service with the highest MRSA prevalence was Community Practice with 21.3% (13/61 samples), followed by ICU (13/97, 13.4%), Surgery (27/243, 11.1%) and Dermatology (9/134, 6.7%), (P = 0.03). It is very important to highlight that MRSA was not detected in any of the surgical suites, with the exception of the doors providing access to that area. All MRSA isolates obtained from the surgery section were found in the pre-surgery area and in their wards.

Pulsotypes belonging to cluster 2 were present in all the services. In particular, P10 (the most prevalent pulsotype) was found in all the services and it was highly
prevalent in the carts/gurneys (considered as general and not linked to any specific service). The only three isolates with SCC\textit{mec} type IV were found in community practice, ICU and surgery.

\textbf{2.4.4 Seasonality}

When analyzing the data by months, June had the highest prevalence at 29.8\% (14/47) MRSA positive (Figure 2.1), followed by February and April both with 21.3\% (10/47), August with 17\% (8/47) and May with 15.6\% (7/45). During March, May and July (months immediately following the top three most prevalent months), it was observed that MRSA prevalence in the environment decreased. Group one included from January to March (14.7\%, 21/143), group 2 from April to June (21.6\%, 30/139), group 3 from July to September (11.3\%, 16/142) and group 4 from October to December (6.9\%, 10/145). The analysis showed that the season of the year was associated with the prevalence of MRSA (P = 0.002), with the highest prevalence in the spring months (April to June).

\textbf{2.4.5 General Prevalence, Phenotyping and Molecular Characterization of Canine Isolates}

A total of 435 dogs were sampled during the MRSA surveillance period and 25 (5.7\%) were found to be MRSA positive. Epidemiological data regarding these dogs was discussed elsewhere [34]. However, the phenotypic and genotypic characterization of the MRSA isolates obtained from dogs is reported here to compare and establish relationships with the isolates found in the hospital environment.
SCCmec typing and PFGE results are summarized in Table 2.5. USA100 was the most prevalent PFGE type with 86.5% (32/37) of the isolates. Other types found were USA500 in 2.7% (1/37) and USA800 in 5.4% (2/37) of the isolates. All USA100 isolates carried the SCCmec type II; in contrast, USA500 and USA800 isolates carried the SCCmec type IV. A dendrogram including only the canine isolates was built; two major clusters and 16 pulsotypes were identified, in which the majority of them (9/16) belong to one cluster. The most prevalent pulsotype in the environment, P10, was isolated from incoming dogs in November, March, April, May and June. Moreover, a dendrogram built with canine and environmental isolates together (Figure 2.4) showed that one pulsotype in particular that was never seen before in the hospital, P9, was apparently introduced by two different dogs on September 9th and 12th (11 months after the surveillance started). On September 15th, P9 was found for the first time in the environment (water bowls) of the service where the dogs were admitted, suggesting that dogs are capable of introducing and contaminating hospital surfaces during their visit (Figure 2.4).

Phenotypically, 11 different antimicrobial susceptibility profiles were found. All of the strains (100%) were classified as MDR MRSA. In addition to the expected resistance to beta-lactams, 92.9% (26/28) of the canine MRSA strains were resistant to Erythromycin, 82.1% (23/28) to Ciprofloxacin and 75.0% (21/28) to Enrofloxacin. Also, inducible resistance to Clindamycin was detected in 78.6% of the strains (22/28) obtained from the dogs. All of the strains (100%) were susceptible to Doxycycline, Chloramphenicol and Vancomycin.
2.5 DISCUSSION

In this study, MRSA was detected in 13.5% of the sampled surfaces across different areas of the OSU VMC. Previous studies have focused on environmental contamination in the veterinary hospital setting during outbreaks, involved only one sampling time, or included repeated sampling but over a very short time interval [29-32]. Nevertheless, these studies reported the presence of MRSA on 1.4% to 10.0% of the surfaces sampled. Similarly, one cross-sectional study performed in the OSU VMC reported the presence of MRSA on 12% of the surfaces during a non-outbreak period, with 2 samplings performed only 1.5 months apart [33]. Collectively, it is clear that MRSA can be present and survive for an extended period of time on multiple surfaces in veterinary settings.

MRSA was isolated from multiple human and animal contact surfaces throughout the OSU VMC. Among the surfaces sampled, carts/gurneys, doors, examination tables and floors were the most prevalent locations. Since MRSA is considered to be a primarily human pathogen [35], we did not expect to find similar levels of contamination on human and animal contact surfaces. Previous studies have also reported positive contamination on animal contact surfaces [29-31, 33], which make them important targets for regular cleaning and disinfection in veterinary settings.

The high MRSA prevalence on the carts/gurneys is one of the most concerning findings of this study. At the OSU-VMC, carts do not belong to any particular service, but they are used to move patients throughout the hospital and between services. These carts are contacted by multiple patients several times per day. If these patients have open wounds or are immunocompromised, this exposure could increase their chances of
developing a nosocomial MRSA infection. Because these carts/gurneys are shared by all the services, they may also serve as a vehicle for dissemination of this pathogen throughout the hospital. This could explain the observed movement of pulsotypes among services with no shared staff or equipment. Finally, even though the carts/gurneys were categorized as an animal contact surface, they are also touched by multiple veterinary personnel and students making them an important occupational health concern.

It was also concerning that the carts/gurneys were found MRSA positive with the same pulsotype over 3 consecutive months. There are two plausible explanations for the detection of the same MRSA strains on such surfaces. First, the lack of or improper application of cleaning and disinfection protocols allowing the maintenance and survival of MRSA for long periods of time. This is critical aspect as previous studies have described how proper cleaning and disinfections of hospital surfaces contributes to the reduction of MRSA in the environment, even during outbreaks [21, 22]. However, complete eradication of this pathogen from surfaces varies depending on the cleaning method used [17, 44-46]. Second, the continuous detection of MRSA in these particular surfaces could be due to their recontamination either by the reintroduction of the same strain into the hospital environment (by animals and visitors), or by recurrent exposure to colonized or infected hospital staff. In any case, it is recommended that all personnel is trained to properly clean and disinfect carts/gurneys between patients. In order to prevent the long term survival of MRSA on this type of mobile surface, protocols should also delegate a staff position or service responsible for ensuring that thorough disinfection of the carts/gurneys occurs routinely.
Across the hospital, eight access doors from the different services were sampled every month. The majority (88%, 7/8) were contaminated with MRSA at least once during the year, with one maintaining the same pulsotype for two consecutive months on two separate occasions (Nov-Dec & Feb-Mar). This finding was not unexpected since door surfaces are contacted by multiple individuals several times per day, and may not be regularly targeted for cleaning and disinfection. Other studies have reported the presence of MRSA on doors of both human and veterinary hospitals [30, 31, 33, 47]. In one case the same MRSA strain was found on the same door during two consecutive samplings fourteen days apart [30]. MRSA is capable of surviving on inanimate surfaces and/or objects [18], especially when these surfaces are not cleaned appropriately; thereby increasing the probability of the environment serving as a reservoir for this nosocomial pathogen. In addition, the persistence of this pathogen on access doors could reflect a lack of or improper compliance with hand hygiene protocols by personnel [17, 19, 48, 49] and/or the incorrect use of gloves [19, 50]. In addition, the presence of MRSA on the doors could be a consequence of colonized or infected personnel working in those areas, who can cross contaminate these surfaces [48]. To ensure that doors do not become a focal point for MRSA maintenance and dissemination in a veterinary hospital, they must be included in the standard operating procedures (SOPs) for regular cleaning and disinfection, and staff compliance with hand washing and proper glove use should be strictly enforced.

When evaluating the molecular epidemiology of the environmental isolates, over 90% were SCCmec type II and USA100. These findings demonstrate that there was little diversity in the MRSA isolates circulating within the hospital. MRSA strains with such
molecular characteristics are frequently classified as hospital acquired strains (HA-MRSA). This type of strain has been reported in the US general population, as well as in companion animals worldwide [31, 51-55]. Interestingly, the same strains present in the environment were also found in the incoming dogs, indicating that patients can also be a source of environmental contamination. This is further supported by our identification of a unique strain not seen before in the hospital arriving with one patient and then subsequently appearing in the environment on animal contact surfaces. Because the personnel and dog owners were not screened, it is not possible to determine how much of the environmental contamination was due to the incoming patients versus other potential sources.

All the isolates from both the environment and the dogs were multidrug resistant; some of them were resistant to 7 different classes of antimicrobial drugs. This observed resistance pattern agrees with the fact that HA-MRSA strains tends to be MDR [56]. Finding that over 90% of the isolates detected in this study were HA-MRSA is an important issue, as treatment and management of this type of infection tends to be more complex and expensive [57, 58].

A trend was observed where MRSA had a higher prevalence in the environment during the warm months of the year, peaking in June. Since there are not other studies reporting surveillance over long periods of time in veterinary hospitals, we cannot draw strong conclusions regarding seasonality. However, reports from human hospital surveillance suggest a seasonality of S. aureus and MRSA infections that most frequently occurred during the spring and summer [59, 60].
Lastly, we acknowledge that our results are limited by our inability to screen hospital personnel (veterinarians, technicians, students and staff) clients, or visitors during the surveillance. This limits our ability to determine the origin of the MRSA isolates contaminating the hospital environment. Further studies will enlighten our understanding of how, when, and where MRSA will contaminate the veterinary environment. Finally, we acknowledge that extrapolation of our results to other veterinary hospitals around the United States is not possible. However, our results may be useful in aiding veterinary hospitals in developing effective surveillance and monitoring programs, as well as biosecurity and biocontainment protocols that will limit the impact of this important nosocomial pathogen in veterinary settings.

2.6 CONCLUSION

Little is known about the ecology of MRSA contamination in veterinary hospital environments during non-outbreak periods. Our results suggest that MRSA is not only present on multiple human and animal contact surfaces throughout the hospital, but that it is also capable of surviving on these surfaces for long periods of time. Some MRSA isolates circulated across multiple surfaces and areas of the hospital for up to 9 months, while continuous introduction of new MRSA strains was observed. In addition, incoming patients carried and contaminated the hospital environment with this bacterium during their visits. Molecular epidemiological analysis revealed that the majority of the environmental and canine MRSA isolates were closely related HA-MRSA strains, showing little diversity. It is clear that environmental contamination plays an important role in the epidemiology and ecology of MRSA in veterinary hospitals, including the
maintenance and transmission of this bacterium. These results have aid in the development of effective programs for the control of this nosocomial pathogen and prevention of its zoonotic transmission in The Ohio State University Veterinary Medical Center.

2.7 ACKNOWLEDGMENT

We wish to thank Duncan MacCannell, from the Center for Disease Control and Prevention (CDC), for facilitating the database containing *S. aureus* strains with the most typical band patterns for each USA type for PFGE characterization. We also want to thank Dr. Herminia de Lencantre, from the Universidade Nova de Lisboa in Portugal, for providing MRSA controls isolates for the standardization of the SCC*mec* type multiplex PCR. Thanks to Amber Reed for her collaboration in the collection and processing of samples in the early stages of this study. We are grateful for the financial support provided for the development of this project by The Ohio State’s Public Health Preparedness for Infectious Diseases (PHPID) research initiative, and the OSU Canine Research funds. Finally, we will like to thank the Network on Antimicrobial Resistance in *Staphylococcus aureus* (NARSA) program for providing several control strains. NARSA is supported under NIAID, NIH contract number HHSN272200700055C.
2.8 LIST OF REFERENCES


<table>
<thead>
<tr>
<th>Hospital Service</th>
<th>Human Contact</th>
<th>Animal Contact</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Doors*</td>
<td>Exam Tables*1</td>
</tr>
<tr>
<td>Community Practice</td>
<td>Computers*1,2</td>
<td>Floor (exam)*1</td>
</tr>
<tr>
<td></td>
<td>Otoscope▲</td>
<td>Muzzles▲</td>
</tr>
<tr>
<td>Dermatology</td>
<td>Doors (ward)*</td>
<td>Cages (ward)*</td>
</tr>
<tr>
<td></td>
<td>Exam lights*</td>
<td>Floors (exam)*</td>
</tr>
<tr>
<td></td>
<td>Fax/phone▲</td>
<td>Muzzles (ward)▲</td>
</tr>
<tr>
<td></td>
<td>Computer*1,2</td>
<td>Water bowls (ward)▲</td>
</tr>
<tr>
<td></td>
<td>Microscope▲</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Otoscope▲</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Paper towel dispenser▲1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Alcohol-gel dispensers▲1</td>
<td></td>
</tr>
<tr>
<td>Intensive Care Unit</td>
<td>Doors*</td>
<td>Cages (2)*</td>
</tr>
<tr>
<td></td>
<td>IV Pumps▲</td>
<td>Muzzles▲</td>
</tr>
<tr>
<td></td>
<td>Computer*1,2</td>
<td>Water Bowls▲</td>
</tr>
<tr>
<td></td>
<td>Laptop▲</td>
<td></td>
</tr>
<tr>
<td>Surgery</td>
<td>Clippers▲</td>
<td>Cages*</td>
</tr>
<tr>
<td></td>
<td>Doors*</td>
<td>Exam Tables*</td>
</tr>
<tr>
<td></td>
<td>Drawer Handles*</td>
<td>Muzzles (ward)▲</td>
</tr>
<tr>
<td></td>
<td>Exam Lights*1</td>
<td>Oxygen Monitors▲</td>
</tr>
<tr>
<td></td>
<td>Light switches*1</td>
<td>Warming Pads*3</td>
</tr>
<tr>
<td></td>
<td>Table Knobs*1</td>
<td>Water Bowls▲</td>
</tr>
<tr>
<td>General</td>
<td>N/A</td>
<td>Carts/Gurney (3)</td>
</tr>
</tbody>
</table>

1 Samples collected as a pool within the same service  
2 Included keyboard and mouse  
3 Multiple warming pads located in the same room were sampled as a pool  
N/A: Not applied

**Table 2.1:** Human and canine contact surfaces sampled with electrostatic cloths (■) or sterile swabs (▲) by service at the Small Animal Hospital in The Ohio State University Veterinary Medical Center during the MRSA Active Surveillance
<table>
<thead>
<tr>
<th>Service</th>
<th>Human Contact MRSA/Samples Collected</th>
<th>Animal Contact MRSA/Samples Collected</th>
<th>Total Contact MRSA/Samples Collected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Community Practice</td>
<td>6/37 (16.2%)</td>
<td>7/24 (29.2%)</td>
<td>13/61 (21.3%)</td>
</tr>
<tr>
<td>Dermatology</td>
<td>1/86 (1.2%)</td>
<td>8/48 (16.7%)</td>
<td>9/134 (6.7%)</td>
</tr>
<tr>
<td>Intensive Care Unit</td>
<td>8/48 (16.7%)</td>
<td>5/49 (10.2%)</td>
<td>13/97 (13.4%)</td>
</tr>
<tr>
<td>Surgery</td>
<td>19/108 (17.6%)</td>
<td>8/135 (5.9%)</td>
<td>27/243 (11.1%)</td>
</tr>
<tr>
<td>General</td>
<td>N/A</td>
<td>15/34 (44.1%)</td>
<td>15/34 (44.1%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>34/279 (12.2%)</strong></td>
<td><strong>43/290 (14.8%)</strong></td>
<td><strong>77/569 (13.5%)</strong></td>
</tr>
</tbody>
</table>

1 Included examination and treatment area of the service
2 Included treatment area and ward of the service
3 Included treatment area of the service
4 Included pre-surgery, surgery rooms and wards of the service
5 Included the carts that transport patients from service to service; since they do not belong to one specific service

Table 2.2: Overall prevalence of MRSA contamination of Human and Animal contact surfaces distributed by services at the Small Animal Hospital in The Ohio State University Veterinary Medical Center during the MRSA active surveillance
<table>
<thead>
<tr>
<th>ENVIRONMENT</th>
<th>By Sample (Total N=77)</th>
<th>By Isolate (Total N=81)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MRSA</td>
<td>%</td>
</tr>
<tr>
<td><strong>SCCmec Typing</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type II</td>
<td>73 / 77</td>
<td>94.8%</td>
</tr>
<tr>
<td>Type III</td>
<td>2 / 77</td>
<td>2.6%</td>
</tr>
<tr>
<td>Type IV</td>
<td>3 / 77</td>
<td>3.9%</td>
</tr>
<tr>
<td><strong>PFGE</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>USA 100</td>
<td>71 / 77</td>
<td>92.2%</td>
</tr>
<tr>
<td>USA 300</td>
<td>2 / 77</td>
<td>2.6%</td>
</tr>
<tr>
<td>USA 500</td>
<td>1 / 77</td>
<td>1.3%</td>
</tr>
<tr>
<td>USA 800</td>
<td>1 / 77</td>
<td>1.3%</td>
</tr>
<tr>
<td>Iberian</td>
<td>1 / 77</td>
<td>1.3%</td>
</tr>
<tr>
<td>Novel Type</td>
<td>2 / 77</td>
<td>2.6%</td>
</tr>
<tr>
<td>No Type(^1)</td>
<td>1 / 77</td>
<td>1.3%</td>
</tr>
</tbody>
</table>

\(^1\) One isolate that did not match to any USA type

**Table 2.3:** Molecular characterization of environmental MRSA isolates from the Small Animal Hospital in The Ohio State University Veterinary Medical Center during the MRSA active surveillance
<table>
<thead>
<tr>
<th>Contact Surface</th>
<th>MRSA / Samples collected</th>
<th>Prevalence per location</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Human Contact Surfaces</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Doors</td>
<td>22/97</td>
<td>22.7%</td>
</tr>
<tr>
<td>Drawer handles</td>
<td>2/12</td>
<td>16.7%</td>
</tr>
<tr>
<td>IV Pumps</td>
<td>2/12</td>
<td>16.7%</td>
</tr>
<tr>
<td>Computers(^1)</td>
<td>7/48</td>
<td>14.6%</td>
</tr>
<tr>
<td>Paper towel and alcohol-gel dispensers</td>
<td>1/12</td>
<td>8.3%</td>
</tr>
<tr>
<td>Clippers</td>
<td>0/12</td>
<td>0.0%</td>
</tr>
<tr>
<td>Exam lights (Dermatology)</td>
<td>0/12</td>
<td>0.0%</td>
</tr>
<tr>
<td>Exam lights, light switches, table knobs</td>
<td>0/24</td>
<td>0.0%</td>
</tr>
<tr>
<td>Fax/phone</td>
<td>0/12</td>
<td>0.0%</td>
</tr>
<tr>
<td>Microscope</td>
<td>0/12</td>
<td>0.0%</td>
</tr>
<tr>
<td>Otoscope</td>
<td>0/24</td>
<td>0.0%</td>
</tr>
<tr>
<td>Misc. locations(^2)</td>
<td>0/2</td>
<td>0.0%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>34/279</strong></td>
<td><strong>12.2%</strong></td>
</tr>
<tr>
<td><strong>Animal Contact Surfaces</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hall carts</td>
<td>15/34</td>
<td>44.1%</td>
</tr>
<tr>
<td>Exam tables and floors</td>
<td>15/48</td>
<td>31.3%</td>
</tr>
<tr>
<td>Water bowls</td>
<td>5/36</td>
<td>13.9%</td>
</tr>
<tr>
<td>Muzzles</td>
<td>4/60</td>
<td>6.7%</td>
</tr>
<tr>
<td>Cages</td>
<td>4/73</td>
<td>5.5%</td>
</tr>
<tr>
<td>Oxygen monitor</td>
<td>0/15</td>
<td>0.0%</td>
</tr>
<tr>
<td>Warming pads</td>
<td>0/24</td>
<td>0.0%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>43/290</strong></td>
<td><strong>14.8%</strong></td>
</tr>
</tbody>
</table>

\(^1\) Included all the keyboards and mice, as well as the laptop in ICU

\(^2\) A few extra locations were suggested for sampling during the duration of the study

**Table 2.4:** Prevalence of MRSA on human and animal contact surfaces at the Small Animal Hospital in The Ohio State University Veterinary Medical Center during the MRSA active surveillance
<table>
<thead>
<tr>
<th>CANINE</th>
<th>By Canine (Total N=24)</th>
<th>By Isolate (Total N=37)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MRSA</td>
<td>%</td>
</tr>
<tr>
<td><strong>SCCmec Typing</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type II</td>
<td>20 / 24</td>
<td>83.3%</td>
</tr>
<tr>
<td>Type IV</td>
<td>3 / 24</td>
<td>12.5%</td>
</tr>
<tr>
<td>No Type²</td>
<td>2 / 24</td>
<td>8.3%</td>
</tr>
<tr>
<td><strong>PFGE</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>USA 100</td>
<td>20 / 24</td>
<td>83.3%</td>
</tr>
<tr>
<td>USA 500</td>
<td>1 / 24</td>
<td>4.2%</td>
</tr>
<tr>
<td>USA 800</td>
<td>2 / 24</td>
<td>8.3%</td>
</tr>
<tr>
<td>No Type³</td>
<td>1 / 24</td>
<td>4.2%</td>
</tr>
</tbody>
</table>

