COMPARATIVE STUDY OF ANTIMICROBIAL RESISTANCE IN COMPANION ANIMALS AND THEIR HEALTHCARE PROVIDERS

By

Elizabeth Hamilton

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Comparative Medicine & Integrative Biology

2011

ABSTRACT

COMPARATIVE STUDY OF ANTIMICROBIAL RESISTANCE IN COMPANION ANIMALS AND THEIR HEALTHCARE PROVIDERS

By

Elizabeth Hamilton

The involvement of companion animals in transmission of resistance within animal and human populations is widely accepted, yet poorly understood. Indicators or risk factors associated with companion animals being hosts or vehicles of transmission have not been fully explored within the context of an animal's interaction with other animals or humans. The objective of this dissertation was to characterize the relationship between companion animals, humans, and environmental surfaces within a veterinary teaching hospital (VTH), as it relates to prevalence, acquisition, persistence, and transmission of resistant bacteria.

Between 2007 and 2009 three distinct groups were enrolled or sampled for this study: (1) animals admitted to the emergency and critical care (ECC), orthopedics (Ortho), soft tissue (ST), and internal medicine (IM) wards; (2) veterinary students going through their clinical rotations and faculty and staff working in the aforementioned wards; and (3) environmental surfaces within the ECC, Ortho, ST, IM wards, and surgery office, prep and recovery areas, and surgical suites. Rectal and nasal samples were collected from animals at admission and discharge; fecal and nasal samples were collected from students at the beginning and end of their clinical rotations; and environmental samples, as well as, fecal and nasal samples were collected from faculty and staff at the beginning of every 4th rotation. All samples were processed for isolation

and identification of enterococci, staphylococci, and *Escherichia coli*. Antimicrobial susceptibility testing was performed and additional molecular tests (pulsed-field gel electrophoresis and multilocus sequence typing) were performed on a selection of isolates. Additionally, epidemiological data were collected by abstraction of animals' charts and questionnaires completed by students, faculty and staff.

These data were analyzed first within each group (animals, humans, and environmental samples) and then globally, with all groups combined. Certain objects within the VTH were identified as more likely to carry organisms. Additionally, factors that increase the risk of an animal either acquiring Methicillin-resistant *Staphylococcus aureus* or having persistence of multi-drug resistant *E. coli* were identified. Clinical procedures performed by students and faculty and staff that would increase the risk of carriage of resistant bacteria were also identified.

From these results, molecularly related isolates were isolated from different environmental sites, different animals, different faculty or staff members, animals and faculty or staff, and students and the environment. By using date of sample collection inferences into route of transmission can be made, however, due to longer time gaps between samples, firm conclusions on direction of transmission cannot be reported. However, these data are applicable to issues of infection control guidelines within VTHs. The VTH patients were not the only possible source for the resistant organisms isolated during this study, which implies that students, faculty and staff share this responsibility. Additionally, special attention should be paid to areas of the VTH that are not a focus of routine cleaning, such as the scale; and specific consideration should be given to patients who visit multiple areas of a VTH during their admission.

ACKNOWLEDGEMENTS

Although many people helped in completion of my doctorate, I would like to take the time to acknowledge my mentor, Professor John B Kaneene. I have always been in awe of his ability to relay his expertise on such a large variety of topics and make each of his students feel as though their project really mattered.

I continued to work at my full-time job at the Michigan Department of Community Health while completing this program. My Division director, Dr. Melinda Wilkins, was such an inspiration during this time. Having seen her not only complete her PhD while working, but also starting her family, gave me the support I needed to return to school while keeping my full time job and growing my family as well. Dr. Wilkins truly taught by example, that you can be a mother, a student and a professional.

I would especially like to acknowledge the following: (i) my guidance committee members, Joseph Gardiner, Julie Wirth, and Shannon Manning, (ii) Katie May for assistance with the laboratory work and sample collection, (iii) The Research and Teaching Technical Support (RATTS) for assistance with recruitment and sample collection, (iv) Roseann Miller, and (v) faculty, staff and students of the VTH for not only participating in my study, but for supporting our study and applying our findings. Financial support for this dissertation was provided by the Center for Comparative Epidemiology and the College of Veterinary Medicine Small Animal Endowment Fund.

Finally, I wish to acknowledge my family, which grew from 2 to 5 members during the course of this program. My husband has given me more support than I could have asked for, which has allowed us to set a positive example for our children on the importance of patience and understanding. I also want to acknowledge, my parents and extended family for their support, whether via email, babysitting, or just listening. Through this experience, I have learned that life rarely occurs the way you expect, but somehow, is always exactly how it should.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

RATIONALE

Antimicrobial resistance in pathogenic bacteria is a public health issue for both humans and animals (Weese et al, 2006). By the end of 2009 there were approximately 77.5 million dogs and 93.6 million cats residing in American households. Additionally dogs and cats lived in almost two-thirds and three-fourths, respectively, of every home in the United States (American Pet Products Association, 2009). These days, many people treat their pets as people, sharing food and sleeping areas. This close contact creates ideal conditions for transmission of resistance and bacteria by direct contact (petting, licking, physical injuries) or through the domestic environment (contamination of food or furnishings) (DeVincent et al, 2005; Guardabassi, et al, 2004). The first report of indistinguishable methicillin-resistant *Staphylococcus aureus* (MRSA) strains isolated from a person and their dog was reported by van Duijkeren et al in 2005. Studies of these types have shown that companion animals and humans can both act as reservoirs for the resistant organisms, completing the loop for re-infection (Weese et al, 2006; Guardabassi, et al, 2004).

When considering the link between human and animal populations, one must consider organisms that are likely to infect both species. *Enterococcus* spp. and *E. coli* are known to be part of the normal flora of the gut of both humans and animals and species of staphylococci are part of the normal flora of skin and nasal areas in humans and animals. However, these organisms can also be pathogenic and cause disease in humans and animals, and specific strains have been shown to more readily develop resistance to specific antimicrobials, thereby complicating treatment protocols.

While it has been suggested that companion animals play a role in the transmission of antimicrobial resistant organisms, little is known about the factors that are important for transmission, persistence, and direction of transmission. Previous studies have compared isolates collected from single points in time, and with the growing evidence that resistant bacteria can pass between humans and animals, specific evidence for direction of transmission is lacking.

RESEARCH QUESTIONS

In this dissertation, the overall goal is to establish clear indications for direction of transmission of resistant bacteria between humans and animals. Application of these findings on infection control guidelines in veterinary medicine could help prevent resistant organisms from infecting already compromised animals and persons. In order to assess this goal, there are key questions that should be answered by the studies conducted. They are:

1. Are resistant bacteria present within the environment of the veterinary teaching hospital, and if so, are certain clones persisting over time?
2. What characteristics of animals being admitted to a veterinary teaching hospital (VTH) are important for that animal acquiring a healthcare associated infection?
3. Does interaction with patients in certain areas of a VTH contribute to healthcare providers acquiring a healthcare associated infection?
4. Are certain clones of bacteria colonizing or infecting both animals and healthcare providers, and if so, are they also found in the VTH environment?