1 Isolate from one canine was lost before phenotypic and genotypic characterization was performed


3 Band pattern did not match with any USA type.

Table 2.5: Molecular characterization of MRSA isolated from canines arriving to the Small Animal Hospital in The Ohio State University Veterinary Medical Center during the MRSA active surveillance
Figure 2.1: Distribution of MRSA prevalence on human and canine contact surfaces at the Small Animal Hospital in The Ohio State University Veterinary Medical Center Section during the MRSA Active Surveillance
Figure 2.2: Prevalence comparison between Pulsotype 10 (P10) and other pulsotypes combined by month during the MRSA Active Surveillance at the Small Animal Hospital in The Ohio State University Veterinary Medical Center
**Figure 2.3:** Prevalence of Pulsotype 10 (P10) between human and animal contact surfaces by month at the Small Animal Hospital in The Ohio State University Veterinary Medical Center during the MRSA Active Surveillance
Figure 2.4: Dendrogram based on the Smal macrorestriction fragment profiles of 118 MRSA isolates obtained from environmental surfaces and canines admitted to the Small Animal Hospital in The Ohio State University Veterinary Medical Center. The percent similarity was calculated with Dice coefficients from the PFGE data. Band position tolerance and optimization were set at 1%.
<table>
<thead>
<tr>
<th>Cluster 1</th>
<th>Cluster 2</th>
</tr>
</thead>
</table>

CHAPTER 3

EPIDEMIOLOGICAL PROFILING OF MRSA POSITIVE DOGS ARRIVING AT A VETERINARY TEACHING HOSPITAL

3.1 SUMMARY

Methicillin Resistant *Staphylococcus aureus* (MRSA) has emerged as an important zoonotic and nosocomial pathogen in veterinary settings. Even though human risk factors for MRSA infection and colonization are well known, this information in animals is lacking. The objective of this study was to identify risk factors associated with MRSA carrier dogs on their arrival at a veterinary teaching hospital. A total of 435 dogs were enrolled in the MRSA active surveillance program at The Ohio State University-Veterinary Medical Center over a one-year period. Dogs were screened for MRSA on arrival, regardless of health status, sex, breed, or age. In addition, an epidemiological survey and medical history were obtained for each dog to identify potential risk factors up to one year prior to the appointment. Of 435 dogs included in the study, 25 (5.7%) were MRSA positive, with 86.5% of the isolates classified SCC*mec* type II and USA100. Four of the 25 MRSA carrier dogs were healthy, 20 had health issues unrelated to MRSA, and 1 had an active MRSA infection. MRSA was detected in the nares (72%, 18/25), skin lesions (24%, 6/25) and the perianal area (16%, 4/25). Except for previous
surgery <90 days (OR: 4.9; 95% CI: 1.4–17.6; p-value 0.01), none of the variables related to the previous medical history, dog’s management, home environment, and other potential exposures were associated with the MRSA carrier status. Although, the profession of the owner was significantly associated; dogs owned by veterinary students were 20.5 times (95% CI: 4.5–93.6; p-value < 0.01) more likely to be MRSA positive than dogs owned by clients with different occupations. MRSA positive dogs were dispersed in different categories preventing the creation of an epidemiological profile that would allow their early recognition upon arrival to a veterinary hospital. However, the association between veterinary students with MRSA positive dogs is a concern that deserves further evaluation.

3.2 INTRODUCTION

Multiple studies have reported or suggested the transmission of Methicillin Resistant Staphylococcus aureus (MRSA) from animals to humans (and vice versa) in veterinary settings [1-5]. Such bidirectional transmission of MRSA in veterinary hospitals has become an important occupational and nosocomial issue as shown by a recent study, which reported that MRSA was the second most common zoonotic pathogen, as well as the second most common nosocomial agent, associated with outbreaks in AVMA accredited veterinary teaching hospitals [6].

Dogs are one of the most common patients that visit veterinary hospitals. Although S. aureus, including MRSA, is not the most common staphylococcal species isolated from dogs [7], in the last 5 years the number of reports of Methicillin susceptible S. aureus (MSSA) and MRSA-associated infections in dogs has grown rapidly [8-10].
Several studies have reported risk factors associated with MSSA/MRSA infections in dogs, including the previous use of antibiotics [11], use of β-lactams or fluoroquinolones [11], previous hospitalization [12], surgical treatment [13], and previous intravenous catheterization [11]. However, these studies were mainly focused on clinical cases. In contrast, risk factors associated with the carriage of these pathogens by healthy dogs may be different. For example, Boost et al. [14] identified the sex of the animal, number of people living in the household, and owner occupation (specifically health care workers) as risk factors associated with MRSA colonized dogs, but reported that the previous use of antibiotics was not.

Therefore, the objective of this study was to identify potential risk factors associated with MRSA carriage in dogs admitted to our veterinary teaching hospital, regardless of their health status, which could allow us to create an epidemiological profile of potential MRSA carrier dogs. This profile may help veterinarians and veterinary personnel to early identify potential MRSA positive dogs upon arrival, so they can be properly handled and segregated to avoid human exposure (decreasing occupational risk), and to prevent contamination of the hospital environment (decreasing nosocomial transmission).

3.3 MATERIALS AND METHODS

3.3.1 Study Population and Sample Collection

This study was conducted as the MRSA active surveillance program at The Ohio State University - Veterinary Medical Center (OSU-VMC). The OSU-VMC is a tertiary referral veterinary medical hospital that is open 24-hours a day. It accepts referral patients
from veterinarians throughout the Midwestern US, but also operates a primary care non-referral community practice. Once each month for one year (November 2007 to October 2008) dogs coming to the OSU-VMC were systematically recruited for participation regardless of their health status, age, sex, or breed. Only patients (coming for the first time or referral clients) that had not visited our hospital in the last 6 months were included in the study.

Each month, dogs presented to the Community Practice and Intensive Care Unit (n=148), Dermatology Service (n=145), and Surgery Services (n=142) of the OSU-VMC were recruited upon admittance to the hospital. These services were targeted for MRSA surveillance as both preliminary cross-sectional data and passive surveillance data from the OSU-VMC (not presented here) indicate that these clinical services have patients with MRSA more frequently compared to other services, which would increase the odds of detecting MRSA cases. In addition, animals attending these services tend to have pre-existing health conditions that could increase their susceptibility to MRSA infections.

When the true prevalence ($p$) of a disease in a population is unknown it is recommended to use a conservative $p$ estimate of 50% [15]. By using this value of $p$, the sample size formula will yield the maximum value of $n$, providing a larger sample size than needed [15]. Therefore, the quota of dogs was obtained based on sample size calculations to detect a higher than expected MRSA prevalence in incoming dogs, with an allowable error of +/-5%, 95% confidence, and 80% power. The result of this calculation suggested that 384 observations were needed to meet our objectives. This number was then divided equally over 12 months and distributed evenly among the different sections of the hospital as the quotas for recruitment of canine patients.
A signed consent form was obtained from the dog’s owner for inclusion into this study. Prior to any clinical examination or intervention the dog was sampled by collecting sterile, pre-moistened cotton swabs in sterile tryptone soy broth. Samples from the nasal cavity, ear channels, external surface of the perianal area, and any skin lesions (if present) were taken in order to maximize the probability of MRSA detection. Samples collection protocols, owner’s consent form and survey administered by veterinarian were evaluated and approved by the Institutional Animal Care and Use Committee (IACUC). Animals with MRSA isolated from one or more anatomical locations were considered MRSA positive.

3.3.2 Epidemiological Survey and Data Collection

An epidemiological survey was administered to the owner by a veterinarian to collect background information about the dog in the year immediately prior to the appointment were the sample was taken. Signalment data about the dog and other relevant epidemiologic information were collected (see Table 3.1). The dog’s medical records at OSU-VMC were also reviewed up to one year prior to study inclusion. The referral medical records used were those previously included in the patient’s chart and/or provided by the owner at the time of his/her visit.

3.3.3 MRSA Isolation, Identification and Staphylococcal chromosome cassette mec (SCCmec) typing

All samples were then taken and processed at the Diagnostic and Research Laboratory for Infectious Diseases (DRLID) at the OSU, College of Veterinary
Swabs were incubated in pre-enrichment media (trypticase soy broth, BD BBL™) for 24 hours at 35°C. Then, an aliquot was plated onto mannitol salt/oxacillin (2µg/ml) agar (BD BBL™), and incubated at 35°C for 24-48 hours. At least three colonies with typical mannitol reaction were selected from each sample and plated on tryptic soy agar with 5% Sheep Blood (Remel®). Bacterial identification was performed by standard colony morphology, size, pigmentation, hemolysis pattern, mannitol fermentation, gram stain reaction, catalase reaction, tube coagulase reaction, anillin reaction, polymyxin B susceptibility, acetoin production, and latex agglutination reaction (Sure-Vue® Color Staph ID). Phenotypic identification of MRSA was confirmed by growth on Oxacillin Screen Agar® plates containing 6µg/ml of oxacillin supplemented with NaCl (BD BBL™) following CLSI protocols [16]. Presence of the mecA gene and SCCmec typing was established using a multiplex PCR[17] with some modifications. Briefly, each PCR mixture with a final volume of 25µl contained 2µl of DNA template, 1x of 2x Multiplex PCR Master Mix (Qiagen®) containing HotStartTaq Polymerase, and 3mM MgCl2 and dNTPs. Each primer concentration was doubled from those stated by Milheirico et al [17]. PCR products were resolved in a 3% Seakem LE (Cambrex) agarose gel with 1x Tris-acetate-EDTA buffer (Promega Corporation) at 100 Volts for 2 hours, and were visualized with ethidium bromide. All assays were performed in a Gradient Thermocycler (Eppendorf). The optimal cycling conditions were the following: 95°C for 15 minutes; 35 cycles of 94°C for 30 seconds, 57°C for 90 seconds and 72°C for 90 seconds; and a final extension of 72°C for 10 minutes. Results of further molecular characterization using the multiplex PCR are not shown.
3.3.4 Pulsed field gel electrophoresis (PFGE)

Digestion of genomic DNA with restriction enzyme \textit{SmaI} was performed following standardized Pulse Net protocols [18], using a CHEF mapper system (Bio-Rad Laboratories, Nazareth, Belgium) to separate restriction fragments for electrophoresis. Salmonella serotype Branderup strain H9812 digested with \textit{XbaI} was used as a molecular size marker. Band pattern and dendrogram analysis were performed in BioNumerics\textsuperscript{®} software (version 6.6, Applied Maths, Ghent, Belgium) using Dice coefficient and Unweighted Pair Group Method using Arithmetic averages (UPGMA) with a 1\% band position tolerance. Isolates with $\geq$98\% similarity were considered indistinguishable and characterized as the same pulsotype. Additionally, pulsotypes with $\geq$80\% similarity were considered to be closely related and grouped in clusters. To establish USA types, each isolate was compared to a CDC database containing 100 \textit{S. aureus} strains with the most typical band patterns using a $\geq$80\% similarity as the cutoff point.

3.3.5 Statistical Analyses

The unadjusted associations between the independent variables and MRSA carrier status was first described by generating odds ratios (OR) and their 95\% confidence intervals (CI) which were assessed using Pearson’s Chi-square or Fisher’s Exact test when appropriate using the EpiInfo software (http://wwwn.cdc.gov/epiinfo/). Independent variables that were associated with MRSA carrier status upon this initial screening at $P < 0.1$ were tested for inclusion in a multivariable logistic regression model using SAS (Proc Genmod, SAS Institute, Cary NC). The final logistic regression model
was obtained using a backward selection procedure with a $P < 0.05$ based on the likelihood ratio chi-square statistic required to remain in the model. Final model goodness-of-fit was assessed using the Hosmer-Lemeshow goodness-of-fit test [19].

### 3.4 RESULTS

During one year of the MRSA Active Surveillance in the OSU-VMC, a total of 435 dogs were sampled. Twenty five dogs (5.7%) were MRSA positive in one or more anatomical locations, and therefore classified as MRSA carriers. The distribution of the screened dogs based on age, sex, reproductive status, breed, coat length, and coat shade is presented in Table 3.2. No association was detected between these variables and the MRSA carrier status.

The nasal cavity was the most common location testing positive for MRSA (72%, 18/25), followed by skin lesions (24%, 6/25), the perianal area (16%, 4/25), and the ears (12%, 3/25). MRSA was found in more than one anatomical location in 20% (5/25) of the canines. Twenty-eight percent (7/25) of the MRSA carrier dogs tested negative in the nose, but positive in other anatomical locations. Interestingly, 50% (3/6) of the dogs that were MRSA positive in a skin lesion were also positive in their nose.

Four of the 25 (16%) MRSA carrier dogs were healthy dogs, 20 (80%) had a health issue unrelated to the presence of MRSA, and only one (4%) had an active MRSA infection (dog with deep pyoderma). A dog was considered healthy if there was no history of current disease or illness and no abnormalities were detected on full physical examination. The dog’s health status was not statistically associated with MRSA carrier status.
Except for previous surgery <90 days (unadjusted OR: 4.68; 95% CI: 1.20 – 16.80; \( P < 0.02 \)), none of the variables from the medical history, management and home environment, and other potential exposures were associated with MRSA carrier status, including hospitalization and previous use of antibiotics.

The profession of the owner was associated (\( P < 0.05 \)) with the MRSA carriage status of the dog. During the interview 44 owners reported to have a profession related to the health care industry, of which six (13.6%) owned a dog that was MRSA positive at the time of their visit. When analyzed further, only one of these six MRSA positive dogs had an owner who was a human health care professional (a nurse). The owners of the remaining dogs in this category of health care professionals were veterinary students (4) and OSU-VMC staff (1). Therefore, the unadjusted odds of a dog owned by a veterinary student testing positive for MRSA on arrival at the OSU-VMC were 8.49 times higher (95% CI: 2.0 – 33.8; \( P < 0.01 \)) than dogs owned by other clients with a different occupation (including human health care workers).

A total of 37 unique isolates were obtained from 24 of the 25 MRSA positive canines. Unfortunately, the only isolate obtained from one canine was lost before any further molecular characterization could be performed. Over 86% of the isolates (32/37) were classified SCC\text{mec} type II, 8.1% (3/37) were type IV and 5.4% (2/37) were not typeable. From the PFGE results, 16 pulsotypes were identified and 11/16 were grouped in two major clusters, with 75.7% (28/37) of the isolates grouped in cluster 1 (Figure 3.1). In addition, 86.5% (32/37) of the isolates were classified as USA100, 2.7% (1/37) as USA500 and 5.4% (2/37) as USA800. Two isolates were not typeable by the protocol used.
3.4.1 Multivariable analysis

After the initial screening, only variables representing age, breed, previous surgery \( \leq 90 \) days, housing, farm contact, and owner’s occupation were associated at \( P \leq 0.1 \) and therefore were tested for inclusion in the final model (Table 3.3). First, dogs owned by veterinary students had a 20.5 (95% CI: 4.5 – 93.6; \( P < 0.01 \)) greater odds of being MRSA positive than dogs with owners from other professions. Second, dogs that have a surgical procedure in the last 90 days before the sample collection had a 4.9 (95% CI: 1.4 - 17.6, \( P < 0.01 \)) greater odds of being MRSA positive than dogs that did not have recent surgery prior to their visit to the OSU-VMC. Finally, indoor housing of dogs appears to be protective against MRSA with an odds ratio of 0.36 (95% CI: 0.13 – 1.05, \( P < 0.06 \)) compared to outdoor housing or a combination of both.

3.5 DISCUSSION

The aim of this study was to identify risk factors for MRSA carriage in dogs presented to a veterinary teaching hospital to create a predictive profile of potential MRSA carrier dogs. This profile may allow veterinary personnel and staff to target these canine patients for segregation and isolation to decrease zoonotic exposure and further contamination of the hospital environment that could lead to nosocomial infections. The targeted year-long surveillance showed that 5.7% of the sampled dogs carried MRSA upon arrival at the OSU-VMC. Other studies have reported MRSA prevalence estimates ranging from 0.5% to 8.9% [8-10, 20, 21]. However, MRSA is not always detected in dogs visiting veterinary hospitals [22].
Even though few studies report MRSA prevalence from specific anatomical locations [23, 24], our identification of the nose as the most common site for MRSA detection was not unexpected as dogs use their noses to interact and recognize their environment. Interestingly, if only the nose had been sampled in this study, 28% of the MRSA positive dogs would not have been identified. Therefore, we recommend that when screening dogs for MRSA carriage to sample more than one anatomical site in order to maximize detection. It was also interesting to observe that half of the dogs with MRSA on their skin lesions were also positive on their nose indicating possibility of self exposure (endogenous source), as has been reported in humans [25].

It is noteworthy that, 16% of MRSA carrier dogs were completely healthy individuals. These dogs would not normally attract suspicion as potential MRSA carriers, highlighting the difficulty of early identification. This also suggests that there is no single physical characteristic of a dog that might increase its likelihood to MRSA colonization, but it is more likely that such colonization results from exposure to infected individuals or contaminated environments. This assertion is in agreement with the results found by others [11], who did not report an association of MRSA colonization with any demographic characteristics of the dogs, except for the use of specific antimicrobials drugs 90 days before the admission. Similarly, of all individual characteristics of the dogs studied by Boost et. al. [26], they only found that female dogs were more likely to be MRSA positive; however, the authors did not provide a biological reason for this association. Therefore, further research will be necessary to determine if there is really an association between these individual dog variables and the MRSA carriage status.
The transmission of MRSA between animals and humans is well described [1, 3, 7, 27-29]. On the human side, health care workers (HCW) and veterinary personnel are considered major risk groups for MRSA colonization due to their frequent exposure to infected patients (human or animals) and/or contaminated environments [30-32]. Therefore, it was not surprising that our results found the owner’s occupation to be associated with the MRSA carrier status of the dogs, especially when the owner was involved in veterinary medicine. It is a major concern that dogs owned by veterinary students are far more likely to be MRSA carriers than dogs owned by other individuals, as these dogs and their owners (who are the most likely source of MRSA to their animals) could act as reservoirs of the bacteria in the community [33]. It has been demonstrated that MRSA positive dogs are not only capable of contaminating household surfaces or transmitting the bacteria to humans (zoonotic exposure) and other animals, but also contaminating veterinary hospital surfaces, thereby increasing the probability of nosocomial infections [1]. Finally, several reports have shown the relationship between human HCW and the colonization of their animals with MRSA [14, 26, 34]. We strongly believe that further research specifically targeting human HCW should be undertaken, and special attention should be paid to veterinary professionals.

There are several possibilities that could explain why dogs owned by veterinary students had a higher risk for MRSA carriage (either contamination or colonization) in this study. First, in veterinary teaching hospitals the students typically perform the physical examination and take care of the patients during the appointment. Therefore, the student’s length of exposure is higher compared to the rest of the hospital personnel (veterinarians, technicians and staff) and they are consequently more likely to
contaminate their clothing or hands, or to even become colonized or infected. Evidence of this increased exposure to patients by veterinary students has been described [35]. Similarly, van Duijkeren et al. reported that personnel, such as veterinary students, who have direct contact with animals were more likely to be MRSA positive (13%) than personnel without such contact (1.6%), therefore identifying direct contact as an important risk factor [2]. Such increased exposure and possibility of contamination or colonization by veterinary students, combined with their limited experience in infectious diseases control practices, could lead to the exposure of their pets by allowing direct contact with the student’s colonized sites or by carrying MRSA home on contaminated clothing, equipment, or their own skin and hair. This role that veterinary students may play in the dissemination of MRSA through their contaminated hands, clothing, or from their colonized sites has been previously suggested [2]. In addition, this pathway of transmission has also been reported in human health care workers [34]. A second possibility is that veterinary students frequently use the service of the OSU-VMC for the medical care of their own pets. However, on some occasions, these animals are not treated as regular patients during their visit, allowing them to have access to multiple sections and locations across the hospital during their stay. This practice could increase the animal’s exposure to contaminated surfaces in the hospital environment and possible colonization to MRSA.

As indicated previously, veterinary students may play an important role in the nosocomial transmission of MRSA in veterinary clinics [35]. Therefore, it is of key importance to emphasize to students and staff that, similar to human hospitals [36], they must pay close attention to the dress code (e.g., not going home with work clothes),
personal hygiene (hand washing), and proper management of their pets during hospital visits. All these biosecurity and biocontainment practices should be applied routinely to decrease the probability of exposing their own animals and contributing to the spread of MRSA inside or outside the hospital.

Our results suggest that surgery is a risk factor for MRSA carriage, and that having a surgery up to 90 days previous to their visit to the hospital increases the odds of being a MRSA positive dog. Transmission of MRSA between colonized or infected veterinary personnel and patients after a surgical procedure has been reported [37, 38], which explains why surgery may be considered a risk factor for nosocomial MRSA infections. However, this connection is not always detected as others have found no association with infections from this pathogen and surgery [9, 11]. Lastly, dogs that lived indoors were less likely to be colonized with MRSA than dogs kept mostly outside. We cannot offer a definitive explanation for this association except that it supports the hypothesis that the dog’s environment plays an important role in the opportunity for MRSA colonization.

Finally, based on the molecular characterization, 86.5% of the isolates were SCCmec type II and USA100, characteristics that are consistent with hospital acquired MRSA strains (HA-MRSA). However, it seems like USA100 strains have slowly migrated into the community, which makes the process of differentiating between hospital acquired and community acquired MRSA more difficult. For example, between 2001-2004, USA100 was the most common strain found in the general US population [39]. This type of MRSA strain has also been describe in companion animals and the people that directly interacts with them (owners and veterinary practitioners) [21, 24, 40-
42], which indicates that what we are finding in the small animal population might be a reflection of what is present in the human population. It is noticeable that even though several MRSA strains are circulating in the human population, only a few are more commonly isolated from companion animals. The possibility of some MRSA strains being able to adapt or survive better in specific animal species should be considered. Conversely, USA500 strains are more commonly associated with horses [43]. The owner of the only dog positive for this type of strain stated to have two horses but did not specify if there was any direct contact between them and the dog. However, it was reported by the owner that the housing of the dog was 50% indoor and 50% outdoor, allowing the possibility of direct or indirect contact between the two animal species. Because of the lack of diversity and very close relatedness of the majority of the MRSA strains detected in the dogs entering the OSU-VMC, the molecular characterization of the MRSA strains did not provide any additional information to the epidemiological profile. However, the information collected through the survey and the medical history did not allow to obtain owner-specific data regarding MRSA exposure to healthcare and community risk factors. Therefore, future studies should expand the epidemiological survey to include as much information about the owner and household members, to identify potential sources of MRSA.

One of the limitations of this study was the fact that we did not follow the dogs over time to determine if they were colonized or just contaminated by MRSA due to a recent exposure to a contaminated surface or infected individual. Colonized dogs without clinical signs are of far greater concern than dogs that are simply contaminated with MRSA but do not have an established subclinical infection. Another limitation was the
fact that even though MSSA strains were also isolated from the dogs included in this study, due to the use of low concentrations of oxacillin in the mannitol salt agar plates during the screening process, it is possible that some MSSA strains were missed. This could lead to an underrepresentation of the true prevalence of MSSA in this population; therefore, they were not included in the analysis. Lastly, due to the fact that the OSU-VMC is a tertiary healthcare facility, our results cannot be literally extrapolated to the general population of dogs and/or every other hospital around the country. However, these results provide valuable information that in combination with other studies in different canine populations, will eventually allow to have a better understanding of the epidemiology of MRSA in dogs.

3.6 CONCLUSIONS

MRSA is currently one of the most important emerging pathogens in human and animal populations. However, risk factors associated with MRSA infections in animals are not well characterized. In this study, we found that owner occupation, previous surgery, and indoor housing were risk factors associated with canine MRSA carriage. Although these characteristics may not be enough to identify an animal as a potential MRSA carrier, factors such as owner’s occupation could be useful to aid this prediction, especially if the owner’s occupation is related to the veterinary medicine field. This study also highlights the possible role that veterinary students could play in recirculating and moving MRSA strains in and out of veterinary hospitals, which should be investigated further. In conclusion, the MRSA status of canine patients arriving at the OSU-VMC is
robably more related to recent contacts and environmental exposures than to any individual characteristic of the animal.

3.7 ACKNOWLEDGMENT

We wish to thank Amber Reed and Christina Jackson for their collaboration in the collection and processing of samples in the early stages of this study, as well as Sarah Baker for her contribution during the data collection from the dogs medical records. We also wish to thank Duncan MacCannell, from the Center for Disease Control and Prevention (CDC), for facilitating the database containing S. aureus strains with the most typical band patterns for each USA type for PFGE characterization; as well as Dr Herminia de Lencantre for providing MRSA controls isolates for the standardization of the SCCmec type multiplex PCR. We are grateful for the financial support provided for the development of this project by The Ohio State’s Public Health Preparedness for Infectious Diseases (PHPID) research initiative, and the OSU Canine Research funds.
3.8 LIST OF REFERENCES


Table 3.1: Variables included in the epidemiological survey used to determine the epidemiological profile of potential MRSA positive dogs arriving at the Ohio State University – Veterinary Medical Center (OSU-VMC)
<table>
<thead>
<tr>
<th>Demographic Characteristics</th>
<th>Medical History</th>
<th>Dog’s Management and House Environment</th>
<th>Other Potential Exposures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Health status at admission</td>
<td>Type of Diet (Commercial, Home Made, Raw food)</td>
<td>Travel history (exhibitions, competitions, pleasure)</td>
</tr>
<tr>
<td>Gender</td>
<td>Reason of visit (current illness)</td>
<td>Used of treats and snacks (frequency and types)</td>
<td>Type of locations visited (parks, playgrounds, nursing homes, hospitals, therapy groups, schools, farms)</td>
</tr>
<tr>
<td>Reproductive status</td>
<td>Hospital section visited</td>
<td>Housing (Indoor, Outdoor)</td>
<td>Grooming practices (professional or at home)</td>
</tr>
<tr>
<td>(spay/neuter or intact)</td>
<td>Referral status</td>
<td>Other pets in the household (species and numbers)</td>
<td>Dog’s role in the household (companion, hunting/sports, work/service)</td>
</tr>
<tr>
<td>Weight</td>
<td>Previous and concurrent illness(es)</td>
<td>Illnesses of other pets in the household</td>
<td>Owner’s Occupation</td>
</tr>
<tr>
<td>Breed</td>
<td>Previous hospitalization</td>
<td>Housing (Indoor, Outdoor)</td>
<td>Human health care worker</td>
</tr>
<tr>
<td>Coat length</td>
<td>Previous surgery</td>
<td>Other pets in the household (species)</td>
<td>Veterinary health care worker</td>
</tr>
<tr>
<td>Coat shade</td>
<td>Previous antimicrobial used</td>
<td>Number of family members in the household</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Previous antimicrobial used by class</td>
<td>Concurrent illnesses in family members</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Use of immunosuppressant drugs</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Use of other drugs</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Analyzed at 30 days, 90 days, and 1 year previous to screening.
<table>
<thead>
<tr>
<th>Variables</th>
<th>N</th>
<th>MRSA (%)</th>
<th>OR</th>
<th>Confidence Interval</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 3 years</td>
<td>142</td>
<td>4 (2.8)</td>
<td>0.38</td>
<td>0.11 – 1.19</td>
<td>0.07</td>
</tr>
<tr>
<td>3 years and older</td>
<td>293</td>
<td>21 (7.2)</td>
<td>1.0</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>233</td>
<td>12 (5.2)</td>
<td>0.79</td>
<td>0.33 – 1.89</td>
<td>0.57</td>
</tr>
<tr>
<td>Female</td>
<td>202</td>
<td>13 (6.4)</td>
<td>1.0</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td><strong>Reproductive Status</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intact</td>
<td>103</td>
<td>6 (5.8)</td>
<td>1.02</td>
<td>0.35 – 2.80</td>
<td>0.97</td>
</tr>
<tr>
<td>Spay/Neuter</td>
<td>332</td>
<td>19 (5.7)</td>
<td>1.0</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td><strong>Breed</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mix</td>
<td>94</td>
<td>2 (2.1)</td>
<td>0.30</td>
<td>0.05 – 1.35</td>
<td>0.09</td>
</tr>
<tr>
<td>Pure</td>
<td>341</td>
<td>23 (6.7)</td>
<td>1.0</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td><strong>Coat Length</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Short</td>
<td>271</td>
<td>19 (7.0)</td>
<td>1.91</td>
<td>0.70 – 5.47</td>
<td>0.17</td>
</tr>
<tr>
<td>Long</td>
<td>158</td>
<td>6 (3.8)</td>
<td>1.0</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td><strong>Color Shade</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>One color</td>
<td>246</td>
<td>15 (6.1)</td>
<td>1.16</td>
<td>0.48 – 2.86</td>
<td>0.72</td>
</tr>
<tr>
<td>2 or more colors</td>
<td>189</td>
<td>10 (5.3)</td>
<td>1.0</td>
<td>–</td>
<td></td>
</tr>
</tbody>
</table>

1 Missing information in 6 dogs, total n=429.

**Table 3.2:** Distribution of positive cases and unadjusted odds for demographic variables of potential MRSA carrier incoming dogs entering the Ohio State University - Veterinary Medical Center (OSU-VMC)
<table>
<thead>
<tr>
<th>Variable</th>
<th>OR</th>
<th>Confidence Interval</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 3 years</td>
<td>0.34</td>
<td>0.11 – 1.09</td>
<td>0.07</td>
</tr>
<tr>
<td>3 years and older</td>
<td>1.00</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td><strong>Breed</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mix</td>
<td>0.22</td>
<td>0.04 – 1.13</td>
<td>0.07</td>
</tr>
<tr>
<td>Pure</td>
<td>1.00</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td><strong>Previous Surgery &lt; 90 days</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>4.90</td>
<td>1.37 – 17.61</td>
<td>0.01</td>
</tr>
<tr>
<td>No</td>
<td>1.00</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td><strong>Housing</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indoor</td>
<td>0.36</td>
<td>0.13 – 1.05</td>
<td>0.06</td>
</tr>
<tr>
<td>Outdoor/Both</td>
<td>1.00</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td><strong>Farm Contact</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>5.29</td>
<td>0.65 – 42.77</td>
<td>0.12</td>
</tr>
<tr>
<td>No</td>
<td>1.00</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td><strong>Owner’s Occupation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Veterinary Students</td>
<td>20.52</td>
<td>4.50 – 93.64</td>
<td>≤0.01</td>
</tr>
<tr>
<td>Other</td>
<td>1.00</td>
<td>–</td>
<td></td>
</tr>
</tbody>
</table>

Table 3.3: Multivariable logistic regression model for estimating the adjusted odds of potential MRSA carrier incoming dogs entering the OSU Veterinary Medical Center (OSU-VMC)
Figure 3.1: Dendrogram based on the Smal macrorestriction fragment profiles of 37 MRSA isolates obtained from canines admitted to the Ohio State University Veterinary Medical Center. The percent similarity was calculated with Dice coefficients from the PFGE data. Band position tolerance and optimization were set at 1%
CHAPTER 4

MOLECULAR EPIDEMIOLOGY OF ENVIRONMENTAL MRSA AT AN EQUINE TEACHING HOSPITAL: INTRODUCTION, CIRCULATION AND MAINTENANCE

4.1 SUMMARY

The role that environmental contamination might play as a reservoir and a possible source of Methicillin-resistant *Staphylococcus aureus* (MRSA) for patients and personnel at equine veterinary hospitals remains undefined, as the environment has only been monitored during outbreaks or for short periods. Therefore, the objectives of this study were to determine the monthly presence, distribution, and characteristics of environmental MRSA at an equine hospital, and to establish patterns of contamination over time using molecular epidemiological analyses. For this purpose, a yearlong active MRSA surveillance was performed targeting the environment and incoming patients. Antimicrobial susceptibility testing, SCC\textit{mec} typing, PFGE typing, and dendrographic analysis were used to characterize and analyze these isolates. Overall, 8.6\% of the surfaces and 5.8\% of the horses sampled were positive for MRSA. The most common contaminated surfaces were: computers, feed-water buckets, and surgery tables-mats. Ninety percent of the isolates carried SCC\textit{mec} type IV, and 62.0\% were classified as
USA500. Molecular analysis showed that new pulsotypes were constantly introduced into the hospital throughout the year. However, maintenance of strains in the environment was also observed when unique clones were detected for 2 consecutive months on the same surfaces. Additionally, pulsotypes were circulating throughout several areas and different contact surfaces of the hospital. Based on these results, it is evident that MRSA is constantly introduced and frequently found in the equine hospital environment, and that some contact surfaces could act as “hot-spots”. These contaminated surfaces should be actively targeted for strict cleaning and disinfection as well as regular monitoring.

4.2 INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a widely spread opportunistic pathogen that has been found circulating on horse farms with prevalences ranging from 0.6 % up to 4.7%; these horses are typically colonized with MRSA without manifesting clinical signs [1-6]. In contrast, a higher prevalence, from 5.8% to 12.0%, has been reported in horses admitted to veterinary hospitals, where the horses are more likely to manifest clinical illnesses mostly associated with joint, skin, traumatic wounds and surgical site infections, among others [7-11]. This pathogen has been isolated from horses worldwide, and the genotypic characteristics of MRSA strains found in the equine population vary within regions [1, 3, 5, 8, 10, 12, 13]. Moreover, certain clones like the well-known USA500, seems to be one of the most prevalent among equidae, and even the emerging LA-MRSA (ST398) has been recently described in horse populations [13-15].

In recent years, MRSA has become one of the most important nosocomial pathogens affecting equine hospitals. An example of this nosocomial transmission and
impact was described in The Netherlands [16], where 33.9% (21/62) of all MRSA equine clinical cases that were managed at a veterinary teaching hospital (VTH) were caused by hospital acquired infections. These horses tested negative for MRSA at their time of arrival and acquired this pathogen during their hospitalization.

When studying the possible sources of MRSA in a veterinary hospital setting, two different scenarios could be considered as potential sources of this bacterium in horses: endogenous and exogenous. An endogenous source refers to animals that are already colonized at their time of arrival and are capable of self-inoculating themselves after a diagnostic or surgical procedure. In contrast, an exogenous source denotes the participation of an external component (human, animal or environment) as the plausible origin of this pathogen. This last scenario has been reported multiple times in which colonized or infected hospital personnel or contaminated hospital environments have been associated with the transmission of MRSA to horses in veterinary settings [8, 17, 18].

Environmental contamination has been considered a possible source of nosocomial MRSA infections that have occurred in equine hospitals [8, 17, 19]. MRSA is capable of surviving up to seven months on inanimate objects and contact surfaces of healthcare facilities [20]. Despite these facts, the presence of MRSA in equine hospital environments has been studied only during outbreak investigations or for very short periods of time [16, 17, 21, 22]. As a result, the role that contaminated surfaces might play as a reservoir and a possible source of MRSA for patients and hospital personnel, as well as the type of strains frequently circulating in equine hospitals remains undefined. Therefore, the objectives of this study were to determine the presence and distribution of
MRSA environmental contamination during one year at an equine teaching hospital; to determine the phenotypic and genotypic characteristics of the MRSA strains circulating in the hospital; as well as to establish patterns and changes of this contamination over time using molecular epidemiological analyses.

**4.3 MATERIALS AND METHODS**

**4.3.1 Active MRSA Surveillance**

This study was conducted at the Galbreath Equine Center (GEC) from The Ohio State University Veterinary Medical Center over a one year period, between September 2009 and August 2010. The GEC is a large tertiary healthcare hospital that receives over 1700 equine patients per year, and provides services to the Ohio equine community as well as referral cases from private practices throughout the Midwestern US.

**4.3.2 Environmental Locations, Surfaces and Sample Collection Method**

Sixty seven environmental samples were collected from the GEC every month from the following services: Internal Medicine (16 samples/month), Intensive Care Unit (14 samples/month) and Surgery (26 samples/month). Samples were also collected from common areas that did not belong to any particular service, and were characterized as General Areas (11 samples/month). The number and locations of the surfaces to be sampled were determined based on the results obtained during a 2007 pilot study performed at GEC [21]. Furthermore, these areas were targeted, because MRSA found in any of these environments would represent a potential nosocomial risk for patients using these services.
Surfaces from the targeted services were categorized as human contact and animal contact surfaces (Table 4.1) as previously described [23]. Since the majority of the spaces around the GEC are very open and large in size, it would have been nearly impossible to individually sample all the surfaces of every room. Therefore, in some cases several surfaces in the same section/room were sampled as a pool (referred as Pool A to Q) to be able to cover as much area of the hospital as possible (see Table 4.1). All areas/rooms of GEC are due to be clean and disinfected at the end of the day, and general equipment (e.g. endoscope, feed & water buckets, surgery tables & mats) are required to be washed and disinfected between each patient. Environmental samplings were performed in late hours of the afternoon, before any cleaning was done by the hospital staff. In the case of the equipment, samples were collected regardless of the last time they were used and/or disinfected.

Every month the same pre-selected surfaces were sampled using dry electrostatic cloths (for large surfaces) and sterile pre-moistened cotton swabs (for smaller surfaces) [23]. In the case of pooled samples, the same electrostatic cloth was used to consistently sample all the surfaces included in the pool. The size and location of the area sampled from each surface were always the same each month. If during the sampling date a pre-selected surface was not available (i.e. the endoscope), it was skipped until the next month. In the case of equipment with numerous units present at the hospital (e.g. twitches, feed & water buckets, and surfaces included in pooled samples O, P and Q), only 3 units were sampled as a pool from those available at the time of sampling. All collected samples were processed at the Diagnostic and Research Laboratory for
Infectious Diseases (DRLID) at the OSU, College of Veterinary Medicine. Upon arrival in the laboratory, electrostatic cloths and swabs were placed in pre-enrichment media and incubated at 35°C for 24 hours [23].

4.3.3 Source of Equine Isolates

To determine the potential role that incoming horses could have in introducing MRSA strains into the hospital, parallel to the monthly environmental surveillance, a convenience sample of equines admitted to the same targeted services of the hospital was performed. Upon arriving to the hospital, a signed consent form was obtained from the horse’s owner. Before any clinical examination was performed on the animal by the hospital personnel, samples were collected from three to four anatomical locations on each horse. These locations were the nares (both sides), armpits (both sides), perianal area, and skin lesions (if any were present). Samples were collected with sterile pre-moistened cotton swabs in TSB (BD BBL™ Trypticase Soy Broth, Becton, Dickinson and Company, Sparks, USA) and were kept at room temperature and away from direct light, until the end of the day when all collected samples were taken to DRLID for further processing. The procedures used to sample the horses were approved by the IACUC (Protocol Number 2010A0000099-R1). In addition to the equine isolates collected during the active surveillance, two specimens from post-surgical MRSA infections that occurred during the study period were also included. Neither one of these horses were sampled at their arrival. These clinical cases were diagnosed by the Clinical Microbiology Laboratory of the OSU Veterinary Medical Center and banked as part of the center’s routine passive surveillance.
4.3.4 Bacterial Isolation and Characterization

Specimen screening was performed as described before [23] using selective and non-selective media. Identification of *S. aureus* colonies was achieved based on colony morphology and reactions to biochemical tests (mannitol fermentation, gram stain, catalase, tube coagulase, latex agglutination [Sure-Vue® Color Staph ID, Biokit USA, inc, Lexington, Mass], anillin fermentation, Polymyxin B susceptibility and acetoin production [Vogues-Proskauer test]). Growth on Oxacillin Screen Agar® (OSA) plates that contained 6µg/ml of Oxacillin supplemented with NaCl (BD BBLTM, Becton Dickinson and Company, Maryland, USA) were used to phenotypically classify isolates as methicillin-susceptible *S. aureus* (MSSA) or MRSA following the Clinical Laboratory Standards Institute protocols [24].

4.3.5 Phenotyping

Antimicrobial susceptibility profiles of 110 environmental isolates were determined by testing against 15 antimicrobials drugs (Amikacin 30µg, Ampicillin 10µg, Amoxicillin with Clavulanic Acid 30µg, Cefpodoxime 10µg, Cephalothin 30µg, Chloramphenicol 30µg, Ciprofloxacin 2µg, Clindamycin 2µg, Doxycycline 30µg, Enrofloxacin 5µg, Erythromycin 15µg, Gentamicin 1µg, Oxacillin 1µg, Sulfamethoxazole with Trimethoprim 25µg and Tetracycline 30µg) using the Kirby-Bauer Disc Diffusion technique following protocols described by CLSI [24]. In addition, Vancomycin resistance was assessed using Vancomycin Screen Agar plates (6 mg/L) (BD BBL™ Vancomycin Screen Agar, Dickinson and Company, Sparks, USA).
Inducible Clindamycin resistance was tested using the D-test [25]. Throughout the text, the term multidrug resistant (MDR) will be used for isolates resistant to 3 or more antimicrobial classes (including beta-lactams after \textit{mecA} gene confirmation).

### 4.3.6 Genotyping: \textit{mecA} gene Confirmation, Staphylococcal Chromosomal Cassette (SCC) \textit{mec} Characterization and Pulsed Field Gel Electrophoresis (PFGE)

Only 71 environmental isolates with unique phenotypic profiles were further characterized. All molecular techniques were performed as previously described [23]. Briefly, SCC\textit{mec} typing (type I to type VI) and confirmation of the presence of the \textit{mecA} gene were assessed using a modified version of a multiplex PCR [26]. All primer concentrations were adjusted by doubling the original concentration. PCR mixture contained 2\mu l of DNA template and 12.5\mu l of 2x Multiplex PCR Master Mix (Qiagen®, Foster City, CA). Primers and molecular grade water were added to reach a final volume of 25\mu l per reaction. A Gradient Thermocycler (Eppendorf, Hamburg, Germany) was used with the following cycling conditions: 95°C for 15 minutes; 35 cycles of 94°C for 30 seconds, 57°C for 90 seconds and 72°C for 90 seconds; and a final extension of 72°C for 10 minutes. Seakem LE (Cambrex, Rockland, ME) 3% agarose gels with 1x Tris-acetate-EDTA buffer were used to resolved PCR products with running conditions at 100 Volts for 2 hours. Gels were visualized with ethidium bromide.