HYPOTHESES TO BE TESTED

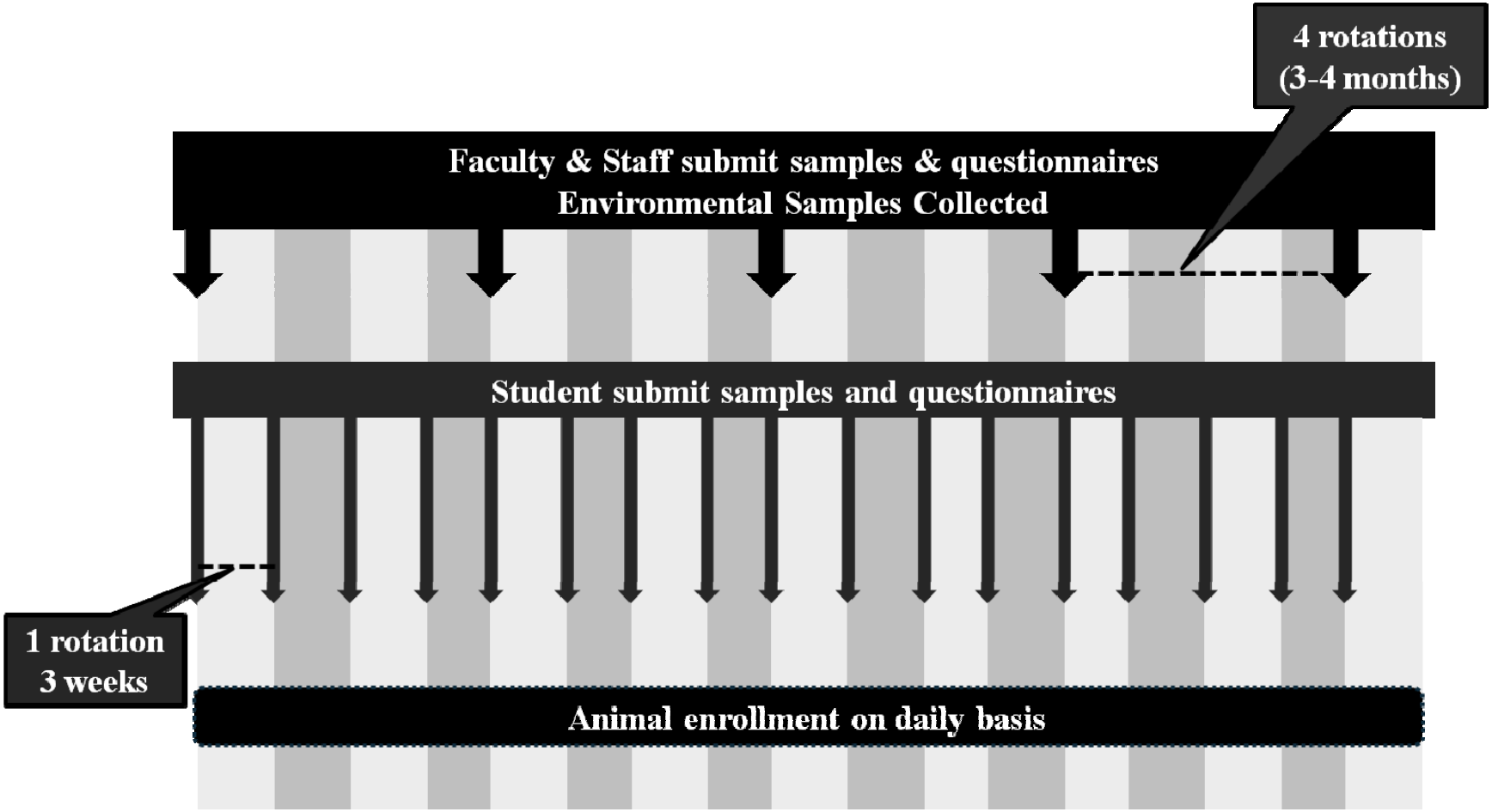
In order to address the above goal and answer the key questions, a number of hypotheses were developed and tested. They are:

1. Prevalence and level of antimicrobial resistance observed from environmental surfaces throughout a veterinary environment will differ from each other over time.
2. Individual animal characteristics and experiences while hospitalized at a veterinary clinic will have impact the likelihood of acquiring a healthcare associated infection.
3. A veterinary healthcare provider's interaction with patients and the clinic environment will be associated with bacterial colonization of that healthcare provider.

OVERVIEW OF RESEARCH

A literature review of the roles that companion animals and environmental surfaces play in transmission of antimicrobial resistance and the transmission of antimicrobial resistance between companion animal and human populations is presented in chapter one. In order to test the stated hypotheses, a longitudinal study (concurrently collecting samples from companion animals and VTH healthcare providers, as well as environmental surfaces through a VTH) was conducted at the Michigan State University (MSU) VTH from 2007 through 2009. All samples were processed for isolation and identification of enterococci, staphylococci and *E. coli.* Additionally, all isolates underwent antimicrobial susceptibility testing and a selection of isolates also underwent molecular analysis via pulsed-field gel electrophoresis (PFGE) and/or mulitlocus sequence typing (MLST).

Figure 1: Overview of enrollment of and sample collection from animals, students, faculty and staff, and environmental surfaces over a 1-year period.



The study was implemented using three groups of subjects: (i) environmental surfaces, (ii) animal patients of the VTH, and (iii) veterinary healthcare providers, consisting of veterinary medical and technical students and faculty and staff.

For the **first group** of subjects (Figure 1, top box), surfaces from the following areas were swabbed: (i) emergency critical care (ECC) ward, (ii) soft tissue/internal medicine (ST/IM) ward, (iii) orthopedic (Ortho) ward, and (iv) surgery prep, office and operating rooms. The same surfaces, such as cages, exam tables, floors, keyboards, telephones and scales, within each of the aforementioned areas were swabbed at the start of every fourth clinical rotation (approximately every three months). **Chapter two** presents details of this part of the study and also addresses hypothesis 1 by describing the prevalence and antimicrobial resistance of enterococci and staphylococci isolated from the different areas and environmental surfaces of a VTH from 2007 to 2009.

The **second group** of subjects (Figure 1, bottom box) focused on dogs and cats admitted to the ECC, ST, IM, or Ortho wards of the MSU VTH. For those companion animals whose owners consented, rectal and nasal/oropharyngeal swabs were collected upon admission and again at discharge. Details of from this part of the study are presented in **chapter three**, which also addresses hypothesis 2 by performing an epidemiological study and analysis of instances animals be admitted to a VTH having persistent multi-drug resistant (MDR) *E. coli* and also acquiring MDR *E. coli* or MRSA.

The **third group** of subjects (Figure 1, middle and top boxes) focused on healthcare providers working in the ECC, ST/IM, Ortho wards, surgery prep, office and operating rooms at the MSU VTH. Veterinary medical and technician students going through their clinical rotations, as well as, faculty and staff working in the aforementioned areas were eligible to

participate. Those students who gave their consent were asked to complete a questionnaire and provide fecal and nasal samples within five days of the start of their rotation and again within five days of the end of their rotation. Thos faculty and staff who gave their consent were asked to complete a questionnaire and provide fecal and nasal samples within five days of the start of every fourth rotation (approximately every three months), to coincide with the environmental surface sampling occurring in the first arm of this study. Findings from this third part of the study are presented in **chapter four**, which also addresses hypothesis 3 by describing instances of VTH healthcare providers experiencing acquisition, persistence, or loss of MRSA or VRE from 2007 to 2009.

CHAPTER ONE

LITERATURE REVIEW

ABSTRACT

The roles that companion animals and veterinary medicine play in development and transmission of antimicrobial resistant organisms have become a point of focus within the scientific community. The objective of this literature review is to not only discuss the impact of interaction between companion animals and humans on antimicrobial resistance, but also present findings that focus on veterinary hospitals and environmental surfaces within veterinary teaching hospitals (VTH) act as vehicles in the transmission.

Literature searches were performed using PubMed, hosted by the National Institutes of Medicine. Although initially, published reports of antimicrobial resistance involving veterinary medicine and companion animals focused on outbreaks of important pathogens, such as MRSA, more recent publications are focusing on veterinary hospitals in the absence of outbreaks. Additionally, more focus is being put on the role of environmental surfaces and effects of infection control guidelines within the VTH. After review of the literature, veterinary personnel hygiene and infection control practices should be the focus, over environmental surfaces, in stopping transmission of healthcare associated infections (HAI) within veterinary medicine.