Macrorestriction digestion of genomic DNA was performed using the enzyme \textit{SmaI}, following PFGE protocols established by the Center for Disease Control and Prevention [27]. \textit{Salmonella} serotype Branderup strain H9812 was digested with \textit{XbaI} and used as a molecular size marker. A CHEF mapper system (Bio-Rad Laboratories,
Nazareth, Belgium) was used to separate DNA fragments, and band patterns were analyzed using BioNumerics® software (version 6.6, Applied Maths, Ghent, Belgium). Dice coefficient and Unweighted Pair Group Method using Arithmetic averages (UPGMA) with 1% tolerance allowed the construction of dendrograms to establish relatedness between strains. Three different dendrograms were created; one including only environmental isolates, one containing only equine isolates (both incoming patients and clinical cases), and one with both environmental and equine isolates. Band patterns with ≥98% similarity were characterized as the same pulsotype. Groups of closely related pulsotypes with ≥80% similarity were classified as clusters. A CDC database containing 100 S. aureus strains with the most typical band patterns for each USA type was used to compare and characterize the environmental isolates (cutoff point of ≥80% similarity).

4.3.7 Statistical Analysis

Comparisons between types of contact surfaces (human vs. animal) and between services (internal medicine, intensive care unit, surgery and general areas) were performed by calculating Chi-square coefficients. Similarly, seasonality was evaluated by comparing results of four groups: data collected from January to March, April to June, July to September, and October to December. Chi-square coefficients were calculated using the statistical software STATA (Small Stata 12.0, StataCorp LP, Texas, USA), and logistic regression models were created (Proc GLIMMIX in SAS version 9.3, SAS Institute Inc, North Carolina, USA) using Tukey-Kramer method for multiple pairwise comparisons. Relationships were considered significant when their $P$-value was ≤0.05.
4.4 RESULTS

4.4.1 General prevalence and characterization of environmental isolates

A total of 770 environmental samples were collected during the year long active surveillance. As it was explained before, if during the sampling date a pre-selected surface was not available it was skipped until the next month; therefore, a total of 34 surfaces were skipped due to this reason. During the twelve month period, 8.6% (66/770) of the overall surfaces sampled were found positive for MRSA. On a monthly basis, MRSA contamination ranged from 0.0% to 18.5% (Figure 4.1). Of the 66 positive surfaces, 71 unique isolates were obtained, indicating that some surfaces were contaminated with two different isolates at the moment they were sampled.

Genotypic characteristics of the 71 environmental isolates can be found in Table 4.3. Over 90% of the isolates carried SCCmec type IV and 62.0% were classified as USA500. Analysis of the dendrogram constructed with only the environmental isolates showed 17 different pulsotypes (P1 – P17) grouped in two clusters; cluster 1 included 83.1% (59/71) of the isolates. Dendrogram analysis of both environmental and equine isolates showed that three specific pulsotypes (P5, P1 and P9) represented the majority of the isolates throughout the year, with 29, 12 and 10 environmental isolates each respectively (Figure 4.2). The most prevalent pulsotype (P5) included isolates characterized as USA500, and was present in the hospital environment for five consecutive months (November 2009 to March 2010). During this period, P5 was evenly distributed on animal and human contact surfaces, and was found in all the hospital services included in the surveillance; in some cases contaminating the same surface for
two consecutive months. In total, 7 and 5 pulsotypes were classified as USA500 and USA300 respectively.

Phenotypically, 21 distinct antimicrobial susceptibility profiles were identified. Based on the combined results of the phenotypic profile, SCCmec type and PFGE pulsotype of the 71 isolates, 34 unique strains (combinations) were found. Over 70% (25/34) of these strains were considered MDR MRSA. Besides beta-lactam resistance, 85.3% (29/34) of the strains were resistant to Gentamicin, 67.3% (23/34) were resistant to Sulfamethoxazole with Trimethoprim and 64.7% (22/34) were resistant to Tetracycline. In addition, all Clindamycin resistant strains (35.3%, 12/34) possessed inducible and not a constitutive resistance. All of the strains (100%) were susceptible to Vancomycin and Amikacin.

4.4.2 MRSA environmental contamination by type of contact surface (human vs. animal)

MRSA monthly prevalence and distribution by surface type is presented in Figure 4.1. Detailed information of MRSA contamination on each contact surface (human and animal) that was sampled during the surveillance is described in Table 4.4. During the surveillance, MRSA contamination of human (9.7%, 43/444) and animal (7.1%, 23/326) contact surfaces were very alike (Table 4.2 and 4.4) ($P = 0.19$). Not including pool samples, the most common human contact surface contaminated with MRSA was the computers (16.7%, 4/24). Among the animal contact surfaces, the feed and water buckets (16.7%, 2/12), followed very closely by surgery tables and mats (15.6%, 7/45), were the most contaminated.
Genotypically, 13 and 7 distinct pulsotypes were found on human and animal contact surfaces respectively; only 3 pulsotypes (P5, P1 and P20) were present on both types of surfaces. It is important to highlight that P5 and P1 were the two most prevalent clones at the hospital and belong to the same clonal cluster (Figure 4.2). Conversely, the third most prevalent pulsotype (P9) was exclusively found on human contact surfaces. Considering P5 alone, 34.8% (16/46) of the MRSA isolates from human contact surfaces and 52.0% (13/25) of the isolates from animal contact surfaces were contaminated by this clone, but no significant difference was detected (P= 0.15). In addition, P5 was found contaminating the same surface for 2 consecutive months on two separate occasions. This was the case on the mats and floors of a surgery recovery room (Nov- Dec) and the computers at the ICU office (Feb- Mar). Conversely, it was also observed that six surfaces were contaminated for 2-3 consecutive months with different pulsotypes, showing a constant reintroduction of strains. Some of these surfaces were the front desk doors, tables and mats, counter tops and cabinets, and computers. It is important to notice that 5 out of the six surfaces continuously contaminated with new strains were human contact surfaces. Pool sample K (which included light switches, counter tops, computers keyboards and mouse, phone and doors of the milk room) was also found positive for 2 consecutive months with 2 different pulsotypes. However, since these samples were collected from several surfaces as a pool, we cannot conclude that it was indeed the same surface that was always contaminated.
4.4.3 MRSA environmental contamination by hospital service

The overall MRSA contamination in each hospital service and the detailed prevalence by type of contact surface is described in Table 4.2. There was not a difference among the prevalences of the 4 services ($P = 0.109$). However, it is important to highlight that the same surfaces were not always sampled in each service (e.g. not all the services had an endoscope or a foal bed) (Table 4.1). Furthermore, the level of exposure (to human or animal contact) varies from surface to surface. Hence, any comparisons among services must be done with care. In any case, the General Areas of the hospital had the most diverse contamination, with 11 different pulsotypes found cycling during the year surveillance. The most prevalent pulsotype (P5) was present contaminating different surfaces from 2 to 5 consecutive months: General Areas for 2 months, Internal Medicine 3 months, ICU 4 months, and Surgery 5 months.

4.4.4 MRSA environmental contamination by season

MRSA environmental contamination was detected in the equine hospital in eleven out of twelve months that were sampled. The months of November and December had the greatest overall (human and animal contact surfaces) MRSA prevalence, as 18.5% (24/66) of the surfaces sampled each month were contaminated (Figure 4.1). The 28 isolates obtained during these 2 months, accounted for 39.4% (28/71) of all the MRSA isolates collected during the surveillance. When analyzing by season, the prevalence of MRSA from Jan-Mar was 10.2% (20/196), Apr-Jun 7.2% (14/194), Jul-Sep 4.3% (8/184) and Oct-Dec 12.2% (24/196). The analysis showed that the season of the year was associated with the prevalence of MRSA ($P = 0.034$) with the highest prevalence during
the fall months (October to December). These results were confirmed by the logistic regression models which showed a significant difference between the summer (lowest contamination) and the fall ($P = 0.04$).

4.4.5 Prevalence and characteristics of MRSA Equine Isolates

A total of 120 incoming horses were sampled in parallel to the active environmental surveillance, and 5.8% (7/120) were MRSA positive in at least one anatomical location. Epidemiological data regarding these horses will not be discussed in this manuscript. The anatomical location that most frequently tested positive for MRSA was the nares (71.4%, 5/7) followed by the armpits (28.6%, 2/7) and the perianal area (14.3%, 1/7). None of the skin lesions sampled were positive for MRSA. Only one horse was positive in two anatomical locations (armpit and perianal); therefore, both isolates (one from each location) were included for further phenotypic and genotypic characterization, resulting in a total of 8 equine MRSA isolates to be analyzed in this study.

Genotypically, 87.5% (7/8) of the isolates were classified as SCCmec type IV, and 12.5% (1/8) as type VI. Fifty percent (4/8) were characterized as USA500, 25.0% (2/8) as USA800, 12.5% (1/8) as USA300 and 12.5% (1/8) as USA100. Dendrogram analysis of only the equine isolates (not shown here) revealed 6 different pulsotypes distributed in two major clusters with only 59.2% similarity among them; one of the isolates was not related to either cluster. The two isolates obtained from different anatomical locations on the same horse were classified as different pulsotypes (one of them was the isolate not included in any cluster), which is indicative that a horse could be
potentially colonized with more than one strain. When compared against environmental isolates, 5/8 equine isolates matched with three environmental pulsotypes (P1, P5 and P20) circulating in the hospital (Figure 4.2). The other 3 equine isolates were characterized as unique pulsotypes that were never detected or previously seen in the environment of the hospital.

Of the 8 equine MRSA isolates, 6 unique strains were identified based on the combined results of phenotypic profile, SCC\textit{mec} type and PFGE pulsortype. Fifty percent of the strains (3/6) were classified as MDR. Similarly to environmental strains, the equine strains were resistant to Gentamicin (100\%, 6/6), Sulfamethoxazole with Trimethoprim (50.0\%, 3/6) and Tetracycline (50.0\%, 3/6). Only one strain was found to have inducible resistance to Clindamycin. All the equine strains (100\%) were susceptible to Vancomycin, Amikacin, Ciprofloxacin, Enrofloxacin and Doxycycline.

4.4.6 Molecular comparison of MRSA isolates from clinical cases and the environment

In February 2010, two equine patients were admitted to the hospital for colic and developed MRSA post-operative infections in their surgical incisions. Neither one of these horses were screened for MRSA at their arrival to the hospital. In both cases the interventions were performed in the same surgery suite (Room B), which was sampled during the monthly environmental surveillance on February 10\textsuperscript{th}. Isolates obtained from these two patients were compared against the environmental isolates using PGFE band patterns and dendrogram analysis. The first patient (patient A) was admitted to the hospital on February 4\textsuperscript{th}. This patient had two surgical procedures on February 4\textsuperscript{th} and
February 9th; both surgeries were performed in the same surgery suite. On February 18th the patient was diagnosed with a MRSA infection of its surgical incision. After molecular analysis, the MRSA isolate from this patient was determined to be identical to one of the most prevalent environmental pulsotypes (P5) circulating in the hospital during that sampling period as well as in previous months (Figure 4.2). The second patient (patient B) was admitted to the hospital on February 8th and underwent surgical procedures on February 8th and February 18th. On February 25th, a specimen collected from the patient’s surgical incision was positive for MRSA. When compared against the environmental isolates, the equine isolate matched with one pulsotype (P11) that was only seen in the hospital environment one time during the whole study (Figure 4.2). This unique pulsotype was found on February 10th on the counter tops and cabinets from the suite where the surgery took place, and it was never seen before or after this event.

4.5 DISCUSSION

No previous reports have been published on estimating the prevalence and distribution of MRSA in an equine veterinary hospital environment over an extended period of time, and regardless of the presence of patients with MRSA infections. In consequence, little is known about the potential that environmental surfaces may play as a source of infections for patients and hospital personnel. Since MRSA can affect humans and horses, the presence of this zoonotic pathogen in the environment may increase the occupational and nosocomial risk for infection. We demonstrated that MRSA was present on 8.6% of the environmental surfaces sampled throughout the yearlong surveillance. Previously only one cross-sectional study has been performed during a non-outbreak
period finding 4.3% MRSA contamination [21]. Other studies have found environmental contamination ranging from 9.6% to 52.7% [16, 17, 22]. However, it is important to highlight that these latter results were obtained when sampling the hospital environment during or after MRSA outbreaks involving equine patients. Our results clearly demonstrate that MRSA is frequently present contaminating the environment throughout the year, not necessarily associated with outbreaks.

When the patterns of MRSA environmental contamination at the hospital were analyzed three important scenarios were noticed: constant introduction and reintroduction of strains, circulation (movement) of clones throughout the hospital services, and maintenance (survival) of strains over time in the environment.

In the first scenario, we noted a continuous introduction of MRSA strains into the hospital, where “new” MRSA clones not previously observed in the surveillance were found on several environmental contact surfaces. Of the 17 pulsortypes that were detected in the environment, 10 of them were observed only once in the 12 months study period. For example, P16 and P4 were only detected in March and July, respectively, and none of them was seen before or after that date (Figure 4.2). Also, reintroduction was observed when a clone was initially detected in the environment, and then disappeared for several months before showing up again in a different area or on another surface later on. For example, P17 was observed in January (surgery) for the first time, it then disappeared for several months, to be detected again in April (ICU and internal medicine), and later in June (general areas) (Figure 4.2). Similar patterns of reintroduction were noticed with other pulsortypes. This scenario of introduction and reintroduction of this pathogen into the environment highlights the importance of performing continuous surveillance and
monitoring to identify the most common strains circulating in the hospital as well as identifying new strains that could represent additional risk.

The second scenario associated with the circulation or movement of MRSA strains across the hospital services, was observed when a unique pulsotype appeared in one specific area or on one surface and was then detected in the continuing months on other surfaces and/or in other sections. This was the case of P5, which was first detected on surfaces from the General Areas and Surgery in November. Then, during the next 4 months, P5 circulated through all the services sampled and by March was mostly detected in the ICU service. After March this pulsotype was never detected again in the environment. Similar scenarios were observed with other pulsotypes, which were detected simultaneously in different services and on surfaces but for shorter periods of time (three consecutive months). These examples reveal the circulation of MRSA clones throughout different areas of the hospital, perhaps carried by hospital personnel, as this has been described in other health care settings [28, 29]. It is also possible that this situation could occur due to the constant reintroduction of the same clone into the hospital environment. However, we cannot reject the possibility of both scenarios (reintroduction and circulation) happening in parallel to each other. Based on these facts, it is highly recommended to emphasize the importance of biosecurity and personal hygiene practices of the hospital personnel, primarily their compliance with hand washing. Contaminated hands and/or the gloves of healthcare workers might be involved in the transmission of nosocomial pathogens like MRSA [28, 30]. On the other hand, it cannot be denied that the equine patients (colonized or infected with MRSA) may have played a role in the movement of this bacterium across the hospital; this issue was not
assessed in this study, but it has been previously suggested [17]. For this reason, the use of biocontainment measures, especially when handling suspicious and/or confirmed MRSA cases, should be strictly implemented.

The third scenario observed was associated with the maintenance of MRSA strains over time in the hospital environment. We detected contamination of the same surface with the same unique pulsotype for two consecutive months. This was the case for the computers of the ICU office, and the mats and floor of a recovery room. We reported similar results in a small animal hospital, where the gurneys used to move patients were found contaminated with the same MRSA pulsotype over 3 consecutive months [23]. The presence over time of the same strain on a specific surface is not surprising, as MRSA has been reported to survive on inanimate surfaces for up to 7 months [20, 30]. In any case, the fact that a MRSA clone was able to survive on a specific surface for up to 2 months in our study, suggest that insufficient cleaning and disinfection protocols are in use, highlighting the importance of identifying and targeting environmental surfaces that are continuously contaminated to be more rigorously addressed.

We identified several surfaces that were contaminated multiple times throughout the year, either with different pulsotypes or in some cases with the same clone. Among the most commonly contaminated human contact surfaces were the computers (16.7%), counter tops and cabinets (10.3%) and doors (9.7%); and among the animal contact surfaces the feed and water buckets (16.7%) and surgery tables and mats (15.6%). All of these surfaces are in contact with many individuals, both human and animal, and thus have a high likelihood of contamination with MRSA. These surfaces might be considered
“hot spots” for MRSA contamination and could potentially become sources of nosocomial infections; therefore, they must be included in any cleaning and disinfection program. Since each practice/hospital is unique, each one should document baseline contamination with MRSA and identify “hot spot surfaces” to target for cleaning and disinfection.

Phylogenetic analysis showed very little diversity of the MRSA strains circulating in the equine hospital (83% of the isolates had ≥80% similarity). Furthermore, the molecular analysis showed that the majority of our isolates carried SCCmec type IV (90.1%) and were identified as USA500 (62.0%). This PFGE clone has been frequently reported in horses as an endogenous MRSA strain and has been classified as a nosocomial or hospital-acquire MRSA (HA-MRSA) [31, 32]. These results provide further evidence that among the constellation of MRSA strains circulating in humans, certain strains are more likely to circulate in horses, their environments and their human contacts as has been previously suggested [4, 6, 32, 33]. In contrast, other strains classified in this study as USA100, USA300 and USA800 are clones frequently reported in the US human population, either as a cause of nosocomial infections (HA-MRSA, USA100 and USA800) or present in the general population (also known as community-acquired MRSA [CA-MRSA], USA300)[34, 35]. The presence of these strains on different surfaces and areas across the hospital highlights the role that humans (personnel and/or clients) could possible play in the contamination of the hospital environment and as a source of nosocomial infections for patients.

We could not definitively identify the source of MRSA infection in the two post-surgical cases in our study. The isolate from patient A was indistinguishable from the P5
isolate present in the hospital environment. Since P5 was present in the hospital 3 months before and 1 month after patient A’s isolate was obtained, we could not establish a temporal relationship. However, the likelihood of acquiring a nosocomial infection with the most common strain circulating the hospital at the time of the surgery is a possibility that could not be discarded. Conversely, the isolate obtained from patient B was indistinguishable from P11, a pulsortype found in the hospital environment only once during the 12 month surveillance. Two possible scenarios can be described. First, it is possible that patient B developed a nosocomial MRSA infection due to indirect contact with contaminated surfaces present in the surgery room during the second intervention (exogenous sources). Second, it is also possible that patient B was already colonized with this particular strain upon arrival to the hospital (endogenous source). Unfortunately, due to the design of the study, we cannot be certain of the source of the MRSA infection in patient B; especially due to the fact that only a representative portion of the hospital environment was sampled. Therefore, it is possible that P11 could have been in other areas/surfaces of the hospital that were not included in this study.

Confirming the actual source of the MRSA strains present in the hospital environment will require further studies to include the sampling of all three components (human, animal and environment) involved in the ecology of this pathogen. Results of the present study suggests that hospital personnel may be important source of MRSA, since the majority (5/6) of the surfaces that were frequently contaminated for 2-3 consecutive month were human contact surfaces. Also there was a higher diversity of clones detected on human contact surfaces (13 clones) than on animal contact surfaces (7 clones). Nonetheless, horses may have still been the primary source responsible for the
contamination detected among the animal contact surfaces. This scenario is even more probable if we consider that 5.8% of the incoming horses were found positive for MRSA upon arriving to the hospital with strains that were also detected in the environment. Moreover, it is important for other veterinary hospitals to consider this possibility, especially since higher prevalences of MRSA in incoming equine patients have been reported in other countries [36]. In any case, since the colonization status of all incoming equine patients and the hospital personnel was not established during the study period, we can only speculate that both groups were involved in the introduction of MRSA to the GEC.

Lastly, we acknowledge that the design used for this environmental surveillance had limitations. First, multiple surfaces were sampled as a pool when necessary due to financial constraints, interfering with our ability to determine in some cases which particular surface was positive. Yet, pooled sampling is a good alternative during routine surveillance as it allows us to establish if a particular area/room is contaminated with MRSA. Second, it is important to recognize that differences among veterinary hospitals in the US and other countries may reduce the ability to extrapolate from the results presented. Nonetheless, this study left no doubt that MRSA is present and circulating in an equine veterinary environment, and these findings can be used during the development of surveillance programs and cleaning and disinfection control plans in other institutions.