INTRODUCTION

In the 1940s, the antimicrobial, penicillin, was considered the 'magic bullet' and was thought to be the solution in man's losing battle against disease and infection. However, resistance to this wonder drug quickly surfaced and the need for alternatives became apparent

almost instantly. Although many antimicrobials have been discovered and introduced to the public since the unveiling of penicillin, corresponding resistance was always close behind. Some resistance is inherent to certain organisms, like cephalosporin resistance in enterococci (Murray, 1998); however, other types of resistant are the product of over and inappropriate use, such as tetracycline in food animals (Silbergeld et al, 2008).

Resistance caused by antimicrobials used as growth enhancers in food animals has been studied at length, and as a result is an accepted path for development of resistance (Silbergeld et al, 2008). However, the role that companion animals and veterinary medicine play in development of antimicrobial resistance is less clear. Interest in this topic is growing, however, the impact has been difficult to quantify as many antimicrobials are used in both human and veterinary medicine and some antimicrobials approved for use in humans only are actually used 'off label' in veterinary medicine (FDA, 2009; AVMA, 1998).

Two important resistant pathogens are a major cause of healthcare associated infections in both human and animal medicine: methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant enterococci (VRE). Although MRSA was detected in humans almost immediately, it took nearly 25 years for MRSA to be isolated from a dog (Guardabassi et al, 2004). Development of VRE occurred much more quickly and was first seen in animals about seven years after the introduction of vancomycin (Bates et al, 1994).

While MRSA has been isolated from both human and animal populations (Weese, et al, 2006), little is known about how interactions between these two populations facilitate transmission. Additionally, Huycke et al (1998) has shown that multi-drug resistant enterococci are among the most important pathogens responsible for HAIs in humans. There has been much evidence (Weese et al, 2008) offered to support the idea that companion animals play an

important role in the transmission of antimicrobial-resistant bacteria. Additionally, previous studies have shown that pathogenic and opportunistic bacteria, such as *Acinetobacter baumannii, S. intermedius, E. faecalis, E. faecium,* MRSA and coagulase-negative staphylococci, are present on objects within veterinary and human hospitals, such as treatment tables and cages (Sidhu et al, 2007), door handles and dry erase boards (Loeffler et al, 2005) and floors (Boerlin et al, 2001). However, there still lacks concrete proof of what precipitates transmission, whether humans or animals are the more likely donor, and what role environmental surfaces play in this transmission.

MATERIALS AND METHODS

An initial search of the literature included the use of PubMed, which is hosted by the National Center for Biotechnology Information, U.S. National Library of Medicine, National Institutes of Health (www.ncbi.nlm.nih.gov), using keyword searches of veterinary hospital, nosocomial infection, environmental surface, enterococci, staphylococci, *E. coli*, antimicrobial resistance, and multiple combinations of these keywords. Additionally, pertinent references cited by authors were also obtained and reviewed. Emphasis was placed on peer-reviewed publications.

ROLES OF COMPANION ANIMALS AND VTH ENVIRONMENTAL SURFACES IN TRANSMISSION OF ANTIMICROBIAL RESISTANCE

The emergence of HAIs with resistant bacteria in veterinary medicine is of concern because, like human patients in hospital settings, animals housed in VTHs are more susceptible to infection (Burke, 2003). Unlike human medicine, there are a limited number of approved antimicrobials for use in companion animals (FDA, 2010). This leaves fewer options when resistance does emerge. Concurrently, the close contact between companion animals and

humans, either at a VTH or in the home, offers favorable conditions for the transmission of resistance and bacteria by direct contact (petting, licking, physical injuries) or through the domestic environment (contamination of food or furnishings) (DeVincent et al 2005; Guardabassi, et al 2004).

Impact of VTH environmental surfaces on transmission. Many cross-sectional studies have reported the presence of bacteria, such as MRSA or VRE, within the VTH environment (Boerlin et al, 2001; Burgess et al, 2004; Ishihara et al, 2010; Loeffler et al, 2005; Sidhu et al, 2007; Weese et al, 2004; Weese et al, 2006). While it is not unexpected to isolate bacteria, even pathogenic bacteria, from any hospital environment, the conclusion of an environmental surface playing a part in the transmission of antimicrobial resistance is almost always based on a breakdown of infection control measures. Persistent breakdown in infection control measures may lead to persistence of pathogens, such as MRSA, within the environment (Murphy et al, 2010a).

One report of an environmental assessment during a MRSA outbreak among horses at a VTH showed that MRSA was recovered from the stalls of MRSA-negative horses (Weese et al, 2004). It was concluded to be the result of either human spread from (i) asymptomatic, colonized veterinary personnel or (ii) by transmission from colonized surfaces or animals as a result of a breakdown in infection control measures. However, it is important to remember that adherence to infection control protocols are imperative in both human and veterinary medicine. There is a documented lack of standard infection control measures for veterinary medicine (Murphy et al, 2010b). With this in mind, Aksoy et al (2010) assessed the environmental cleanliness and hygiene in a VTH, using standards proposed for human hospitals. Overall

findings were similar to what is currently being found in human hospitals, in that samples were more likely to fail the cleanliness standards when collected from the floors vs. doors.

The accessibility of contaminated or colonized environmental surfaces within a VTH helps with making conclusions about routes of transmission. For example, the presence of pathogenic bacteria in areas where animal patients are unlikely to touch, such as computer keyboards and door handles, is most likely the result of contact with veterinary personnel (Heller et al, 2009). Conclusions are less clear when pathogenic bacteria are isolated from locations with equal accessibility, such as cages, leashes, or exam tables. A recent study on the bacteria isolated from the environment of a VTH show that MRSA was most likely to be isolated from the floor and equipment of x-ray computed tomography (CT) room and a the office floor of veterinary personnel (Ishihara et al, 2010). Their reported findings that suggest MRSA is being transmitted between animals and veterinary personnel with or without an environmental surface vehicle, which are similar to that seen in human hospitals.

Documented transmission between people and animals. Because the first accounts of MRSA in companion animals were linked to an owner either being infected or working in a health care setting, it was originally hypothesized that MRSA in animals were mainly transmitted from humans. However, transmission can potentially occur in both directions, and direct exposure to MRSA-positive animals can lead to transmission to humans and potential infections (Moodley et al, 2008), especially within a veterinary setting.

Studies of MRSA infections have shown that companion animals and humans can both act as reservoirs for the resistant organisms, completing the loop for re-infection (Guardabassi et al, 2004; Lanz et al, 2003). Specifically, van Duijkeren et al (2005) reported on a woman with repeated MRSA infections, only to finally conclude that her son and family dog were carriers.

Additionally, Weese et al (2006) described MRSA infections in household pets and the transmission between these pets and their household contacts, concluding that household pets are more likely to acquire MRSA from their owners and household contacts, rather than from a VTH or from the general companion animal community.

While the importance of MRSA as a pathogen in both human and veterinary medicine has been described, a newer pathogen is stepping into the light. *Staphylococcus pseudintermedius* is commonly isolated from dogs and is rarely isolated from humans without routine contact with companion animals (Duquette et al, 2004). As with *S. aureus,* resistance to methicillin limits the ability to treat infections caused by methicillin-resistant *S. pseudintermedius* (MRSP). With *S. pseudintermedius* being a commensal organism of dogs, resistance could make infection from this organism difficult treat, however, this issue is also important in human medicine. Frank et al (2009) assessed the risk of MRSP colonization in owners of actively infected dogs, which they concluded to be low. However, Paul et al (2011) assessed the prevalence of MRSP among small animal veterinarians, and found that MRSP was more prevalent then MRSA. This study concluded that MRSP is an emerging zoonotic pathogen.