4.6 CONCLUSION

In conclusion, this is the first report of a yearlong environmental surveillance performed at a large equine hospital, and it was confirmed that MRSA is present on
different contact surfaces during a non outbreak period. We observed that different MRSA strains were not only constantly introduced and/or reintroduced into the hospital, but they were also moved among and maintained in the environmental surfaces of different sections of the hospital. The presence of MRSA in all but one of the 12 months of the surveillance, the detection among human and animal contact surfaces across multiple services, and the presence of MDR profiles are all causes of concern from the point of view of occupational safety as well as control and prevention of nosocomial infections. These findings highlight the necessity of maintaining effective cleaning and disinfection protocols at all times, as well as the importance of performing continuous surveillance to identify strains circulating the hospital as well as the surfaces that could act as “hot spots” and reservoirs for this zoonotic and nosocomial pathogen.

**4.7 ACKNOWLEDGEMENTS**

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4.8 LIST OF REFERENCES


Table 4.1: Contact surfaces sampled with electrostatic cloths (■) or sterile swabs (▲) at an equine teaching hospital
<table>
<thead>
<tr>
<th>Hospital Service</th>
<th>Human Contact</th>
<th>Animal Contact</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Internal Medicine</strong></td>
<td>Twitches - Handle 1</td>
<td>Stocks 1</td>
</tr>
<tr>
<td>(Triage Room, Med Room, Ward, and Aisles)</td>
<td>Computers 2</td>
<td>Railing 2</td>
</tr>
<tr>
<td></td>
<td>Counter Tops &amp; Cabinets 1</td>
<td>Floor, Drain &amp; Stall Mat 1</td>
</tr>
<tr>
<td></td>
<td>Doors 1</td>
<td>Twitches - Chain 1</td>
</tr>
<tr>
<td></td>
<td>Ultrasound - Controls 1</td>
<td>Ultrasound - Probe 1</td>
</tr>
<tr>
<td></td>
<td>Endoscope - Controls 1</td>
<td>Endoscope 1</td>
</tr>
<tr>
<td></td>
<td>Pool A (Triage) 4</td>
<td>Pool O (Ward) 5</td>
</tr>
<tr>
<td><strong>Intensive Care Unit</strong></td>
<td>Doors 1</td>
<td>Feed &amp; Water Buckets 1</td>
</tr>
<tr>
<td>(Ward, ICU Radiology, ICU Office, and Aisles)</td>
<td>Carts 1</td>
<td>Foal Bed 1</td>
</tr>
<tr>
<td></td>
<td>Charts &amp; Files 1</td>
<td>Foal Cart 1</td>
</tr>
<tr>
<td></td>
<td>Computers 2</td>
<td>Floor, Drain &amp; Stall Mat 1</td>
</tr>
<tr>
<td></td>
<td>Supply Cart 1</td>
<td>Foal Watch Mats 1</td>
</tr>
<tr>
<td></td>
<td>Pool B (Aisles) 4</td>
<td>Pool P (Ward) 5</td>
</tr>
<tr>
<td></td>
<td>Pool C (Aisles) 6</td>
<td></td>
</tr>
<tr>
<td><strong>Surgery</strong></td>
<td>Doors 1</td>
<td>Stocks 1</td>
</tr>
<tr>
<td>(Scrub Room, Prep Room, Surgery Suites, Recovery Rooms, and Orthopedic Ward)</td>
<td>Counter Tops &amp; Cabinets 1</td>
<td>Surgery Table &amp; Mats 1</td>
</tr>
<tr>
<td></td>
<td>Hoist Controls 1</td>
<td>Recovery – Mats &amp; Floor 1</td>
</tr>
<tr>
<td></td>
<td>Pool D (Suite B) 1</td>
<td>Pool Q (Ward) 5</td>
</tr>
<tr>
<td></td>
<td>Pool E (Suite C) 4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pool F (Suite E) 4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pool G (Ward) 6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pool H (Scrub R.) 6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pool I (Prep R.) 6</td>
<td></td>
</tr>
<tr>
<td><strong>General Areas</strong></td>
<td>Counter Tops 1</td>
<td>Door, Wall &amp; Floor 7</td>
</tr>
<tr>
<td>(Treadmill Room, Breezeway, Office/Front Desk, Isolation Stalls, Milk Room, LA Radiology and CT Room)</td>
<td>Doors 2</td>
<td>Floor 7</td>
</tr>
<tr>
<td></td>
<td>Counter tops, cabinets &amp; sink 7</td>
<td>CT Table 8</td>
</tr>
<tr>
<td></td>
<td>Pool J (Breezeway) 8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pool K (Milk R.) 8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pool L (Treadmill) 8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pool M (LA Radiology) 8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pool N (CT Room) 8</td>
<td></td>
</tr>
</tbody>
</table>

1 Three units of this type of equipment were sampled as a pool
2 Two computers (keyboards and mouse) were sampled as a pool
3 Surfaces sampled in three stalls as a pool
4 Pool samples A, B, D, E, and F included: Light switches, phone, oxygen and suction valves, radio, microwave, lamps and medical carts within the same area or room
5 Pool samples O, P, and Q included: Halters, hay bags, and muzzles (4 of each) within the same ward
6 Pool samples C, G, H, I, L, M, and N included: Counter tops, cabinets and other contact surfaces within the same area or room
7 Surfaces sampled from four isolation stalls as a pool
8 Pool samples J and K included: Light switches, counter tops, computers (keyboards and mouse), phone and doors within the same area or room
<table>
<thead>
<tr>
<th>Service</th>
<th>Human Contact MRSA/Samples Collected</th>
<th>Animal Contact MRSA/Samples Collected</th>
<th>Total Contact MRSA/Samples Collected</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Internal Medicine</strong></td>
<td>5/84 (6.0%)</td>
<td>5/103 (4.9%)</td>
<td>10/187 (5.3%)</td>
</tr>
<tr>
<td><strong>Intensive Care Unit</strong></td>
<td>8/94 (8.5%)</td>
<td>5/72 (6.9%)</td>
<td>13/166 (7.8%)</td>
</tr>
<tr>
<td><strong>Surgery</strong></td>
<td>15/172 (8.7%)</td>
<td>11/115 (9.6%)</td>
<td>26/287 (9.1%)</td>
</tr>
<tr>
<td><strong>General</strong></td>
<td>15/94 (16.0%)</td>
<td>2/36 (5.6%)</td>
<td>17/130 (13.1%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>43/444 (9.7%)</td>
<td>23/326 (7.1%)</td>
<td>66/770 (8.6%)</td>
</tr>
</tbody>
</table>

**Table 4.2:** Overall prevalence of MRSA contamination distributed by services at an equine teaching hospital
<table>
<thead>
<tr>
<th>ENVIRONMENT</th>
<th>By Surface (Total N=66)</th>
<th></th>
<th>By Isolate (Total N=71)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MRSA</td>
<td>%</td>
<td>MRSA</td>
<td>%</td>
</tr>
<tr>
<td><strong>SCCmec Typing</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type II</td>
<td>2 / 66</td>
<td>3.0 %</td>
<td>2 / 71</td>
<td>2.8 %</td>
</tr>
<tr>
<td>Type IV</td>
<td>60 / 66</td>
<td>90.9 %</td>
<td>64 / 71</td>
<td>90.1 %</td>
</tr>
<tr>
<td>Type V</td>
<td>1 / 66</td>
<td>1.5 %</td>
<td>1 / 71</td>
<td>1.4 %</td>
</tr>
<tr>
<td>Type VI</td>
<td>4 / 66</td>
<td>6.1 %</td>
<td>4 / 71</td>
<td>5.6 %</td>
</tr>
<tr>
<td><strong>PFGE</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>USA 100</td>
<td>4 / 66</td>
<td>6.1 %</td>
<td>4 / 71</td>
<td>5.6 %</td>
</tr>
<tr>
<td>USA 300</td>
<td>16 / 66</td>
<td>24.2 %</td>
<td>16 / 71</td>
<td>22.5 %</td>
</tr>
<tr>
<td>USA 500</td>
<td>44 / 66</td>
<td>66.7 %</td>
<td>44 / 71</td>
<td>62.0 %</td>
</tr>
<tr>
<td>USA 800</td>
<td>7 / 66</td>
<td>10.6 %</td>
<td>7 / 71</td>
<td>9.9 %</td>
</tr>
</tbody>
</table>

* Some surfaces were contaminated with two different isolates at the moment they were sampled; therefore, the sum of surfaces by SCCmec and PFGE will add up to more than 66.

Table 4.3: Molecular characterization of environmental MRSA isolates obtained from an equine teaching hospital
Table 4.4: Prevalence of MRSA contamination on human and animal contact surfaces at an equine teaching hospital
### Human Contact Surfaces

<table>
<thead>
<tr>
<th>Contact Surface</th>
<th>MRSA / Samples collected</th>
<th>Prevalence per surface</th>
</tr>
</thead>
<tbody>
<tr>
<td>Computers (keyboards and mouse)</td>
<td>4/24</td>
<td>16.7%</td>
</tr>
<tr>
<td>Counter Tops &amp; Cabinets</td>
<td>6/58</td>
<td>10.3%</td>
</tr>
<tr>
<td>Doors</td>
<td>10/103</td>
<td>9.7%</td>
</tr>
<tr>
<td>Charts / Files</td>
<td>1/12</td>
<td>8.3%</td>
</tr>
<tr>
<td>Endoscope (Controls)</td>
<td>1/12</td>
<td>8.3%</td>
</tr>
<tr>
<td>Ultrasound (Controls)</td>
<td>1/12</td>
<td>8.3%</td>
</tr>
<tr>
<td>Carts</td>
<td>0/22</td>
<td>0.0%</td>
</tr>
<tr>
<td>Counter tops, cabinets &amp; sink²</td>
<td>0/12</td>
<td>0.0%</td>
</tr>
<tr>
<td>Hoist Controls</td>
<td>0/12</td>
<td>0.0%</td>
</tr>
<tr>
<td>Twitches (Handle)</td>
<td>0/12</td>
<td>0.0%</td>
</tr>
<tr>
<td>Pool Samples J and K³</td>
<td>5/24</td>
<td>20.8%</td>
</tr>
<tr>
<td>Pool Samples C, G, H, I, L, M, and N⁴</td>
<td>10/83</td>
<td>12.0%</td>
</tr>
<tr>
<td>Pool Samples A, B, D, E, and F⁵</td>
<td>5/58</td>
<td>8.6%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>43/444</strong></td>
<td><strong>9.7%</strong></td>
</tr>
</tbody>
</table>

### Animal Contact Surfaces

<table>
<thead>
<tr>
<th>Contact Surface</th>
<th>MRSA / Samples collected</th>
<th>Prevalence per surface</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed &amp; Water Buckets</td>
<td>2/12</td>
<td>16.7%</td>
</tr>
<tr>
<td>Surgery Table &amp; Mats</td>
<td>7/45</td>
<td>15.6%</td>
</tr>
<tr>
<td>CT Table</td>
<td>1/12</td>
<td>8.3%</td>
</tr>
<tr>
<td>Floor</td>
<td>1/12</td>
<td>8.3%</td>
</tr>
<tr>
<td>Foal Bed</td>
<td>1/12</td>
<td>8.3%</td>
</tr>
<tr>
<td>Foal Cart</td>
<td>1/12</td>
<td>8.3%</td>
</tr>
<tr>
<td>Railing</td>
<td>1/12</td>
<td>8.3%</td>
</tr>
<tr>
<td>Twitches (Chain)</td>
<td>1/12</td>
<td>8.3%</td>
</tr>
<tr>
<td>Ultrasound (Probe)</td>
<td>1/12</td>
<td>8.3%</td>
</tr>
<tr>
<td>Mats &amp; Floor⁶</td>
<td>3/47</td>
<td>6.4%</td>
</tr>
<tr>
<td>Door, Wall &amp; Floor²</td>
<td>0/12</td>
<td>0.0%</td>
</tr>
<tr>
<td>Endoscope</td>
<td>0/12</td>
<td>0.0%</td>
</tr>
<tr>
<td>Floor, Drain &amp; Stall Mat⁷</td>
<td>0/12</td>
<td>0.0%</td>
</tr>
<tr>
<td>Foal Watch Mats</td>
<td>0/12</td>
<td>0.0%</td>
</tr>
<tr>
<td>Stocks</td>
<td>0/12</td>
<td>0.0%</td>
</tr>
<tr>
<td>Pool Samples O, P, and Q⁸</td>
<td>4/36</td>
<td>11.1%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>23/326</strong></td>
<td><strong>7.1%</strong></td>
</tr>
</tbody>
</table>

---

¹ Two computers (Keyboards and mouse) were sample as a pool
² Surfaces sampled from four isolation stalls as a pool
³ Pool samples J and K included: Light switches, counter tops, computers (keyboards and mouse), phone and doors within the same area. These Pool samples were collected from General Areas
⁴ Pool samples C, G, H, I, L, M, and N included: Counter tops, cabinets and other contact surfaces within the same area. These Pool samples were collected from Intensive Care Unit, Surgery and General Areas
⁵ Pool samples A, B, D, E, and F included: Light switches, phone, oxygen and suction valves, radio, microwave, lamps and medical carts within the same area. These Pool samples were collected from Internal Medicine, Intensive Care Unit and Surgery
⁶ Samples collected in the surgery recovery rooms
⁷ Surfaces sampled in three stalls as a pool
⁸ Pool samples O, P, and Q included: Halter, hay bags, and muzzles (3 of each) within the same ward. These Pool samples were collected from Internal Medicine, Intensive Care Unit and Surgery
Figure 4.1: Monthly distribution of environmental MRSA prevalence during one-year of active surveillance at an equine hospital
Figure 4.2: Dendrogram analysis of environmental and equine MRSA isolates obtained at an equine teaching hospital
<table>
<thead>
<tr>
<th>Key</th>
<th>Abbreviation</th>
<th>Species</th>
<th>Source</th>
<th>Isolate Number</th>
<th>Location</th>
<th>Date of Isolation</th>
<th>Pathogenic Profile</th>
<th>BGC Type</th>
<th>USA Type</th>
<th>Surfaces Described</th>
</tr>
</thead>
<tbody>
<tr>
<td>210.0</td>
<td>ETH 149</td>
<td>Surgery</td>
<td>November 2009</td>
<td>Amplified rpoC2 + spaType (8347)</td>
<td>ETH</td>
<td>V</td>
<td>USA Type 300</td>
<td>MDR</td>
<td>-</td>
<td>Mouse - founder</td>
</tr>
<tr>
<td>213.0</td>
<td>ETH 149</td>
<td>Surgery</td>
<td>September 2009</td>
<td>Amplified rpoC2 + spaType (8347)</td>
<td>ETH</td>
<td>V</td>
<td>USA Type 300</td>
<td>MDR</td>
<td>-</td>
<td>Mouse - founder</td>
</tr>
<tr>
<td>317.0</td>
<td>ETH 179</td>
<td>Surgery</td>
<td>November 2009</td>
<td>Amplified rpoC2 + spaType (8347)</td>
<td>ETH</td>
<td>N</td>
<td>USA Type 100</td>
<td>Surgery Room - Surgical table &amp; Mats</td>
<td></td>
<td></td>
</tr>
<tr>
<td>241.0</td>
<td>ETH 177</td>
<td>Surgery</td>
<td>November 2009</td>
<td>Amplified rpoC2 + spaType (8347)</td>
<td>ETH</td>
<td>N</td>
<td>USA Type 100</td>
<td>Surgery Room - Surgical table &amp; Mats</td>
<td></td>
<td></td>
</tr>
<tr>
<td>255.0</td>
<td>ETH 179</td>
<td>Surgery</td>
<td>November 2009</td>
<td>Amplified rpoC2 + spaType (8347)</td>
<td>ETH</td>
<td>N</td>
<td>USA Type 100</td>
<td>Surgery Room - Surgical table &amp; Mats</td>
<td></td>
<td></td>
</tr>
<tr>
<td>258.0</td>
<td>ETH 179</td>
<td>Surgery</td>
<td>April 2010</td>
<td>Amplified rpoC2 + spaType (8347)</td>
<td>ETH</td>
<td>N</td>
<td>USA Type 100</td>
<td>Surgery Room - Surgical table &amp; Mats</td>
<td></td>
<td></td>
</tr>
<tr>
<td>271.0</td>
<td>ETH 179</td>
<td>Surgery</td>
<td>July 2010</td>
<td>Amplified rpoC2 + spaType (8347)</td>
<td>ETH</td>
<td>N</td>
<td>USA Type 100</td>
<td>Surgery Room - Surgical table &amp; Mats</td>
<td></td>
<td></td>
</tr>
<tr>
<td>273.0</td>
<td>ETH 177</td>
<td>Surgery</td>
<td>August 2010</td>
<td>Amplified rpoC2 + spaType (8347)</td>
<td>ETH</td>
<td>N</td>
<td>USA Type 100</td>
<td>Surgery Room - Surgical table &amp; Mats</td>
<td></td>
<td></td>
</tr>
<tr>
<td>461.0</td>
<td>ECOH 274</td>
<td>Surgery</td>
<td>June 2010</td>
<td>Amplified rpoC2 + spaType (8347)</td>
<td>ETH</td>
<td>N</td>
<td>USA Type 100</td>
<td>Surgery Room - Surgical table &amp; Mats</td>
<td></td>
<td></td>
</tr>
<tr>
<td>572.0</td>
<td>ETH 177</td>
<td>Surgery</td>
<td>July 2010</td>
<td>Amplified rpoC2 + spaType (8347)</td>
<td>ETH</td>
<td>N</td>
<td>USA Type 100</td>
<td>Surgery Room - Surgical table &amp; Mats</td>
<td></td>
<td></td>
</tr>
<tr>
<td>572.0</td>
<td>ETH 177</td>
<td>Surgery</td>
<td>July 2010</td>
<td>Amplified rpoC2 + spaType (8347)</td>
<td>ETH</td>
<td>N</td>
<td>USA Type 100</td>
<td>Surgery Room - Surgical table &amp; Mats</td>
<td></td>
<td></td>
</tr>
<tr>
<td>575.0</td>
<td>ETH 177</td>
<td>Surgery</td>
<td>July 2010</td>
<td>Amplified rpoC2 + spaType (8347)</td>
<td>ETH</td>
<td>N</td>
<td>USA Type 100</td>
<td>Surgery Room - Surgical table &amp; Mats</td>
<td></td>
<td></td>
</tr>
<tr>
<td>575.0</td>
<td>ETH 177</td>
<td>Surgery</td>
<td>July 2010</td>
<td>Amplified rpoC2 + spaType (8347)</td>
<td>ETH</td>
<td>N</td>
<td>USA Type 100</td>
<td>Surgery Room - Surgical table &amp; Mats</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CHAPTER 5

UNDERSTANDING THE INTRODUCTION AND CIRCULATION OF
ENVIRONMENTAL MRSA IN A LARGE METROPOLITAN HOSPITAL
DURING A NON-OUTBREAK, YEARLONG PERIOD

5.1. SUMMARY

Even though methicillin-resistant \textit{Staphylococcus aureus} (MRSA) is one of the most important pathogens related to severe nosocomial infections and outbreaks, the transmission of this pathogen within healthcare settings is still not fully understood. In particular, the hospital environment has been suggested as a possible source of MRSA for patients, but it has been studied mostly during outbreak investigations. Improved understanding of MRSA in the environment is needed in order to develop effective programs for the control and prevention of environmental contamination. Therefore, an active environmental surveillance including staff and general public contact surfaces was performed for 12 consecutive months in two general medicine wards during a non-outbreak period. Concurrently, aggregated data of MRSA patient infection burden was analyzed as a plausible indicator of the level of environmental contamination. Overall, 23.7\% of the surfaces were positive for MRSA. Analysis of contamination patterns showed the introduction and/or reintroduction of clones into the environment and the
frequent contamination of surfaces (2-3 consecutive months with different pulsotypes) that were labeled as hot spots (e.g. chart holders, handrails and elevators). In addition, MRSA pulsotypes appeared to be dynamic with no particular prevalent clone establishing over time (absence of long term contamination). When compared to the levels of MRSA patient infection burden, there was no association between the numbers of clinical cases present in the wards and the level of surface contamination on common areas outside patient rooms over time. These results demonstrate that MRSA frequently contaminates staff and general public contact surfaces during non-outbreak periods, regardless of the number of patients with MRSA infections present in the hospital. Consequently, continuous and thoroughly cleaning and disinfection of high-contact surfaces is needed to prevent the long term establishment and survival of MRSA in the hospital environment.