TRANSMISSION OF ANTIMICROBIAL RESISTANT ORGANISMS BETWEEN COMPANION ANIMAL AND THEIR HEALTHCARE PROVIDERS

The nature of housing within a VTH (i.e., cages and runs) limits the opportunity for direct interaction of its patients, thereby lessening the opportunity for direct transmission of organisms between patients. Thus, acquisition of an HAI is more likely to occur via direct transmission from a veterinary professional or indirect transmission via veterinary professionals or environmental surfaces, equipment and supplies. Additionally, VTH patients could perpetuate

transmission without involvement of healthcare providers, as MDR *E. coli* from dogs has been shown to directly contaminate environmental surfaces, which could serve as a transmission vehicle to healthcare providers and other patients (Warren et al, 2001; Trott et al, 2004).

Experiences learned from human hospitals can and should be applied to VTHs. Boyce et al (1997) reported that the gloves of 42% of nurses who performed activities in the rooms of MRSA-infected patients, but did not actually touch the patients, were contaminated with MRSA. Additionally, vancomycin-resistant *Enterococcus* spp. was transferred to 10.6% of previously disinfected sites after being touched by a nurse during routine tasks (Duckro et al, 2005). These studies exemplify how the environment combined with interaction of healthcare providers could sustain nosocomial transmission of drug-resistant bacteria.

Impact of veterinary personnel on transmission. Whether human or animal medicine, the types of on-the-job exposures that medical personnel have with regard to potentiating resistance are quite different from non-medical personnel. Studies on the carriage of MRSA among veterinary staff, which is an important pathogen in both human and animal populations, have shown that staff with direct contact with animals have a higher chance of carrying MRSA (Moodley et al, 2008); and also that this carriage has been associated with an increase of HAIs in small animal hospitals (Walther et al, 2009). Since MRSA is common to both human and animal populations, one cannot directly conclude that veterinary personnel are the source of these HAIs, however, adherence to infection control is the responsibility of medical staff and any lapse in this effort could cause direct or indirect transmission to patients and other staff.

The issue of infection control is also of concern with the evidence that bacteria not common to humans, such as *S. pseudintermedius*, have been shown to be prevalent in veterinary staff in constant contact with dogs (Guardabassi et al, 2004; Paul et al, 2011). MRSP has been

previously reported in dogs (Perreten et al, 2010, Hanselmen et al, 2008). However, a recent study provides evidence of MRSP being transmitted between animals and humans (Vincze et al, 2010). MRSP infection in veterinary medicine cripples the effectiveness of available treatments, but the presence of MRSP in either both human and veterinary medicine provides an opportunity for transmission of resistance to other pathogens.

While the mere presence of veterinary personnel at a VTH put them at an increased risk for colonization with organisms such as MRSA or MRSP, adherence to infection control practices, or lack there of, can affect one's risk. Without a standard for infection control practices in veterinary medicine (Benedict et al, 2008), there is no gold-standard with which to measure effectiveness of veterinary personnel's actions. Aksoy et al, 2010 assessed VTH cleanliness using standards set for human hospitals and found the VTH to be in line with these standards. However, Murphy et al (2010b) performed an infection control survey on Canadian veterinary clinics, finding that while most reported enhanced infection control measures for animal patients with known infections or obvious symptoms, there was a significant lack of infection control measures in handling other, less obvious infectious patients (e.g. rabies).

SUMMARY

There has been much evidence offered to support the idea that companion animals play an important role in the transmission of antimicrobial resistant bacteria. However, there still lacks concrete proof of directionality, and the notion that inanimate objects play a role in transmission must also be further investigated. Evidence is being brought to light to support the idea of VTHs playing a part with antimicrobial resistance (Boerlin et al, 2001; Burgess et al, 2004; Loeffler et al, 2005; Sidhu et al, 2007; Weese et al, 2004). Not only do VTHs house and use antimicrobials, but the nature of their services encourage close interaction between humans

and animals. This sets the stage for the development and transmission of antimicrobial resistance.

Much evidence has been presented to indicate that patients, environmental surfaces, and healthcare providers of a VTH all have a role to play in transmission of HAIs in the veterinary setting. While infection control within human medicine is widely practiced, definitive standards for infection control in VTHs are lacking. A study of AVMA-accredited VTHs showed that although infection control is a stated priority, formalized training and education are lacking and staff have reported instances of gravitating towards procedures for their convenience and not necessarily for their effect on infection control (Benedict et al, 2008). Additionally, Bartley et al. (2008) states that although environmental surfaces are important vectors in transmission of pathogens, healthcare worker hygiene should be the focus, over environmental surface disinfection.

HAIs are detrimental to all, whether human or animal medicine. And while animals definitely play an important role in the spread of HAIs, even in a VTH, they are still components of a human world. A qualitative risk assessment of acquisition of MRSA in a VTH found that staff pose the greatest risk, followed by environmental surfaces (Heller et al, 2010). While there are a variety of avenues in which veterinary personnel can affect transmission of organisms within a VTH. Direct and indirect contact are the easiest to monitor, but use of biological sampling and molecular techniques, however, adherence to infection control practices, or a breakdown of these, also impact the likelihood of transmission.

CONCLUSION

Previous studies in this area have either assessed a single part of this transmission, such as the environmental surfaces only. Additionally, while more recent studies are being reported

that have incorporated multiple sources (animals, environment and/or humans), these lack either longitudinal data collection, power and sample size, or molecular techniques with which identical strains could be identified.

Regardless of the origin, human interaction with patients and environmental surfaces are the most important vehicles of HAI transmission. This is due to the limited ability for direct interaction of VTH patents as well as infection control measures expected to take place among veterinary personnel. More longitudinal studies involving all aspects of transmission are needed in order to come to conclusions regarding risk of acquiring HAIs in veterinary medicine and most likely direction of transmission. Therefore, the studies presented in this dissertation were longitudinal in nature and were designed to provide further insight into (i) the prevalence and level of antimicrobial resistance observed from environmental surfaces throughout the VTH, (ii) the impact that individual animal patient characteristics and experiences while admitted to the VTH have on the likelihood of acquiring a healthcare associated infection, (iii) the effect that a VTH healthcare provider's interaction with patients and the environment of the VTH have on bacterial colonization of that healthcare provider, and (iv) molecular relatedness of bacteria isolated from animal patients, healthcare providers and environmental surfaces of a VTH.

CHAPTER 2

Prevalence and Antimicrobial Resistance of *Enterococcus* spp and *Staphylococcus* spp isolated from repeated sampling of surfaces within a Veterinary Teaching Hospital 2007 – 2009

STRUCTURED ABSTRACT

Objective – Determine the prevalence and antimicrobial resistance of enterococci and staphylococci from environmental surfaces of a VTH from 2007 to 2009.

Design – Longitudinal

Sample Population –Surfaces from the ECC, ST/IM, Ortho wards, surgery prep, office and operating rooms at a VTH, including cages, exam tables, floors, keyboards, telephones and scales.

Procedures – Surfaces within the VTH were swabbed every three months between 2007 and 2009. Resulting isolates of enterococci and staphylococci were tested for antimicrobial susceptibility using microbroth dilution. A subset of isolates was analyzed using PFGE.

Results – From 430 samples, 88 enterococci and 110 staphylococci were obtained. Isolation of enterococci and staphylococci were significantly associated with samples from the cage. Almost one-third of enterococci showed pentaresistance, however, pentaresistance was less common among staphylococci, with the exception of coagulase-negative staphylococci (CoNS). Over the course of this study, repeated sampling from the scale showed progressively more resistant *E. faecium* and CoNS. Identical PFGE clones were isolated from samples collected from different surfaces on the same day.

Conclusion and Clinical Relevance – Overall, the level of resistance increased during the course of this study. While not surprising, the high levels of resistance observed among *E. faecium* is of concern due to the organism's ability to serve as a source of resistance for other pathogens. This study provides data that can be used to create evidence-based infection control practices within VTHs, and identify critical control steps that could be used to control the spread of resistant pathogens.

INTRODUCTION

Companion animals play an important role in the occurrence of antimicrobial resistant bacteria in human and veterinary medicine (Boerlin et al, 2001; Burgess et al, 2004; Hanselman et al, 2008; Weese et al, 2004; Weese et al, 2008). Bacteria typically found in dogs, such as *Staphylococcus pseudintermedius*, have the ability to carry and transfer resistance to a host of other pathogenic bacteria (Frank et al, 2009), and the hospital environment can act as a reservoir for these resistance elements and/or pathogens (Burgess et al, 2004; Weese et al, 2004; Sidhu et al, 2007).

To date, few studies have explored the diversity of bacteria present on environmental surfaces within a VTH (Sidhu et al, 2007; Aksoy et al, 2010; Fraser et al, 2009; Loeffler et al, 2005). Certain objects in the clinical setting are ideal to serve as vehicles in transmission of organisms between humans and animals. There have been previous reports of isolation of pathogenic and non-pathogenic organisms from objects within VTHs, such as treatment tables and cages (Sidhu et al, 2007), door handles (Aksoy et al, 2010; Loeffler et al, 2005) and floors (Boerlin et al, 2001).

The role of the veterinary hospital environment in transmission of pathogens should be at the forefront of studies in healthcare associated infections. As with any hospital or clinical setting, the emergence of healthcare associated infections with resistant bacteria is of concern because patients are already at increased risk for infection and may return home harboring resistant bacteria. Although infection control practices within VTHs have not been widely studied, a recent article detailed application of human-hospital standards for infection control within a VTH[8]. Studies such as these are vital considering that interactions between health care providers and patients within VTHs are quite different from human hospitals.

With increasing frequency, evidence is being presented to support the idea of veterinary hospitals playing a part in the transmission of antimicrobial resistant organisms (Boerlin et al, 2001; Burgess et al, 2004; Weese et al, 2006; Sidhu et al, 2007; Loeffler et al, 2005). Not only do veterinary hospitals supply and use antimicrobials, but there are ample opportunities for close interaction between humans and animals. This provides opportunities for the development of antimicrobial resistance and transmission of resistant organisms. The objective of this study was to determine the prevalence and antimicrobial resistance of *Enterococcus* spp and *Staphylococcus* spp within a VTH from 2007 to 2009.

MATERIALS AND METHODS

Study Design: A repeated cross-sectional study of selected areas in the Michigan State University VTH was conducted from February 2007 through December 2009 resulting in 13 rounds of sample collection. Samples were collected at the beginning of every fourth clinical rotation, which was approximately every three months, from the same sites and locations throughout the VTH.

Sample Collection: Five areas were chosen for inclusion in this study. These included the ECC, ST/IM and Ortho wards, surgery prep and recovery rooms, and surgery office and operating rooms. Within each area chosen for inclusion in this study, the following sites were identified for sample collection: animal cages and runs (including the door and floor), door knobs, exam tables, floor area (area of the floor where animals laid while being cared for), floor drains, hose ends/connectors, computer keyboards, telephones, leashes, scales, sinks and sink drains, suction canisters, suction and tank control knobs, IV poles, storage cabinet handles, light switches and water blankets. All sites were not present in each area of the VTH; for example, cages were present in the ECC, ST/IM, Ortho and surgery recovery areas (one cage was sampled per area), however keyboards were only present in the Ortho and surgery office areas.

Samples were collected between 2 and 3 pm. This time of day was chosen because by the afternoon, patients would have been examined and/or treated in the areas selected for sampling. General site-specific cleaning occurs as patients are seen, however more thorough cleaning by janitorial staff occurs after 4pm. Samples were collected with a sterile swab and transport tube containing Stuart's transport medium (Becton, Dickinson and Company). Each sample was collected by running a moistened swab over the surface area of each site while simultaneously twirling the swab tip. For example, when sampling a keyboard, the swab was run/twirled over all keys of the keyboard. When sampling a cage, the swab was run/twirled over the latching mechanism, the portion of the door that was adjacent to the floor, the opening of the cage and the front edge of the bottom surface of the cage. Collected samples were then taken to the Center for Comparative Epidemiology-Microbial Epidemiology Laboratory to be processed.

Laboratory Isolation and Identification: Swabs were streaked onto Columbia CNA plates and were incubated for 48 hours at 37°C and inspected for typical morphology. Up to five isolates

demonstrating typical *Enterococcus* spp and *Staphyloccocus* spp morphology were chosen for identification.

(i) Identification of enterococci was completed using API 20 Strep identification strips, as directed by the manufacturer (bioMérieux, Inc.) and specific speciation was performed using the API 20 Strep Analytical Profile Index.

(ii) Identification of staphylococci was completed using a collection of biochemical tests, including inoculation of P agar, Voges-Proskauer (VP), trehalose, maltose and urea medium. A coagulase test was also performed. Positive P agar (growth), positive coagulase, positive VP (color change), positive trehalose (color change), positive maltose (color change), and positive urea (color change) were used to identify *S. aureus*. Negative P agar (no growth), mixed coagulase, negative VP (no color change), positive trehalose (color change), positive or negative maltose (color change or no color change), and positive urea (color change) were used to identify *S. intermedius*. During this study, evidence was published concerning misclassification of *S. intermedius* (Sasaki et al, 2007) thus for the remainder of this paper, we will use *S. intermedius* Group, made up of *S. intermedius, S. pseudintermedius, S. delphini, S. schleiferi* subsp. *coagulans, S. hyicus* or *S. lutae* and abbreviated "SIG", in place of *S. intermedius*. Any isolates not typical for *S. aureus* or SIG were then tested further via API Staph Identification strips as directed by the manufacturer (bioMérieux, Inc.) and specific speciation was performed using the API Staph Analytical Profile Index. After speciation, these isolates were further grouped into SIG or as coagulase-negative staphylococci (CoNS) for purposes of analysis.

Each positively identified isolate of *Enterococcus* spp or *Staphylococcus* spp was suspended in TSB, 0.5 ml of the suspension was added to 0.5 ml 65% glycerol solution, and the

mixture was frozen at -70°C. Additionally, the five isolates were stabbed onto TSA and stored at room temperature, until antimicrobial susceptibility testing was performed.

Antimicrobial susceptibility testing. The Sensitire ® microdilution system (Trek Diagnostics, Inc.) was used to perform antimicrobial susceptibility testing on a commercially prepared plate (GPN3F, Trek). Antimicrobials included ampicillin, ceftriaxone, ciprofloxacin, clindamycin, daptomycin, erythromycin, gatifloxacin, gentamicin and high-level gentamicin, levofloxacin, linezolid, oxacillin, penicillin, quinupristin/dalfopristin, rifampin, streptomycin, tetracycline, trimethoprim/sulfamethoxazole and vancomycin. These antimicrobials were chosen in order to ensure inclusion of antimicrobials used in both human and animal medicine. *E. faecalis* (ATCC 29212), *S. aureus* (ATCC 29213) and *E. coli* (ATCC 25922) were used as the quality control organisms. Quality control results were reviewed for each batch of tests, all of which were within acceptable limits. We did not address inducible clindamycin resistance.