5.2. INTRODUCTION

In healthcare settings, methicillin-resistant *Staphylococcus aureus* (MRSA) is a well-known pathogen responsible for causing severe hospital associated infections worldwide [1-3]. Even though invasive MRSA infections in healthcare settings seem to be declining in the United States (US) [4, 5], nosocomial outbreaks are continuously reported [6-12]. In most cases, the origin of the outbreak strain cannot be determined. These circumstances highlight the importance of implementing effective infectious diseases control and prevention programs, with consideration for all possible sources of MRSA. Such sources include patients, visitors, hospital personnel and the hospital environment.
In the case of environmental contamination, numerous researches have been performed studying the presence of MRSA on contact surfaces present inside the patients’ rooms and/or general areas of the healthcare facilities [13-16]. Moreover, some of these studies suggested that environmental contamination might be involved in the transmission of MRSA within the hospital. For example, a prospective study conducted in a 9-bed intensive care unit (ICU) collected samples from patients alongside with four environmental areas adjacent to their bed space [14]. MRSA was found in 21.8% (188/864) of the surfaces sampled and pulsed-field gel electrophoresis (PFGE) was used to establish clonality between the isolates. In 35.7% (20/56) occasions indistinguishable MRSA isolates were obtained from patients and their nearby environment concurrently. In addition, 42.6% (26/61) of the MRSA positive patients acquired the microorganism during their stay in the unit; interestingly, 14/26 patients acquired MRSA strains that were distinct from the ones found in other patients. Particularly, the authors found 3 cases that were colonized with MRSA strains that were detected in the ICU environment within 3-10 days before the patients were found positive. The authors hypothesized that contaminated contact surfaces were the source of MRSA for these nosocomial cases, especially since no other patients were found positive (with the same strain) 7 days (2/3) and 54 days (1/3) before these cases were diagnosed with MRSA. Unfortunately, other plausible sources (e.g. hospital personnel) were not evaluated during the study period. Nonetheless, these findings demonstrate that even if environmental surfaces were not the main (direct) source of MRSA for these 3 patients, they still played a big role in the transmission of this pathogen within the ICU unit [14].
Improved understanding of MRSA in the environment is needed in order to develop effective programs for the control and prevention of environmental contamination. It has been suggested that contact surfaces could serve as a reservoir of MRSA in healthcare settings and as a source of nosocomial infections [14, 17]. Yet, the majority of the studies that evaluated MRSA environmental contamination in hospitals have been performed during outbreaks investigations or for very short periods. There is little information about the presence of environmental MRSA over extended periods of time during non-outbreak phases. Since patients with active MRSA infections are admitted to the hospital each day, and hospital personnel and/or visitors who are colonized with MRSA may visit the hospital as well, the potential contact with environmental surfaces leading to contamination of the hospital environment can occur. Hence, MRSA can be found on contact surfaces in healthcare settings and could survive on inanimate objects from weeks to months [18] during outbreak and non-outbreak periods. Therefore, in order to understand the impact of environmental contamination in the transmission of MRSA within hospitals, it is necessary to determine the prevalence of MRSA in the environment during non-outbreak periods and to establish if other factors, like the presence of MRSA positive patients, seasonal variations, and types of unit are capable of influencing the baseline rate of environmental contamination in the hospital. The present study describes the results obtained after one year of environmental monthly samplings in a large metropolitan teaching hospital, and its correlation with MRSA patient infection burden. Furthermore, the molecular and epidemiological characterization of environmental MRSA isolates, its location and potential transmission patterns will also be studied.
5.3. MATERIALS AND METHODS

5.3.1 MRSA Surveillance: Location, environmental surfaces and sample collection method

This study was performed at The Ohio State University Wexner Medical Center (OSU-WMC) for twelve consecutive months, from March 2009 until February 2010. This tertiary healthcare facility is located in Midwestern United States, and it is considered one of the largest and most diverse academic medical centers in the country. The surveillance was carried out in two general medicine wards (Ward 1 and Ward 2). Ward 1 consisted of 18 patient rooms (9 single-including 4 negative airflow isolation infection rooms and 9 double patient rooms) housing 27 patients. Ward 2 consisted of 27 rooms (15 single and 12 double patient rooms) housing 39 patients. Ward 1 and Ward 2 are interconnected with a hallway bridge, which allows the staff, patients and general public to move between them. However, in the case of hospital staff, each ward has their own assigned personnel and only under certain circumstances (e.g. patient overflow) the staff could cover both wards. The Infectious Diseases Service’ patients were generally admitted to Ward 1, where the team conference room was located at the end of the hallway. Other internal medicine services such as hematology/oncology, nephrology including hemodialysis patients, intensive care units patient and other services such as surgical patients were usually admitted to other units. Our hospital has a policy for MRSA contact isolation: If a patient is diagnosed with active MRSA infection or is colonized with MRSA (history of positive MRSA screen), the patients are placed either in a single room or roomed with another patient with a known MRSA infection or positive MRSA screen. It is important to note that during the study period, no MRSA
outbreaks were reported by the OSU-WMC Infection Control on either of the two wards sampled. Each month 38 representative high contact surfaces, twenty-two surfaces from Ward 1 and sixteen surfaces from Ward 2, were selected from the staff and general public areas of these wards (see Table 5.1 for specific areas sampled), which were pre-selected prior to the onset of the study and consistently sampled each month. The criteria used for inclusion of the surfaces consisted of: Surfaces that are frequently touched, that are contacted by multiple individuals, and/or have been suggested as potential sources of contamination in a hospital setting. No areas inside any of the patient rooms were included in this study.

During the time of the study, the OSU-WMC Environmental Services staff was in charge of cleaning the common areas of these patient care units (staff and general areas) every evening with a hospital grade phenolic disinfectant.

Surfaces sampled were classified as “staff contact” and “general public” contact surfaces (Table 5.1). Staff contact surfaces were located within nursing stations and/or enclosed areas usually off limits to the general public. Although some surfaces may be within reach of the public, the majority of the touches are made by the healthcare workers. Contrary, general public surfaces were those touched primarily by the general public and occasionally by hospital staff.

Sampling collection was performed as previously described [19]. Briefly, depending on the size of the surface, dry electrostatic clothes/Swiffer® (for larger surfaces), and sterile cotton swabs pre-moistened in sterile TSB (BD BBL™ Trypticase Soy Broth, Becton, Dickinson and Company, Sparks, USA) (for smaller surfaces) were used. Some samples were collected as a pool to minimize culture costs and increase
likelihood of positivity; in these cases two or more surfaces (within the same area/ward) were collected using the same cloth or swab (Table 5.1). Immediately after sampling, all samples collected were taken to the Diagnostic and Research Laboratory for Infectious Diseases (DRLID) for further processing. Pre-enrichment media (TSB) was added to bags containing electrostatic cloths. Bags and tubes (containing sampling swabs) were incubated at 35°C for 18-24 hours.

5.3.2 MRSA Isolation and Identification

Isolation process was performed as previously described [19]. All samples incubated in pre-enrichment media were transferred onto mannitol salt/oxacillin (2µg/mL) agar (BD BBL™ Mannitol Salt Agar, Dickinson and Company, Sparks, USA) and incubated at 35°C for 24-48 hours. Based on phenotypic characteristics and mannitol reaction, 1-3 unique plausible staphylococcal colonies were selected and streaked onto blood agar plates (Remel®, Blood Agar (TSA with 5% Sheep Blood), Lenexa, USA). *S. aureus* identification was performed by standard colony morphology (including size, pigmentation and hemolysis pattern), mannitol fermentation, gram stain, catalase, tube coagulase, anillin fermentation, Polymyxin B susceptibility, acetoin production (Vogues-Proskauer test), and latex agglutination (Sure-Vue® Color Staph ID, Biokit USA, Inc, Lexington, Mass). Oxacillin Screen Agar® (OSA) plates containing 6µg/ml of Oxacillin supplemented with NaCl (BD BBLTM, Becton Dickinson and Company, Maryland, USA) were used to phenotypically confirm MRSA colonies [20].
5.3.3 Antimicrobial Susceptibility (Phenotyping)

At least one isolate per positive sample was selected for phenotypic characterization. If two isolates from the same sample were morphologically different (colony size, color, and hemolysis), phenotypic characterization was performed in both of them. Antimicrobial susceptibility testing was performed following the Kirby-Bauer Disc Diffusion technique [20]. Antibiotics included were: Amikacin 30µg, Ampicillin 10µg, Amoxicillin with clavulanic acid 30µg, Cefpodoxime 10µg, Cephalotin 30µg, Chloramphenicol 30µg, Ciprofloxacin 2µg, Clindamycin 2µg, Doxycycline 30µg, Enrofloxacin 5µg, Erythromycin 15µg, Gentamicin 1µg, Oxacillin 1µg, Sulfamethoxazole Trimethoprim 25µg and Tetracycline 30µg. Vancomycin resistance was tested using Vancomycin Screen Agar plates (6 mg/L) (BD BBL™ Dickinson and Company, Sparks, USA). Six strains were used for quality control purposes: *S. aureus* (ATCC 43300), *S. aureus* (ATCC 29213), *S. aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212), *Enterococcus faecalis* (ATCC 51299), *Escherichia coli* (ATCC 25922), and *Pseudomonas aeruginosa* (ATCC 27853). Isolates that were resistant to three or more classes of antimicrobials (including beta-lactams after confirmation of the *mecA* gene) were considered multidrug resistant (MDR). Inducible Clindamycin resistance was tested based on bacterial growth pattern consistent with the D-test when placing the Erythromycin disc next to the Clindamycin disc [21].
5.3.4 *mecA* Confirmation, Staphylococcal Chromosome Cassette *mec* (SCC*mec*) Typing and Pulsed-field Gel Electrophoresis (PFGE)

Based on the phenotyping results, one unique colony from each surface sampled was genotyped. If two colonies from the same source had different susceptibility profiles, and both were selected for further characterization. Confirmation of the presence of the *mecA* gene and staphylococcal chromosomal cassette *mec* (SCC*mec*) typing was performed as previously described [22] with some modifications. The DNA used for polymerase chain reactions (PCR) was extracted by boiling 100µl of bacterial suspension for 7 minutes at 95°C [23]. Each PCR mixture with had final volume of 25µl containing 2µl of DNA template; 12.5µl of 2x Multiplex PCR Master Mix (Qiagen®, Foster City, CA) including HotStartTaq Polymerase, 1.5mM MgCl2 and dNTPs; and primers (with doubled concentrations compared to the original protocol). All assays were performed in a Gradient Thermocycler (Eppendorf, Hamburg, Germany) with the following cycling conditions: 95°C for 15 minutes; 35 cycles of 94°C for 30 seconds, 57°C for 90 seconds and 72°C for 90 seconds; and a final extension of 72°C for 10 minutes. PCR products were resolved in a 3% Seakem LE (Cambrex, Rockland, ME) agarose gel with 1x Tris-acetate-EDTA buffer (Promega Corporation, Madison, WI) at 100 Volts for 2 hours, and were visualized with ethidium bromide.

Macrorestriction by pulsed-field gel electrophoresis of MRSA confirmed isolates was performed as previously described [24]. In brief, bacterial genomic DNA was digested using SmaI following the standardized protocols established by the Centers for Disease Control and Prevention [25]. A CHEF mapper system (Bio-Rad Laboratories, Nazareth, Belgium) was used to resolve restriction fragments. *Salmonella* serotype
Branderup strain H9812 was digested with *Xba*1 and used as a molecular size marker. The resulting band patterns were analyzed by BioNumerics® software (version 6.6, Applied Maths, Ghent, Belgium) to determine the relatedness between strains by using Dice coefficient and Unweighted Pair Group Method using Arithmetic averages (UPGMA) to achieve dendrograms with a 1% band position tolerance [26]. Interpretation of the dendrograms was performed as previously described [24], were each band pattern (with ≥98% similarity) represented a pulsotype. Groups of pulsotypes with ≥80% similarity were considered to be closely related and therefore classified as a cluster. To establish USA types, a CDC database containing 100 *S. aureus* strains with the most typical band patterns for each USA type was used. Isolates obtained from the hospital environment were compared against the database, and those with ≥80% similarity were classified as the same USA type.

### 5.3.5 Strain Characterization

Each isolate was characterized and classified as a strain if it possessed a unique combination of phenotypic profile (antimicrobial drug susceptibility test profile), SCC*mec* type, and PFGE pulsotype.

### 5.3.6 MRSA patient infection burden

Aggregate data for the number of patients with MRSA infections during the study period was provided by the OSU-WMC infection control records. This was collected to assess if there was an association between the number of MRSA patients on a ward and the level of contamination detected in the environment. Therefore, the number of patients
with a positive diagnosis of MRSA at any time during their stay on the wards sampled was recorded. Each individual patient was counted as one case and cases were totaled for the month in which they were admitted to the ward. Only the aggregated number of patients for the month was provided. No identifying information was collected on the patients, and the investigators did not access any patient medical records. Total aggregated census counts (patient days) of the two wards studied during our time period were also provided without direct access to any medical records. Unit censuses were taken each night at midnight to determine how many patients were in each ward and compiled for monthly data reports. Clinical MRSA isolates from patients were not characterized by phenotype or genotype; hence, they were not included in the molecular epidemiology analysis performed in this study.

5.3.7 Statistical Analysis

Surface location, date of collection, and molecular strain characterization results were organized and stored. Chi-square coefficients were calculated to make comparisons between types of contact surfaces (staff contact vs. general public), and also to compare results between the two wards. To establish a seasonality pattern, the year was divided into four quarters, each with three months (January to March, April to June, July to September, and October to December). Chi-square coefficients were then used to make comparisons among these four groups. These analyses were performed using the statistical software STATA (Small Stata 12.0, StataCorp LP, College Station, TX) and the Epi Info™ software (http://www.cdc.gov/epiinfo/). To determine if environmental contamination and MRSA patient load were correlated, data were analyzed using
Microsoft Excel and SPSS Statistics software (version 17.0, IBM Corporation, New York, USA). The data were analyzed separately by ward, surface and time. Infection burden proportions were calculated for standardization. Burden of MRSA infection was calculated as the ratio of number of patient days infected per 1,000 patient days per month. Chi-square tests were conducted to compare relationships between the wards and surfaces. Fisher’s exact were used to ensure validity when data counts were low. Correlation coefficients were calculated to determine the association of patient infection burden and environmental contamination burden. Relationships were considered significant when their p-value was ≤ 0.05.

5.4 RESULTS

5.4.1 General environmental prevalence and isolates characterization

A total of 448 surfaces were sampled during the yearlong surveillance, and 23.7% (106/448) were found positive for MRSA (Table 5.2). The overall monthly MRSA contamination ranged from 10.5% to 36.8% (Figure 5.1). The number of samples collected per month varied from 36 to 38 surfaces, depending on their availability at the time of sampling. Of the 106 positive surfaces, 109 MRSA isolates were phenotypically and genotypically characterized; indicating that 3 individual surfaces were contaminated with 2 different strains (each) at the moment the sample was collected.

Detailed genotypic characteristics of all the environmental isolates can be found in Table 5.3. Almost half of the characterized isolates were classified as SCCmec type II (48.6%, 53/109) and USA100 (49.5%, 54/109), traits that are known as hospital-acquired MRSA (HA-MRSA). Dendrogram analysis showed that the majority of the isolates
(100/109) were grouped in 5 different clusters; two of them (C3 and C4) were closely related (79.6% similarity) and included most of the SCCmec type II / USA100 isolates (Figure 5.2). In addition, 34 pulsotypes (P1-P34) were identified. During the first six months of the surveillance (March to August) 18 pulsotypes (represented by 43 isolates) were identified. Similarly, throughout the last six months of the surveillance (September to February) 20 pulsotypes (represented by 66 isolates) were observed. However, only four pulsotypes (P4, P12, P16 and P20) were found in the first and second half of the yearlong program (Figure 5.3), showing a clear shift over time of the pulsotypes present in the hospital environment (lack of long term contamination).

During the surveillance, the four most prevalent pulsotypes (P4, P17, P33, and P30) were found in both wards for short periods of time, either continuously (maximum of two consecutive months) or intermittently contaminating a variety of surfaces (Figure 5.3). For instance, P17 was present for two consecutive months in the environment, going from only being detected on 2 surfaces (11.8%, 2/17 isolates) in November to later represented 71.4% (10/14) of the MRSA isolates found in December. Another example of continuous presence over time is the case of P30; this pulsotype was found contaminating 1 - 4 surfaces per month in April, May, July and August to later disappear for the rest of the study period. In the case of intermittent presence, P4 was scarcely observed on 3 contact surfaces (30.0%, 3/10 isolates) in March; then it was not detected for 7 months until November, were 70.6% (12/17) of the isolates found during this month were characterized as P4.

In regards to the patterns of environmental contamination, in several occasions specific surfaces were contaminated with different MRSA pulsotypes for 2-3 consecutive
months. Such was the case of the chart holders, coffee machine, elevators, handrails, hand sanitizers, doors, laptops, medicine carts (drawers and laptops), and the medicine room (Figure 5.3). Contrary, there was only one particular case were the exact same pulsotype (P17) was found contaminating the same surface (handrails) for two consecutive months (November-December) (Figure 5.2 and 5.3).

Based on the different result combinations for phenotypic profile, SCC\textit{mec} type and PFGE pulsotype from each isolate, 52 strains were identified from the 109 isolates. Phenotypically, 76.9\% (40/52) of the strains were classified as multidrug resistant (MDR). Other than the expected beta-lactam resistance, 96.2\% (50/52) of the strains were resistant to Erythromycin, 73.1\% (38/52) to Clindamycin, and 69.2\% (36/52) to Ciprofloxacin. Only 15.8\% (6/38) of the Clindamycin resistant strains showed an inducible mechanism of resistance. One strain was found phenotypically susceptible to Oxacillin; however, the presence of the \textit{mecA} gene was later confirmed by PCR. Therefore, such isolate was classified as MRSA. All strains were susceptible to Chloramphenicol and Vancomycin.

5.4.2 MRSA environmental contamination by Wards

In Ward 1, MRSA was found contaminating one or more surfaces every month of the yearlong surveillance. It had an overall surface contamination of 21.8\% (Table 5.2), with monthly prevalence ranging from 13.6\% to 61.9\% (Figure 5.1). Table 5.1 shows the contamination rates for each surface sampled in this ward. Genotypically, 58/109 isolates, represented by 28 pulsotypes, were found in Ward 1. The majority of the isolates were classified as SCC\textit{mec} type II – PFGE USA100 (50.0\%, 29/58 isolates) or HA-MRSA,
and SCCmec type IV – PFGE USA300 (36.2%, 21/58) or community-acquired MRSA (CA-MRSA).

Ward 2 had an overall MRSA contamination of 26.2%, difference that was not statically significant when compared to Ward 1 \( (P = 0.28) \). In contrast, during 3 months of the surveillance no surfaces were found positive (Figure 5.1). In addition, the highest rate of monthly contamination (62.5%) was found in three different months (Figure 5.1). Table 5.1 shows the contamination rates for each surface sampled in this ward. Some surfaces (e.g. chart holders, computers and medicine carts) were present in both Ward 1 and 2 and showed different MRSA contamination prevalences. However, none of these differences were statically significant. Genotypically, 51/109 isolates, represented by 16 pulsotypes, were found in this ward. Similarly to Ward 1, the majority of the isolates from Ward 2 were classified as SCCmec type II – PFGE USA100 (43.1%, 22/51) and SCCmec type III – PFGE Novel Type (31.4%, 16/51), which are frequently classified as HA-MRSA strains. In contrast, only 9.8% (5/51) of the strains found in this ward where classified as SCCmec type IV – USA300 or CA-MRSA.

Throughout the study period, only 29.4% (10/34) of the identified pulsotypes were found contaminating both wards, in some cases during the same month.

5.4.3 MRSA environmental contamination by type of contact surface (Staff vs General Public)

A total of 305 staff contact surfaces were sampled and 23.3% (71/305) were positive for MRSA (Table 5.2). Similarly, 24.5% (35/143) of the general public contact surfaces sampled were found MRSA positive (Table 5.2), difference that was not
statistically significant ($P = 0.78$). Detailed contamination rates for each staff and general public contact surfaces sampled are shown in Table 5.1. The medicine room, chart holders, access doors and medicine cart were the most frequently contaminated staff contact surfaces. Handrails, coffee machine and elevators were the most prevalent general public contact surfaces.

Genotypically, 73/109 MRSA isolates (represented by 29 pulsotypes) were obtained from staff contact surfaces, compared to 36/109 isolates (19 pulsotypes) from general contact surfaces. Of the 34 pulsotypes found in the hospital, 41.2% (14/34) were shared between the two types of contact surfaces.

When combining the SCCmec and PFGE type to further characterize the strains, the majority of the isolates obtained from staff contact surfaces (50.7%, 37/73) were characterized as SCCmec type II – PFGE USA100 or HA-MRSA, and only 19.2% (14/73) were classified as SCCmec IV – PFGE USA300 or CA-MRSA. In contrast, in general public contact surfaces, almost the same number of isolates were classified as SCCmec type II – PFGE USA100 (38.9%, 14/36) and SCCmec type IV – PFGE USA300 (33.3%, 12/36) HA-MRSA and CA-MRSA respectively.

5.4.4 Seasonality

The presence of MRSA on environmental surfaces varied between the four seasonal quarters. In Quarter 1 (January to March), MRSA was present in 25.8% (89/112) of the surfaces sampled. Quarter 2 (April to June) had the lowest prevalence with 18.9% (18/113) positive surfaces. In the case of Quarter 3 (July to September) and Quarter 4 (October to December), MRSA was detected in 33.7% (28/111) and 49.3% (37/112) of
the surfaces sampled, respectively. Statistical analysis showed that the season of the year was significantly associated with the presence of MRSA in the environment ($P = 0.019$), where the highest prevalence was noted during the fall months.

**5.4.5 MRSA in Clinical Patients**

During the study period, both wards held patients for 19,613 patient days, of which positive cultures for MRSA infection were accumulated throughout 133 patient days. The infection burden of MRSA among these patients was 6.78 infections per 1,000 patients in internal medicine wards. The infection burden was at its lowest in May 2009, at 2.44 infections and was the highest in December 2009 at 8.60 infections per 1,000 patients in the listed wards. This data is summarized in conjunction with the surface contamination data (Figure 5.4). Despite similarities in graphical modeling, regression statistics indicated no significant association between MRSA patient infection burden and surface contamination with a Pearson coefficient of 0.3 for each one of the wards analyzed (Ward 1, $P = 0.40$; and Ward 2, $P = 0.42$). For example, in Ward 2 there were 3 months (Apr, Aug and Sep) where MRSA was not detected in any of the surfaces. However, during the same months a high burden of MRSA infections per 1,000 patients was observed (8.86, 8.11, and 7.32, respectively).

**5.5 DISCUSSION**

The present study described the results obtained after one year of monthly environmental samplings in two general internal medicine wards from a large metropolitan teaching hospital. The goal of the study was to establish patterns of MRSA
environmental contamination and its correlation (if any) with MRSA patient infection burden during a non-outbreak period. In average, MRSA was found on 23.7% of the surfaces sampled. Similarly to our research, one study in particular collected monthly samples from 3 different hospitals for 9 consecutive months, with an overall MRSA environmental prevalence of 1.3% [27], which is significantly lower compared to the rate reported in this study. Other studies have reported prevalences ranging from 0.0% to 24.0% [9, 28-33]; however, all of these studies were performed during a 15 week period or less and three of them were carried out during outbreak investigations. Variations in environmental MRSA prevalence can be expected, especially if factors like geographical location (prevalence of MRSA colonization in local population), hospital’s characteristics (size, patient load), hospital’s cleaning/disinfection protocols, study design (surfaces sampled, sampling technique used), and surface’s characteristics (type of material and texture) are taken into consideration. Therefore, it is important for each institution to determine its own baseline prevalence rates. Nevertheless, our results showed that MRSA is capable of frequently contaminate hospital contact surfaces even in the absence of any reported or known outbreak.

Throughout the year, a wide variety of pulsotypes were isolated from the hospital environment. A “turnover” or continuous change of MRSA clones appeared to occur as only 4 of the 18 pulsotypes found in the first six months of the study were still present in the last months of the yearlong surveillance. The pulsotypes appear to be dynamic with no particular prevalent clone establishing over time. Regardless this diversity, MRSA was constantly present on contact surfaces every month of the year, which highlights the need of continuous and thoroughly cleaning and disinfection of the hospital environment.
It is important to note that when environmental sampling has been performed during outbreak investigations, generally only one or two predominant clones are observed on hospital’s surfaces (especially those near positive patients), matching with those found in the clinical patients or healthcare workers [7, 34, 35]. Therefore, it is safe to assume that if during routine environmental surveillance or monitoring we do not observed high diversity of MRSA clones it is likely that we are facing an outbreak. It is the authors’ believe that since the MRSA strains found in the environment usually reflects the clones present in the population in contact with such surfaces, the high diversity of clones observed each month during our environmental surveillance reflects that no outbreak occurred during the sampling period. Moreover, every institution should monitor their own environments to establish their “baseline MRSA background”. Consequently, an increase in their baseline environmental contamination by a unique clone could lead to an early outbreak identification or even a potential detection of a “silent” outbreak. A “silent” outbreak is when there is a higher than expected number of cases heavily contaminating the environment, but an epidemiological connection among them has not yet been established yet.

With the exception of P17, which was present on the handrails for two consecutive months, no other particular pulsotype(s) were found persistently contaminating a particular contact surface. These results could indicate that current cleaning and disinfection (C&D) protocols have been effective in preventing the long term establishment and survival of MRSA in such surfaces. During this time frame the OSU-WMC Environmental Services workers cleaned all high touch surfaces on the ward with a standard hospital grade phenolic disinfectant on the evening shift each day.
However, several surfaces were identified as hot spots for MRSA contamination, as they were frequently contaminated with different pulsotypes. Examples of these surfaces included chart holders (positive 6 of 12 months), handrails (6/12), elevators (5/12), hand sanitizer dispensers (4/12), medicine carts (4/12), and medicine room’s cabinets (3/12). Some of these surfaces have also been found as highly contaminated by other authors [28, 36]. A possible explanation for the frequent presence of MRSA in these hot spots is the fact that these surfaces are touched numerous times during the day by patients, visitors and hospital personnel, and in many cases they are not included in routine protocols of C&D. Even though it may not be feasible to clean such surfaces after each contact, Environmental Services needs to be aware that surface MRSA contamination can act as a potential source for nosocomial infections. Targeted cleaning of these hot spots should be incorporated into future C&D to decrease surface transmission. Since this study, the OSU-WMC has transitioned to a full electronic medical record system and has eliminated medical charts and chart holders in the wards. Future studies will be necessary to determine if the environmental contamination load found on the chart holders would now shifts to the computers, laptops, and mobile computers on wheel units, as the hospital personnel spends more time now using these equipment.

Even though no significant difference was detected in the rate of positivity between general public and staff contact surfaces, or in the diversity of strains between them, a clear disparity was observed in the characteristics of MRSA strains found on each type of surface. Our results revealed that HA-MRSA (38.9%, SCCmec type II – USA100) and CA-MRSA (33.3%, SCCmec type IV – USA300) strains were almost equally detected on general public surfaces. In contrast, in staff contact surfaces the majority of
the strains were classified as HA-MRSA (50.7%, SCCmec type II – USA100), and very few as CA-MRSA (19.2%, SCCmec type IV – USA300). This could be explained by the fact that general public contact surfaces (compared to staff contact) are commonly touched by a larger variety of people (patients, visitors and hospital personnel), with different lifestyles, risk factors and levels of exposure to different MRSA clones in the community. These results provide further evidence that patients might not be the only ones responsible for contaminating the hospital environment.

When analyzing and comparing the data obtained in each ward, two major scenarios or patterns of contamination were observed. In the first scenario we noticed unique pulsotypes independently circulating (never shared) in each ward, which shows that each location has their own “environmental MRSA ecology”. Such was the case of P32 and P33 in Ward 1, and P3 and P24 in Ward 2 (Figure 5.2). On the contrary, the second scenario showed that a predominant pulsotype could be co-circulating in both wards at the same time. An example of this was observed with P4 and P17 (Figure 5.2). A plausible explanation for this movement of strains could be people (either hospital personnel or patients) in contact with contaminated surfaces in Ward 1 that later move to Ward 2 (vice versa) carrying these strains with them. Or, these MRSA strains could be one of the most prevalent present in the community who are visiting the hospital; consequently, it is possible that the MRSA strain has not been moved between the wards but is continuously introduced and/or reintroduced by visitors, patients and hospital personnel. Even though only 10/34 pulsotypes were shared between the wards and the exact source of these strains cannot be determined, our results still show that the
circulation of MRSA strains between wards or areas that are physically apart could happen on a regular basis.

These patterns of contamination clearly show that MRSA strains are constantly introduced (and/or reintroduced) and spread in the environment of hospitals serving populations in which MRSA circulates. The presence of this “baseline MRSA background” highlights the importance of frequent and appropriate C&D protocols, which includes targeting hot spots surfaces daily and managing the flow of personnel and equipment between locations. Nonetheless, long term contamination of environmental contamination was not observed, which confirms that current C&D protocols efficiently prevent the maintenance of MRSA strains over time on the hospital environment.

The burden of MRSA in patients was 6.78 infections per 1,000 patients in the internal medicine wards studied. Recent published reports indicate the range of MRSA burden to be from 3.95 infections to as high as 8.02 infections per 1,000 patients, illustrating that our patient infection burden fell within an expected and published range [37, 38]. Overall MRSA patient infection burden was not associated with surface contamination on common areas outside patient rooms over time. Similarly, the two measurements do not appear to be linearly correlated as the Pearson coefficient is 0.2 with a $P=0.55$. These results further demonstrate that the variability in MRSA environmental contamination is determined by multiple factors and not just the presence of MRSA positive patients, including but not limited to application and enforcement of C&D protocols as well as compliance of hospital personnel with biosecurity measures and personal hygiene.
Lastly, we acknowledge that our results are also limited in that only two general medicine wards were included in the study and the prevalence of contamination in other sections of the hospital cannot be established. In addition, we recognize that extrapolation of our results to other hospitals and healthcare settings is not possible. Nonetheless, this is the first published long term environmental study that illustrates the presence and change in circulating MRSA strains in the hospital environment.

5.6 CONCLUSION

In conclusion, this study identified several environmental surfaces in the hospital frequently contaminated over time, which could act as potential sources of nosocomial infections. Further longitudinal molecular and epidemiological studies combining environmental sampling with clinical patient MRSA isolates, healthcare workers and patient screening are needed to determine sources and directionality of environmental contamination as well as the ecology of this important nosocomial pathogen in healthcare settings.

5.7 ACKNOWLEDGEMENT

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PCR. Finally, we will like to thank the Network on Antimicrobial Resistance in *Staphylococcus aureus* (NARSA) program for providing several control strains. NARSA is supported under NIAID, NIH contract number HHSN272200700055C.
5.8 LIST OF REFERENCES


Table 5.1: Contact surfaces sampled with electrostatic cloths (■) or sterile swabs (▲) in two General Medicine Wards at The Ohio State University Wexner Medical Center during the yearlong Active MRSA Surveillance
<table>
<thead>
<tr>
<th>HOSPITAL AREA</th>
<th>STAFF CONTACT</th>
<th>MRSA / Surfaces Collected</th>
<th>GENERAL PUBLIC CONTACT</th>
<th>MRSA / Surfaces Collected</th>
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<td>Surface Description</td>
<td></td>
<td>Surface Description</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chart holders$^1$</td>
<td>11/24 (45.8%)</td>
<td>Elevators$^2$</td>
<td>6/24 (25.0%)</td>
</tr>
<tr>
<td>Ward 1</td>
<td>Computers$^3$</td>
<td>3/36 (8.3%)</td>
<td>Hand sanitizer dispensers$^7$</td>
<td>4/24 (16.7%)</td>
</tr>
<tr>
<td></td>
<td>Laptop$^5$</td>
<td>3/21 (14.3%)</td>
<td>Telephone$^8$</td>
<td>4/24 (16.7%)</td>
</tr>
<tr>
<td></td>
<td>Medicine carts (drawers)$^9$</td>
<td>5/24 (20.8%)</td>
<td>Coffee machine$^9$</td>
<td>4/12 (33.3%)</td>
</tr>
<tr>
<td></td>
<td>Medicine cart laptops$^8$</td>
<td>3/24 (12.5%)</td>
<td>Handrails$^9$</td>
<td>7/12 (58.3%)</td>
</tr>
<tr>
<td></td>
<td>Copy machine$^5$</td>
<td>2/12 (16.7%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Markers$^7$</td>
<td>1/12 (8.3%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Doors$^{10}$</td>
<td>4/12 (33.3%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ward 2</td>
<td>Chart holders$^1$</td>
<td>9/24 (37.5%)</td>
<td>Elevators$^{11}$</td>
<td>4/12 (33.3%)</td>
</tr>
<tr>
<td></td>
<td>Computers$^{12}$</td>
<td>3/12 (25.0%)</td>
<td>Hand sanitizer dispensers$^7$</td>
<td>6/23 (26.1%)</td>
</tr>
<tr>
<td></td>
<td>Laptops$^5$</td>
<td>3/20 (15.0%)</td>
<td>Telephone$^9$</td>
<td>0/12 (0.0%)</td>
</tr>
<tr>
<td></td>
<td>Medicine carts (drawers)$^9$</td>
<td>10/24 (41.7%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Medicine cart laptops$^8$</td>
<td>4/24 (16.7%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Copy machine$^9$</td>
<td>1/12 (8.3%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Medicine room$^{13}$</td>
<td>6/12 (50.0%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dirty room button$^9$</td>
<td>3/12 (25.0%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Two pool samples were collected per ward; each pool sample contained four pre-selected chart holders from the same area.
2 Two pool samples were collected in this ward. The first sampled contained all the call buttons of 3 adjacent elevators servicing this ward. The second sample contained the call button of one elevator servicing another area of this ward.
3 Three pool samples were collected in this ward; each pool sample contained two computers (keyboard and mouse) located in the nurses’ station.
4 Two pool samples were collected per ward; each pool sample contained four dispensers from the same area.

Continued
Table 5.1: Continued

5 Two laptops (keyboard and mouse) for general use were sampled separately from each ward. These laptops are placed in a mobile unit that hospital staff could move across the ward.
6 Two telephones were sampled separately from this ward.
7 Two medicine carts were sampled separately from each ward. These carts are mobile units that nurses move from room to room.
8 Two medicine cart laptops (keyboard and mouse) were sampled separately from each ward. These laptops are attached to the medicine cart unit.
9 Three to four of the available markers at the main nurses’ board were sampled as a pool.
10 Access doors to office’s hall of faculty and healthcare professionals (including door handles and door handicap buttons).
11 One pool sample was collected in this ward; this sample contained all the call buttons of the 4 elevators servicing such ward.
12 One pool sample was collected in this ward; this sample contained two computers (keyboard and mouse) located in the nurses’ station.
13 Drawer handles and keyboards from the medical supply management system were sampled as a pool. The room includes several medication storage units that are stationary.
<table>
<thead>
<tr>
<th>Hospital Area</th>
<th>Staff Contact MRSA / Samples Collected</th>
<th>General Public Contact MRSA / Samples Collected</th>
<th>P-value</th>
<th>Total Contact MRSA / Samples Collected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ward 1</td>
<td>32/165 (19.4%)</td>
<td>25/96 (26.0%)</td>
<td>0.21</td>
<td>57/261 (21.8%)</td>
</tr>
<tr>
<td>Ward 2</td>
<td>39/140 (27.9%)</td>
<td>10/47 (21.3%)</td>
<td>0.37</td>
<td>49/187 (26.2%)</td>
</tr>
<tr>
<td>Total</td>
<td>71/305 (23.3%)</td>
<td>35/143 (24.5%)</td>
<td>0.78</td>
<td>106/448 (23.7%)</td>
</tr>
</tbody>
</table>

**Table 5.2:** Overall prevalence of MRSA contamination of staff and general public contact surfaces distributed in two General Medicine Wards at The Ohio State University Wexner Medical Center during the yearlong active MRSA surveillance.
Table 5.3: Molecular characterization of environmental MRSA isolates from two General Medicine Wards at The Ohio State University Wexner Medical Center during the yearlong active MRSA surveillance

<table>
<thead>
<tr>
<th>ENVIRONMENT</th>
<th>SCCmec Typing</th>
<th>PFGE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MRSA (%)</td>
<td>By Type of Surface</td>
</tr>
<tr>
<td></td>
<td>(Total N=109)</td>
<td>MRSA (%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Staff (N=73)</td>
</tr>
<tr>
<td>Type II</td>
<td>53 / 109 (48.6%)</td>
<td>38 / 73 (52.1%)</td>
</tr>
<tr>
<td>Type III</td>
<td>21 / 109 (19.3%)</td>
<td>16 / 73 (21.9%)</td>
</tr>
<tr>
<td>Type IV</td>
<td>34 / 109 (31.2%)</td>
<td>18 / 73 (24.7%)</td>
</tr>
<tr>
<td>Not Typeable</td>
<td>1 / 109 (1.0%)</td>
<td>1 / 73 (1.4%)</td>
</tr>
<tr>
<td>USA 100</td>
<td>54 / 109 (49.5%)</td>
<td>39 / 73 (53.4%)</td>
</tr>
<tr>
<td>USA 300</td>
<td>28 / 109 (25.7%)</td>
<td>15 / 73 (20.5%)</td>
</tr>
<tr>
<td>Novel Type</td>
<td>21 / 109 (19.3%)</td>
<td>15 / 73 (20.5%)</td>
</tr>
<tr>
<td>USA 800</td>
<td>1 / 109 (1.0%)</td>
<td>0 / 73 (0.0%)</td>
</tr>
<tr>
<td>Not Typeable</td>
<td>5 / 109 (4.6%)</td>
<td>4 / 73 (5.5%)</td>
</tr>
</tbody>
</table>

1 One isolate could not be characterized with the methods used in this study
2 Five isolates that did not match to any USA type
Figure 5.1: Distribution of monthly environmental MRSA prevalence in two General Medicine Wards at The Ohio State University Wexner Medical Center during the yearlong active MRSA surveillance
Figure 5.2: Dendrogram analysis of environmental MRSA isolates obtained from two General Medicine Wards at The Ohio State University Wexner Medical Center during the yearlong active MRSA surveillance
Table 5.3: Examples of presence and distribution (by ward and type of contact surface) of environmental contamination represented by twenty-five MRSA pulsotypes during the yearlong MRSA active surveillance in two General Medicine Wards at The Ohio State University Wexner Medical Center

<table>
<thead>
<tr>
<th>Ward 1 General</th>
<th>Ward 2 General</th>
<th>Chart Holders</th>
<th>Doors</th>
<th>Handrails</th>
<th>Elevator</th>
<th>Hand sanitizer dispensers</th>
<th>Coffee Machine</th>
<th>Medicine Room</th>
<th>Chart Holders</th>
<th>MedCart (Group 1)</th>
<th>MedCart (Group 2)</th>
<th>Elevator</th>
<th>Hand sanitizer dispensers</th>
</tr>
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<tr>
<td></td>
<td></td>
<td>P5</td>
<td>P6</td>
<td>P30</td>
<td>P28</td>
<td>P20</td>
<td>P21</td>
<td>P15</td>
<td>P4</td>
<td>P12</td>
<td>P12</td>
<td>P28</td>
<td>P1</td>
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<td>P29</td>
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<td>P9</td>
<td>P21</td>
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<td>P11</td>
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<td>P33</td>
<td>P17</td>
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<td>P17</td>
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<td>P12</td>
<td>P17</td>
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<td>P23</td>
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<td>P4</td>
<td>P17</td>
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<td>P18</td>
<td>P17</td>
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<td>P18</td>
<td></td>
<td></td>
<td>P18</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 5.3:** Examples of presence and distribution (by ward and type of contact surface) of environmental contamination represented by twenty-five MRSA pulsotypes during the yearlong MRSA active surveillance in two General Medicine Wards at The Ohio State University Wexner Medical Center
MRSA patient infection burden is calculated as per 1,000 patient days
Environmental contamination rate is calculated as per 10 surfaces

**Figure 5.4:** Analysis of overall MRSA environmental contamination and patient infection burden by month during the yearlong active MRSA surveillance at The Ohio State University Wexner Medical Center
CHAPTER 6

THE HOSPITAL ENVIRONMENT: A RESERVOIR FOR METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS WITHIN HEALTHCARE SETTINGS

Within healthcare settings, three main components interact in the transmission cycle of nosocomial pathogens like methicillin-resistant *Staphylococcus aureus* (MRSA): patients, healthcare workers (HCWs) and the hospital environment. The involvement of patient and HCWs has been studied and reported for several decades, mostly in human hospitals, but the role that inanimate surfaces might play in the transmission of MRSA is still poorly understood [1]. Therefore, active long term environmental surveillances were established in veterinary and human tertiary healthcare facilities to improve our current knowledge in environmental MRSA contamination and its plausible significance in the development of nosocomial infections and occupational exposure of HCWs.

The results presented in this dissertation clearly show that MRSA is frequently present in the environments of human and animal hospitals in the absence of an active outbreak. It was shown that this pathogen is constantly introduced and/or reintroduced into the hospital likely by patients (human or animal), visitors and hospital personnel. Such influx of MRSA strains, along with lack or inadequate cleaning and disinfection
protocols, promoted the persistence over time of this bacterium within the hospital environment. Most importantly, MRSA strains were moved across different areas of the hospitals (by HCWs and/or patients) allowing the spread of this pathogen throughout each facility. Regardless of how and when contact surfaces became contaminated, it was demonstrated that the hospital environment plays a key role as a reservoir and as a plausible source of MRSA within human and veterinary healthcare settings.