The MIC value at which no growth occurred was measured using the Trek AutoReader, which utilizes fluorescence technology, and an antimicrobial susceptibility/resistance profile was generated. Susceptibility, intermediate susceptibility and resistance (SIR) were determined by applying breakpoints as published by the Clinical and Laboratory Standards Institute (Performance Standards for Antimicrobial Susceptibility Testing: Informational Supplement M100, CLSI). Enterococcal resistance to gentamicin, ceftriaxone, clindamycin, trimethoprim-sulfamethoxazole and oxacillin were not interpreted, and high-level aminoglycoside resistance was not interpreted for staphylococci. Pentaresistance was defined as an isolate being resistant to five or more antimicrobials.

Pulsed-field gel electrophoresis. In order to determine the relatedness between different isolates of the same species, PFGE was performed. As a pilot, nine isolates were chosen (seven *E. faecium* and two *E. faecalis*) and PFGE was performed based on Michigan State University, Diagnostic Center for Population and Animal Health's Standard Operating Procedures # SPECIAL.001.02. Restriction enzyme, SmaI, was used and electrophoresis was performed using a CHEF unit (model CHEF-DRIII), achieved by ramping the switch times from 4 seconds to 35 seconds. The overall run time was 20 hours. PFGE clone groupings were determined according to the standard of Tenover et al (1995).

Statistical Analysis. Because up to 5 typical colonies of each *Enterococcus* spp and *Staphylococcus* spp were chosen from each sample, we wanted to ensure that we were not unnecessarily over-counting the organisms isolated from each site. In order to accomplish this, the susceptibility pattern produced by applying CLSI breakpoints to all antimicrobials tested were compared for each group of species isolated at each site for each sampling event. Any species with identical susceptibility patterns, sample collection dates and sample sites were restricted, and one isolate was randomly chosen for inclusion in the analysis.

Prevalence: Proportion of organisms recovered by site and area were calculated using SAS 9.1.3 (SAS Institute). A Fisher's exact test (two-tailed) was performed to compare the proportion of organisms recovered from each site and area to all other sites and areas, for each organism. Univariate analyses using chi-square were performed for the two independent variables site and area. These categorical variables were transformed into dummy variables. For site, the dummy variables were cage, exam table, floor area, floor drain, keyboard, phone, scale and all other sites, with the door knob being referent. For area, the dummy variables were ECC, ST/IM, and Ortho wards and surgery office and operating rooms, with the surgery prep and recovery rooms

being referent. Both variables (site and area) had a p value < 0.2 and were included in two multivariate logistic regression models using isolation of enterococci or staphylococci as the outcome variables. Odds ratios and 95% confident intervals (CI) were reported for each and significance was determined by a 95% CI that did not cross the null of 1.0.

Antimicrobial Resistance: Proportion of species isolated, along with proportion of antimicrobial resistance and pentaresistance were calculated. The MIC_{50} and MIC_{90} were obtained by reporting the MIC at which 50% and 90% of isolates scored and were reported for selected sites and antimicrobials. Finally, the MIC_{50} for isolates recovered from scale during repeated sampling events were also presented.

RESULTS

Prevalence. A total of 430 samples from surfaces throughout the VTH were collected. Of these samples, *Enterococcus* spp was isolated from 41 sites and *Staphylococcus* spp was isolated from 68 sites (Table 1). Bacteria were isolated from all areas sampled throughout the VTH; however, not all sites within those areas were contaminated. Enterococci and staphylococci were not isolated from the sink/sink drains.

The ST/IM ward had the highest proportion of both *Enterococcus* spp and *Staphylococcus* spp isolated (17.7% and 31.6%, respectively), and the Surgery OR/office had the lowest proportions. These two areas were the only ones that were significantly associated with the isolation of *Enterococcus* spp and *Staphylococcus* spp (Table 1).

Table 1: Numbers and percentages of samples taken and occurrences of isolation of *Enterococcus* spp. and *Staphylococcus* spp. from selected sites throughout the VTH, 2007 - 2009

Category	Total Samples[a]		*Enterococcus* spp.			*Staphylococcus* spp.		
	n	*% of total*	*n*	*% of site total*	*p*[b]	*n*	*% of site total*	*p*[b]
Site								
Cage	66	15.3	13	19.7	**0.002**	16	24.2	**0.041**
Floor Area	13	3.0	4	30.8	**0.027**	6	46.2	**0.002**
Door Knob	39	9.1	3	7.7	1.0	2	5.1	0.064
Exam Table	15	3.5	0	--	--	6	40.0	**0.009**
Floor Drain	26	6.0	3	11.5	0.727	1	3.8	0.099
Keyboard	26	6.0	5	19.2	0.082	9	34.6	**0.007**
Leash	13	3.0	1	7.7	1.0	3	23.1	0.442
Phone	65	15.1	6	9.2	0.928	12	18.5	0.525
Scale	13	3.0	5	38.5	**<0.001**	9	69.2	**<0.001**
Sink/Sink Drain	52	12.1	0	--	--	0	--	--
Surgery Prep Items[c]	41	9.5	0	--	--	1	2.4	**0.0114**
Surgery OR Items[c]	61	5.6	1	1.6	**0.018**	3	4.9	**0.0124**
Area								
Soft Tissue/Internal Medicine Ward	79	18.4	14	17.7	**0.006**	25	31.6	**<0.001**
ECC Ward	65	15.1	10	15.4	0.081	10	15.4	0.918
Orthopedic Ward	91	21.2	12	13.2	0.182	15	16.5	0.844
Surgery Prep/Recovery	73	17.0	3	4.1	0.123	6	8.2	0.051
Surgery OR/Office	122	28.4	2	0.5	**<0.001**	12	2.8	**0.033**
Season								
Winter	133	30.9	11	8.3		27	20.3	
Spring	99	23.0	10	10.1		10	10.1	
Summer	99	23.0	14	14.1		15	15.2	
Fall	99	23.0	6	6.1		16	16.2	
TOTAL	**430**		**41**	**9.5**		**68**	**15.8**	

Table 1 (cont'd)

[a] Represents specimens from 13 different sampling events.

[b] Use of Chi Square and Fisher's Exact (for cells less than 5) analysis to assess association between isolation of *Enterococcus* spp. or *Staphylococcus* spp. from each site and area.

[c] Surgery prep items include sterile capnograph connectors, hose ends and IV poles; Surgery OR Items include wall light switch, cabinet handles, water blanket and control knobs within a surgical suite.

The sites with the highest proportion of *Enterococcus* spp were the floor area and the scale, and the sites with the highest proportion of *Staphylococcus* spp were the floor area, exam table and scale. Univariate analysis (Table 2) of the variable site (door handle was referent) revealed that isolation of *Enterococcus* spp was significantly associated with samples obtained from the cage, floor area, and the scale. Isolation of staphylococci, however, was significantly associated with all sites, except for the phone and 'other' sites. Notably, the likelihood of isolating either organism from the scale was quite high (enterococci OR = 10.625 [2.116, 53.356] and staphylococci OR = 41.625 [6.564, 263.957]). Univariate analysis of area (surgery prep/recovery was referent) revealed that isolation of enterococci was significantly associated with the ST/IM and ECC wards, however isolation of staphylococci was only significant for the ST/IM ward.

Table 2: Univariate Odds Ratios and 95% Confidence Intervals for recovery of *Enterococcus* spp. or *Staphylococcus* spp. from select sites throughout the VTH, 2007 - 2009

Site	*Enterococcus* spp.				*Staphylococcus* spp.			
	OR	*95% CI*		*p*	*OR*	*95% CI*		*p*
Site								
Door Handle		-ref-		<0.001		-ref-		<0.001
Cage	**4.17**	1.12	15.50		**5.92**	1.28	27.35	
Exam Table	--	--	--		**12.33**	2.13	71.57	
Floor Area	**7.56**	1.44	39.59		**15.86**	2.64	95.23	
Floor Drain	2.22	0.42	11.83		0.74	0.06	8.61	
Keyboard	4.05	0.89	18.49		**9.79**	1.91	50.30	
Phone	1.73	0.41	7.27		4.19	0.89	19.83	
Scale	**10.625**	2.116	53.356		**41.625**	6.564	263.957	
Other	0.206	0.034	1.267		0.809	0.162	4.056	
Area								
Surgery Prep/Recovery		-ref-		<0.001		-ref-		<0.001
Surgery OR/Office	0.389	0.063	2.384		1.218	0.437	3.398	
Soft Tissue/Internal Medicine Ward	**5.026**	1.381	18.291		**5.17**	1.979	13.507	
ECC Ward	**4.242**	1.113	16.164		2.03	0.694	5.937	
Orthopedic Ward	3.544	0.961	13.076		2.204	0.809	6.003	

When the model controlled for site, none of the areas were significantly associated with isolation of either organism (Table 3). Significant associations observed between staphylococci and site were retained and became more precise. The association between isolating enterococci from the cage and scale were retained and were more precise as well; however the floor area was no longer significantly associated and was replaced by a significant association with the keyboard.

Table 3: Multivariate logistic regression model of risk factors associated with recovery of *Enterococcus* spp. or *Staphylococcus* spp. from select sites throughout the VTH, 2007 - 2009

Site	*Enterococcus* spp.			*Staphylococcus* spp.		
	OR	*95% CI*		*OR*	*95% CI*	
Site						
Door Handle		-ref-			-ref-	
Cage	**3.89**	1.03	14.62	**5.30**	1.09	25.78
Exam Table	--	--	--	**9.24**	1.35	63.11
Floor Area	5.84	0.96	35.43	**26.70**	3.54	201.61
Floor Drain	2.09	0.34	12.86	0.48	0.04	5.82
Keyboard	**6.90**	1.23	38.70	**7.07**	1.28	39.13
Phone	2.02	0.48	8.60	3.80	0.78	18.64
Scale	**8.328**	1.463	47.392	**30.798**	4.024	235.698
Other	0.395	0.061	2.558	0.715	0.13	3.919
Area						
Surgery Prep/Recovery		-ref-			-ref-	
Surgery OR/Office	0.419	0.056	3.112	1.451	0.453	4.643
Soft Tissue/Internal Medicine Ward	2.335	0.579	9.415	1.591	0.521	4.857
ECC Ward	2.368	0.543	10.328	0.699	0.18	2.714
Orthopedic Ward	1.931	0.444	8.388	1.827	0.608	5.494

Antimicrobial Resistance. Resistance observed for *Enterococcus* spp was highly prevalent and was most commonly observed to rifampin (46%) and quinupristin-dalfopristin (50%) (Table 4). Nearly all isolates of *Enterococcus* spp (95.4%) showed resistance to at least one antimicrobial and 18.1% showed pentaresistance (Table 5).

Staphylococci most frequently showed resistance to Beta-lactam antimicrobials (Table 4). Resistance in staphylococci was uncommon for other classes of antimicrobials, with the exception of gatifloxacin, a quinolone (66%). Pentaresistance was observed in 22.9% of isolates, which was largely driven by resistance seen among CoNS (Table 5).

Table 4: List of antimicrobials assessed for *Enterococcus spp.* and *Staphylococcus spp.* isolates and results of antimicrobial susceptibility testing

Class	Antimicrobial	*Enterococcus spp.* (N = 90)			*Staphylococcus spp.* (N = 110)		
		S	I	R (%)	S	I	R (%)
Aminoglycocide	Gentamicin	€	€	€	99	7	4 (4)
	Gentamicin 500[a]	76	--	14 (16)	€	€	€
	Streptomycin 1000[a]	85	--	5 (6)	€	€	€
Cephalosporin	Ceftriaxone	€	€	€	94	12	4 (4)
Fluoroquinolone	Ciprofoxacin	51	20	19 (21)	95	4	11 (10)
	Gatifloxacin	76	3	11 (12)	--	97	73 **(66)**
	Levoflaxacin	76	1	13 (14)	96	1	--
Glycopeptide	Vancomycin	87	3	--	110	--	--
Lincosamide	Clindamycin	€	€	€	88	8	14 (13)
Lipopeptide	Daptomycin	90	--	--	110	--	--
Macrolide	Erythromycin	36	32	22 (24)	66	4	40 (36)
Oxazolidinone	Linezolid	90	--	--	110	--	--
Penicillin	Ampicillin	59	--	31 (34)	83	--	27 (25)
	Oxacillin	€	€	€	74	--	36 (33)
	Penicillin	58	--	32 (36)	64	--	46 (42)
Rifampin	Rifampin	37	12	41 **(46)**	109	1	--
Streptogramin	Quinupristin-dalfopristin	20	25	45 **(50)**	109	1	--
Sulfonamide	Trimethoprim-sulfamethoxazole	€	€	€	107	--	3 (3)
Tetracycline	Tetracycline	50	6	34 (38)	93	1	16 (15)

[a]S: Synergy and R: No Synergy

€MIC were not interpreted

Table 5: Numbers and percentages of species of *Enterococcus spp. and Staphylococcus spp.* isolates and their antimicrobial susceptibility

Species		Number of Isolates		Numer of Isolates Resistant to at least one antimicrobial		Isolates Resistant to 5 or more antibiotics	
		n	%	*n*	%	*n*	%
***Enterococcus* spp. (total)**		***87***		***83***	***95.4***	***15***	***18.1***
E. faecium	49	49	56.3	47	95.9	14	29.8
E. faecalis	35	35	40.2	34	97.1	1	2.9
E. durans	3	3	3.4	2	66.7	0	--
***Staphylococcus* spp. (total)**		***110***		***70***	***63.6***	***16***	***22.9***
S. aureus	8	8	7.3	2	25.0	1	50.0
SIG[a]	37	37	33.6	19	51.4	0	--
CoNS[b]	65	65	59.1	49	75.4	15	30.6

[a]SIG: *Staphylococcus intermedius* Group (*S. pseudintermedius, S. intermedius, S. delphini*) and could also include other coagulase-positive species: *S. schleiferi* subsp. *coagulans, S. hyicus, S. lutae.*

[b]CoNS: Coagulase negative species: *S. epidermidis, S. haemolyticus, S. hominis, S. warneri, S. chromogenes, S. xylosus, S. saprophyticus, S. caprae, S. cohnii* subsp. *ureolyticus, S. sciuri, S. lugdunensis*

Table 6 details the occurrence of samples in which *E. faecium, E. faecalis, S. aureus,* SIG and CoNS were isolated, and the proportion of those samples in which a pentaresistant organism was identified. The occurrence of pentaresistance among enterococci was most common in *E. faecium* (56%). A pentaresistant isolate was recovered from every sample in which *E. faecium* was isolated, with the exception of the door knob. Overall, samples collected from the cage had the highest proportions of pentaresistance, however, pentaresistant CoNS isolated from the phone (43%) was more prevalent than pentaresistance in CoNS isolated from the cage (25%). Very few samples contained pentaresistant isolates of *E. faecalis, S. aureus* or SIG.