6.1 MRSA IN HUMANS AND ANIMALS VS MRSA IN THE ENVIRONMENT

Previous studies have described the molecular characteristics of MRSA isolates present in humans and animal populations [2-5]. Based on this information, two scenarios have been described. In the first case, it has been portrayed that animals and humans that are in close contact to them are more likely to be colonized and/or infected with the same MRSA clones [3, 5-7]. Second, within the animal kingdom, the prevalence of certain MRSA strains varies by species, suggesting the possibility of an evolutionary adaptation of a MRSA strain(s) to a specific animal population [2, 3, 5]. In this light, the results described throughout this dissertation are further proof of these phenomenon; for scenario one, we found that all strains present in dogs and their related environments were human associated strains. For scenario two, we found a unique strain in the equine hospital environment and its patients that even though is frequently present in horses, it is scarcely observed in humans or other animal species. This highlights the fact that not only S. aureus is plausibly transmitted between species but also that is exceptionally adaptable to a wide range of hosts, even though is considered a bacteria of human origin. This
zoonotic transmission could enhance the probability of bacterial spread within the community, and also allows animals to become a reservoir of this pathogen [8, 9].

Distinct differences were observed between MRSA strains found in canines and equine. In dogs, the most prevalent MRSA strain isolated was classified as USA100 / SCCmec type II (Chapter 3). Among the hospital-acquired MRSA (HA-MRSA) clones present in the U.S. human general population, the USA100 / SCCmec type II strain is the most prevalent one [10]. Therefore, it is reasonable to assume that transmission or a spillover of strains from human origin to dogs has occurred where canines can act as temporary reservoirs (in the absence of an active infection), and bidirectional transmission is equally observed. In the case of horses, USA500 / SCCmec type IV was the strain most commonly isolated from these animals (Chapter 4), which is also the most prevalent and adapted MRSA clone in different equine populations [3, 5, 7, 11]. Contrary to the canine case, this clone has been identified with very low prevalence in the human general population [12]. Nonetheless, inter-species transmission is still suggested, especially since USA500 seems to be particularly prevalent in humans that are in close contact with equine populations (e.g. veterinarians and horse owners) [2, 3, 7]. Consequently, it could be assume that even though this clone is from human origin, it is the animal reservoir (horses) that generally spreads the bacterium to their human caregivers.

Interestingly, a mirror effect or reflection symmetry was also observed between the MRSA clones found in the veterinary hospital environments during non-outbreaks periods and the patients that concurrently visited such settings. For example, USA100 / SCCmec type II was the most predominant strain contaminating the small animal hospital.
(incoming canines and environment), and USA500 / SCCmec type IV was the most common clone isolated from the equine hospital (incoming horses and environment). Moreover, in the case of the human general medicine wards, the most prevalent clone found contaminating the environment (USA100 / SCCmec type II) was also the most prevalent strain among the patients with MRSA bloodstream infections treated in this unit [13]. Therefore, environmental contamination could be a precise representation of the MRSA clones present in the patients utilizing such settings, information that could be useful for infectious control teams in healthcare settings. For example, a sudden increment in the level of environmental contamination with one particular clone could plausibly represent the early detection of an outbreak, especially in those cases where an active surveillance of patients is not performed.

6.2. THE BATTLE AGAINST ENVIRONMENTAL MRSA CONTAMINATION DOES NOT TAKE PLACE EVERYWHERE

Even though the results described in this dissertation revealed that MRSA can be frequently found contaminating hospital contact surfaces during non-outbreak periods, other studies performed by the same research group have found different results.

The first case study was related to a possible MRSA outbreak in an animal shelter in Ohio after six dogs that were adopted developed MRSA infections within days of leaving the facility. Among the affected canines, the only thing they had in common was the shelter from where they were adopted. Therefore, the shelter authorities decided to perform an investigation to determine if the establishment was indeed the source of the bacterium. For 3 consecutive months, 15 randomly selected and 40 pre-selected surfaces
were sampled every 4 weeks (data not published). Surfaces were collected from the main lobby, surgery suite, recovery room, adoption ward, isolation ward and bonding room, based on their contact type (human or animal) and frequency (high). After screening 120 environmental samples and 45 dogs, 86.7% (104/120) of the surfaces and 64.4% (29/45) of the dogs sampled were positive for methicillin-resistant coagulase negative staphylococci, but no coagulase positive staph (including *S. aureus*) were found in neither the dogs nor the environment. Unfortunately, the shelter personnel were not included in the study. Nonetheless, based on these results, and since the MRSA isolates from the affected dogs were not available to establish if they were all the same clone, it was concluded that the shelter was not likely the source of the MRSA strains affecting the adopted pets, and no further actions were taken by the shelter authorities or the new owners.

Similarly to the yearlong active surveillance established at the Small Animal Hospital (Chapter 2), a second study was performed in an Animal Emergency Hospital in Ohio during their opening year (data not publish). This referral hospital is open 24 hours and it specializes in emergency, intensive care, surgery and diagnostic imaging. Since the facility was new, an active surveillance was established to monitor trends of MRSA environmental contamination while their number of patients increased during their first year in service. Every 3 months for one year an average of 70 environmental samples were collected from human and animal contact surfaces across the different areas of the hospital (intensive care unit, internal medicine, surgery, diagnostic imaging and general areas). After collecting samples from 279 surfaces, both, methicilli-susceptible *S. aureus* (MSSA) and *S. pseudintermedius* (MSSP) were found contaminating 18.6% (52/279) and
12.0% (36/279) contact surfaces respectively across different areas of the hospital during the year (Table 6.1). However, even after increasing their patient’s admissions from 0 to 608 (by the end of the study), only 0.7% (2/279, a computer and an exam table) of the surfaces sampled were positive for MRSA, prevalence that is noticeably different from the 13.5% found in the Small Animal Teaching hospital reported in this dissertation (Chapter 2). Due to the design of the study, it was not possible to establish a reason(s) to explain such results. However, even though in principle, both veterinary hospitals receive similar clients/patients (e.g. mostly referral cases with serious health complications), the veterinary teaching hospital described in chapter 2 has a large body of veterinary students among their staff. In contrast, the emergency hospital is a smaller facility where fewer people (beside veterinarians, technicians and administrative personnel) are present. In addition, factors like cleaning and disinfection protocols, and the colonization status of clinical personnel and patients could have been associated to the lower environmental contamination.

A third study was performed in a neonatal intensive care unit (NICU) in a large tertiary healthcare facility [14]. Since MRSA has been frequently associated with outbreaks and nosocomial infections in ICUs worldwide [15-19], the purpose of this study was to establish an active surveillance to detect the presence of MRSA in a NICU environment and it’s correlation with the colonization status of nurses concurrently working in the same unit. During one year, quarterly samplings were performed on an average of 63 pre-selected surfaces (present in bedside areas or pods, isolations rooms, breast feeding room and general areas) and nurses that volunteered to be sampled. At the end of the study, 254 surfaces and 220 nurses were screened. Only 0.8% (2/254, a
recliner chair and a pooled sample from doors, chairs and microwave from the breast feeding room) of the contact surfaces and 1.4% (3/220) of the nurses were positive for MRSA (Table 6.2). However, MSSA was found contaminating 27.2% (69/254) of the surfaces sampled in different areas, equipment and furniture, including the recliner chairs that were adjacent to each patient’s bed. Currently, molecular characterization and further analysis of the MRSA isolates obtained it’s been performed. Nonetheless, preliminary data showed that the level of *S. aureus* environmental contamination was significantly associated with the location of the surfaces. Contact surfaces present in areas where clinical personnel were required to apply hand sanitizer at the entrance and before they left the area had lower levels of environmental contamination compared to those that did not require such intervention. Furthermore, general public contact surfaces were more contaminated than clinical personnel contact surfaces. It was concluded that the implementation of infectious disease control and prevention measures successfully contributes in the control of environmental contamination. Moreover, cleaning and disinfection protocols should target surfaces in contact not only with the clinical personnel, but with the general public as well to minimize the presence of *S. aureus* and potentially MRSA in the NICU environment.

Ultimately, MRSA environmental contamination is usually detected in facilities with high population density where infected and susceptible individuals are in close contact, such as tertiary healthcare hospitals and nursing homes [20-25]. However, the results from the three studies previously described in this section demonstrate that even in environments where it is expected to find MRSA contamination (e.g. shelters, veterinary clinics and intensive care units), it can be absent or in very low prevalence. Moreover,
these results reveal that the presence or absence of MRSA in a particular environment will depend on several circumstances or factors, including the colonization prevalence in the population in contact with the environment, number of infected people and/or animals in contact with the surfaces, type of material that the surfaces are made off, cleaning and disinfections protocols, among others. In the case of non-healthcare related facilities, like animal shelters, the frequent presence of MRSA could represent a potential risk for the spread of this bacterium into the community, by contaminating and/or colonizing healthy individuals that eventually could develop infections at the household level. In the case of healthcare settings, like the Emergency Animal Hospital and the NICU, having environmental contact surfaces contaminated with MRSA could represent a higher risk of developing nosocomial infections in susceptible patients, as well as a plausible increase in the occupational risk for HCWs.

The findings described in this section provide reassurance that the battle against MRSA does not take place everywhere, but at the same time, it should not give us a false sense of security and we cannot bring our guard down. Furthermore, appropriate cleaning and disinfection has proven to be one of the best tactics to control MRSA environmental contamination, as it been described in studies where MRSA outbreaks could not be controlled until rigorous cleaning and disinfection interventions were implemented in the affected units/wards [22, 26, 27]. Therefore, protocols should be constantly evaluated and updated to control and prevent the level of MRSA environmental contamination.
6.3 THE ENVIRONMENTAL CONTAMINATION DILEMMA: FUTURE DIRECTIONS

Questions regarding how much environmental MRSA contamination can be present on a surface, and how it establishes and persists over time in healthcare settings still remain unanswered. Therefore, some of the future research should be focused on quantifying the level of contamination on inanimate surfaces and identifying characteristics that allow certain strains to prevail and survive on contact surfaces over long periods of time.

Currently, most of the studies performed to evaluate MRSA environmental contamination focus solely on establishing the presence or not of the bacterium (qualitative method). However, the number of viable bacteria contaminating a surface, or the number of colony forming units (CFUs), will make a significant difference on the level of exposure that a susceptible individual has to the bacterium. For example, the number of MRSA CFUs required to potentially initiate an infection in a susceptible individual varies from ten to millions, mostly depending on the presence of lesions or open wounds around the skin area that is in contact with the contaminated surface, and/or the use of devices that breach the skin (e.g. catheters), which facilitates the inoculation of the bacteria [1]. Regardless, it is not the same risk to be exposed to a surface contaminated with 10 CFUs than one contaminated with 1,000,000 CFUs; the higher the number of CFUs present on a surface, the greater the risk of acquiring the microorganism (colonization and/or infection) and vice versa. The problem is that by using the current qualitative contamination assessment methods both of these surfaces will test positive. Therefore, it would not be possible to measure the risk level that a susceptible individual
in contact with the positive surface could have. This highlights the importance of developing protocols that allows the quantification of bacteria present on a surface.

Unfortunately, some of the problems that researchers are currently facing in regards to the quantification of environmental contamination are the variability in the total surface area of an item (e.g. an examination table vs a computer keyboard) and which segment of the surface to sample. Since each surface will have a unique size and shape, and probably there will be some parts that are more frequently “touched” than others, the MRSA contamination might not be evenly distributed across the whole surface. Therefore, is it important to use a sampling method that allows us to cover and “capture” as much of the bacterial contamination as possible. For this purpose, one of our future directions to continue our environmental research includes the adaptation and standardization of our current sampling technique (using electrostatic cloths) to quantify the level of contamination on surfaces with a quantitative polymerase chain reaction (qPCR). By using electrostatic cloths, we attempt to first ensure that the entire area of the surface is covered and sampled (something that is limited by using other methods like swabs, especially on larger surfaces). Second, we rely on the electrostatic charge present in the cloth to maximize the number of bacteria recovered from the surface to accurately quantify the level of contamination.

Ultimately, a standardized quantification method to measure bacterial environmental contamination will have at least two advantages over the currently used qualitative techniques. First, a quantitative technique will allow us to detect not only if a surface if positive or not (qualitative method) but to also measure the level of risk that the surface might pose to susceptible individuals based on the number of CFUs present on
the surface. Second, the efficacy of cleaning and disinfection protocols could be evaluated by quantifying the level of contamination before and after a surface is cleaned/disinfected, which also gives the opportunity to establish quantitative standards for surface cleanliness [1]. Ideally, a minimum number of CFUs or cut-off point representing a very low risk for developing an infection should be established. In this case, that cut-off point could become the target to reach after cleaning and/or disinfection of contact surfaces.

The second forthcoming project involves the study of the biofilm production capability present in the MRSA clones isolated from the hospital environments that seem to be more capable of establishing and prevail on contact surfaces. A biofilm is “a microbially derived sessile community characterized by cells that are irreversibly attached to a substratum or interface or to each other, are embedded in a matrix of extracellular polymeric substances that they have produced, and exhibit an altered phenotype with respect to growth rate and gene transcription” [28]. The formation of this structure confers a level of protection to the bacteria that lives inside the biofilm against external harmful circumstances, mainly extreme environmental conditions (e.g. oxygen and iron limitation, and high osmolarity), attack by host immune cells, the effect of antimicrobials and the effect of cleaning and disinfection products [29, 30]. The significance of biofilm production in the hospital environment has been recently described [31, 32]. A study performed in an ICU showed that even after terminal cleaning of the equipment and furnishings of the unit (which included cleaning with a neutral detergent followed by disinfection with chlorine), biofilms containing viable multidrug resistant bacteria were detected in 4/6 surfaces evaluated. Moreover, to further prove that
the bacteria present in the biofilms were viable, MRSA was grew on agar plates inoculated from 2/4 surfaces (a venetian blind cord and a curtain) that were covered in biofilms [31]. Another study evaluated the in vitro effect of three commonly used biocides containing benzalkonium chloride (1% w/v), chlorhexidine gluconate (4% w/v) and triclosan (1% w/v) over MRSA and Psedomonas aeruginosa biofilms growing in materials frequently used in hospital environments. The authors found that all three biocides were ineffective against the biofilms at the concentrations recommended for use on the label of the product [32]. Both of these studies are proof of how biofilm production could facilitate the prolonged survival of bacterial pathogens like MRSA in the hospital environment, even after cleaning and disinfection. The question of whether certain MRSA clones detected in hospitals are more efficient in producing biofilms and therefore be more frequently isolated from contact surfaces remains to be answer.

6.4 CONCLUSIONS

Since MRSA was discovered over 50 years ago, numerous efforts have been implemented to successfully treat and prevent infections cause by this pathogen in healthcare settings and the community. Much is known about the microorganism itself as well as how it interacts with its host (human or animal) and its related risk factors. In contrast, clinicians and researchers still debate whether the environment is a figure that should be considered to prevent and control MRSA nosocomial infections. This dissertation describes the original results obtained from yearlong active MRSA surveillances performed in 3 different hospitals. These prospective studies were the first (now published) to implement continuous monthly samplings of the hospital environment.
(veterinary and human settings) concurrently with incoming patients (in the veterinary settings). The study design allowed us to bring the medical community one step closer to elucidating the role that contact surfaces play in the transmission cycle of MRSA within healthcare settings, particularly during non-outbreak investigations.

After collecting data from 3 different hospitals (human and veterinary) it was concluded that MRSA was frequently present throughout these facilities, contaminating a wide variety of surfaces in the absence of any known outbreaks. In some cases, this presence was due to the constant introduction of MRSA clones (by humans and animals) into the hospital. In others, it was associated to the maintenance or survival over time (up to 5 consecutive months) of certain strains on the same contact surfaces. These findings highlight the importance of developing and upholding effective cleaning and disinfection protocols targeting hospital surfaces that could act as hot spot of MRSA contamination and as a source for nosocomial infections.

The impact of the studies described in this dissertation could be measured by the changes implemented in 2/3 settings evaluated after our results were shared with hospital’s representatives. For example, at the Small Animal Hospital not only were the hand sanitizer manual dispensers replaced by automatic ones, but the hospital also increased the number of dispensers present across different sections to make them more accessible for the hospital personnel. Additionally, spray bottles with disinfectant were attached to all of their gurneys (which were frequently positive for MRSA) to facilitate the cleaning and disinfection of the surface between patients. Lastly, the veterinary hospital recently changed the disinfectant used during their daily cleaning and disinfection routine. Future studies will be needed to evaluate if the level of MRSA
environmental contamination present in the facility is reduced after such action was implemented. In the case of the Wexner Medical Center, their chart holders were among the most prevalent surfaces in the hospital. It is expected with the replacement of all medical charts and their respective holders with an electronic medical record system to decrease or minimize the presence and maintenance of MRSA in the general medicine wards. Furthermore, similarly to the veterinary hospital, all hand sanitizer dispensers were replaced with automatic units, since their manual version was found positive 17% of the occasions they were sampled. In addition to the employed control and preventive measures, MRSA outbreaks have not been reported in any of the 3 hospitals evaluated since their corresponding studies ended.

Further studies will be needed to obtain evidence that is beyond reasonable doubt of the actual MRSA transmission from the hospital environment to patients, and not just a random occurrence in one or two specific cases. Nonetheless, the information described and discussed throughout this dissertation adds one more piece to the epidemiological puzzle that MRSA represents as a nosocomial and zoonotic threat within healthcare facilities.
6.5 LIST OF REFERENCES


<table>
<thead>
<tr>
<th>SAMPLING DATE</th>
<th># Surfaces Sampled</th>
<th>MRSA (%)</th>
<th>MSSA (%)</th>
<th>MRSP (%)</th>
<th>MSSP (%)</th>
</tr>
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<tbody>
<tr>
<td>April 2013</td>
<td>67</td>
<td>1 (1.5)</td>
<td>17 (25.4)</td>
<td>1 (1.5)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>July 2013</td>
<td>71</td>
<td>0 (0.0)</td>
<td>10 (14.1)</td>
<td>0 (0.0)</td>
<td>6 (8.5)</td>
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<tr>
<td>October 2013</td>
<td>73</td>
<td>0 (0.0)</td>
<td>8 (11.0)</td>
<td>0 (0.0)</td>
<td>12 (16.4)</td>
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<tr>
<td>January 2014</td>
<td>68</td>
<td>1 (1.5)</td>
<td>17 (25.0)</td>
<td>0 (0.0)</td>
<td>18 (26.5)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>279</td>
<td>2 (0.7)</td>
<td>52 (18.6)</td>
<td>1 (0.4)</td>
<td>36 (12.9)</td>
</tr>
</tbody>
</table>

MRSA: Methicillin-resistant *Staphylococcus aureus*
MSSA: Methicillin-susceptible *S. aureus*
MRSP: Methicillin-resistant *S. pseudintermedius*
MSSP: Methicillin-susceptible *S. pseudintermedius*

**Table 6.1:** Coagulase positive Staphylococci environmental contamination during a year-long MRSA active surveillance at an Animal Emergency Hospital in Ohio
<table>
<thead>
<tr>
<th>SAMPLING DATE</th>
<th># Surfaces Sampled</th>
<th>MRSA (%)</th>
<th>MSSA (%)</th>
<th># Nurses Screened</th>
<th>MRSA (%)</th>
<th>MSSA (%)</th>
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<tr>
<td>May 2012</td>
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<td>0 (0.0)</td>
<td>18 (28.1)</td>
<td>100</td>
<td>2 (2.0)</td>
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<td>20 (30.0)</td>
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<td>1 (1.0)</td>
<td>27 (27.0)</td>
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<tr>
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<td>62</td>
<td>0 (0.0)</td>
<td>16 (25.8)</td>
<td>20</td>
<td>0 (0.0)</td>
<td>3 (15.0)</td>
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<tr>
<td>January 2012</td>
<td>62</td>
<td>1 (1.6)</td>
<td>15 (24.2)</td>
<td>0</td>
<td>N/A</td>
<td>N/A</td>
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<td>TOTAL</td>
<td>254</td>
<td>2 (0.8)</td>
<td>69 (27.2)</td>
<td>220</td>
<td>3 (1.4)</td>
<td>58 (26.4)</td>
</tr>
</tbody>
</table>

MRSA: Methicillin-resistant *Staphylococcus aureus*
MSSA: Methicillin-susceptible *S. aureus*
N/A: Not Applied

**Table 6.2:** *S. aureus* environmental contamination during a year-long MRSA active surveillance at a Neonatal Intensive Care Unit (NICU)
REFERENCES


Faires, M., D. Pearl, et al. (2013) "The identification and epidemiology of meticillin-resistant *Staphylococcus aureus* and Clostridium difficile in patient rooms and the ward environment." BMC Infectious Diseases Volume, 342 DOI:

Faires, M., D. Pearl, et al. (2012) "A prospective study to examine the epidemiology of meticillin-resistant *Staphylococcus aureus* and Clostridium difficile contamination in the general environment of three community hospitals in southern Ontario, Canada." BMC Infectious Diseases Volume, 290 DOI:


"Staphylococcus aureus." Biomedical papers of the Medical Faculty of the University Palacký, Olomouc, Czechoslovakia 152(2): 191-202.


Murphy, C., V. Quan, et al. (2012) "Nursing home characteristics associated with methicillin-resistant *Staphylococcus aureus* (MRSA) Burden and Transmission." BMC Infectious Diseases Volume, 269 DOI: 10.1186/1471-2334-12-269