Table 6: Occurrences of isolation of resistant *E. faecium, E. faecalis, S. aureus,* SIG and CoNS from selected sites throughout the VTH, 2007 - 2009

Category	*E. faecium*			*E. faecalis*			*S. aureus*			SIG[a]			CoNS[b]		
	n	(*res*[5])		*n*	(*res*[5])		*n*	(*res*[5])		*n*	(*res*[5])		*n*	(*res*[5])	
Site															
Cage	7	4	57%	5	0	--	3	1	33%	7	0	--	8	2	**25%**
Floor Area	3	3	100%	1	1	100%	1	0	--	5	0	--	3	0	--
Door Knob	2	0	--	1	0	--	0	--		1	0	--	1	0	--
Exam Table	0	--		0	--		1	0	--	3	0	--	2	1	50%
Floor Drain	3	2	67%	1	0	--	0	--		0	--		1	1	100%
Keyboard	5	2	40%	0	--		1	0	--	1	0	--	7	1	14%
Leash	1	1	100%	0	--		0	--		2	0	--	1	0	--
Phone	2	1	50%	3	0	--	1	0	--	0	--		14	6	**43%**
Scale	4	2	50%	3	1	33%	0	--		7	1	14%	5	0	--
Other Sites[c]	0	--		1	0	--	0	--		1	0	--	3	0	--
Area															
Soft Tissue/Internal Medicine Ward	7	3	43%	7	1	14%	2	0	--	15	1	7%	11	2	18%
ECC Ward	8	6	75%	2	1	50%	2	1	50%	6	0	--	6	1	17%
Orthopedic Ward	11	6	55%	3	0	--	2	0	--	4	0	--	10	1	10%
Surgery Prep/Recovery	0	--		2	0	--	0	--		1	0	--	5	2	40%
Surgery OR/Office	1	0	--	1	0	--	1	0	--	1	0	--	13	5	38%
TOTAL	**27**	**15**	**56%**	**15**	**2**	**13%**	**7**	**1**	**14%**	**27**	**1**	**4%**	**45**	**11**	**24%**

Footnotes for Table 6 continued on next page.

Table 6 (cont'd)

(res^5) = number and percent of samples in which the named isolate was resistant to 5 or more antibiotics.

[a]SIG: *Staphylococcus intermedius* Group (*S. pseudintermedius, S. intermedius, S. delphini*) and could also include other coagulase-positive species: *S. schleiferi subsp. coagulans, S. hyicus, S. lutae*.

[b]CoNS: Coagulase negative species: *S. epidermidis, S. haemolyticus, S. hominis, S. warneri, S. chromogenes, S. xylosus, S. saprophyticus, S. caprae, S. cohnii subsp. ureolyticus, S. sciuri, S. lugdunensis*.

[c]Other Sites includes sink/sink drains, sterile capnograph connectors, hose ends, IV poles, wall light switch, cabinet handles, water blanket and control knobs within a surgical suite.

The levels of resistance to certain antimicrobials were detailed for organisms isolated from a selection of sites throughout the VTH (Table 6). Overall, the MIC_{90} for *E. faecium* was the highest dilution measured. Organisms isolated from the cage, floor area and floor drain had MIC_{50} values at the highest dilution measured for tetracycline and ampicillin (Table 7a). Isolates of *E. faecalis* were overall less resistant than *E. faecium*, and resistance to ampicillin was not observed at all for this species.

Resistance to SIG and CoNS are detailed in Table 7b. Resistance among isolates of SIG was not frequent, regardless of sample site and antimicrobial. The MIC_{90} of oxacillin in SIG isolated from the scale was in the resistant range, however the MIC_{50} for this site and antimicrobial was still in the sensitive range (MIC_{50} = 2 μg/ml). Additionally, all isolates of SIG showed intermediate resistance to gatifloxacin, regardless of sample site. Resistance observed among CoNS had no apparent pattern, however those sites with the most isolates (cage, exam table and phone) also had the highest levels of resistance to gatifloxacin, erythromycin and oxacillin (Table 7b). Additionally, regardless of sample site, isolates of CoNS showed high

levels of resistance to erythromycin (MIC_{50} and MIC_{90} of >4 µg/ml). Resistance observed in isolates of CoNS sampled from the scale did not show high levels of resistance, this is contrary to what was observed in all other species of enterococci and staphylococci isolated from the scale. Although MRSA was recovered, there was not enough resistance of *S. aureus* to warrant including in our data table.

Table 7a: Minimum inhibitory concentrations of tetracycline and ampicillin for *E. faecalis* and *E. faecium* isolated from selected sites at the VTH

Organism	Selected Sites	Number of Isolates	MIC (ug/ml)					
			Tetracycline			Ampicillin		
			Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}
E. faecium								
	Scale	8	2 to ≥16	9	≥16	0.25 to >16	8.5	>16
	Cage	19	≤2 to ≥16	≥16	≥16	0.5 to >16	>16	>16
	Floor Area	5	<2 to ≥16	≥16	≥16	0.5 to >16	>16	>16
	Keyboard	6	2 to >16	2	>16	1 to >16	1	>16
	Floor Drain	4	8 to >16	>16	>16	1 to >16	>16	>16
	Phone	2	≤2 to >16	9	>16	2 to >16	9	>16
E. faecalis [a]								
	Scale	5	2 to >16	2	>16	1*	1	1
	Cage	17	≤2 to ≥16	2	≥16	0.5 to 1	1	1
	Floor Area	1	>16*	>16	>16	1*	1	1
	Floor Drain	4	2*	2	2	1*	1	1
	Phone	6	2 to >16	2	>16	1*	1	1

[a] *E. faecalis* was not isolated from the keyboard

*All values were the same, there was no range

Table 7b: Minimum inhibitory concentrations of gatifloxacin, erythromycin and oxacillin for SIG and CoNS isolated from selected sites at the VTH

Organism	Selected Sites	Number of Isolates	MIC (ug/ml)								
			Gatifloxacin			Erythromycin			Oxacillin		
			Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}
SIG[a]											
	Scale	15	≤1*	≤1	≤1	≤0.25 to 4	<0.25	0.25	≤0.25 to 8	2	8
	Cage	7	≤1*	≤1	≤1	≤0.25*	≤0.25	≤0.25	≤0.25*	≤0.25	≤0.25
	Floor Area	5	≤1*	≤1	≤1	≤0.25 to 4	<0.25	4	≤0.25 to 0.5	≤0.25	0.5
	Exam Table	3	≤1*	≤1	≤1	≤0.25 to 4	<0.25	4	≤0.25*	≤0.25	≤0.25
	Keyboard	1	<1*	<1	<1	<0.25*	<0.25	<0.25	<0.25*	<0.25	<0.25
CoNS[b]											
	Scale	5	≤1*	≤1	≤1	≤0.25 to 0.5	0.5	0.5	≤0.25*	≤0.25	≤0.25
	Cage	13	≤1 to 4	2	4	≤0.25 to ≥4	0.5	>4	≤0.25 to ≥8	≤0.25	≥8
	Floor Area	3	≤1*	≤1	≤1	0.25 to >4	>4	>4	<0.25 to 2	0.5	2
	Exam Table	2	<1 to 8	1.5	8	<0.25 to >4	2.125	>4	<0.25 to 2	1.125	2
	Keyboard	11	≤1 to 2	≤1	2	≤0.25 to >4	>4	>4	<0.25 to >8	4	>8
	Floor Drain	2	<1*	<1	<1	>4*	>4	>4	1*	1	1
	Phone	25	≤1 to >8	1	4	<0.25 to >4	>4	>4	<0.25 to ≥8	1	>8

*All values were the same, there was no range

[a]SIG: *Staphylococcus intermedius* Group (*S. pseudintermedius, S. intermedius, S. delphini*) and could also include other coagulase-positive species: *S. schleiferi* subsp. *coagulans, S. hyicus, S. lutae.*

[b]CoNS: Coagulase negative species: *S. epidermidis, S. haemolyticus, S. hominis, S. warneri, S. chromogenes, S. xylosus, S. saprophyticus, S. caprae, S. cohnii* subsp. *ureolyticus, S. sciuri, S. lugdunensis*

Changes in prevalence in resistance over time. The longitudinal nature of this study enabled comparisons of antimicrobial resistance over time. Table 8 shows the MIC_{50} from isolates recovered from repeated sampling of the scale. Because not all species were isolated at all sampling events, only *E. faecium, E. faecalis*, and SIG are displayed in this table. Between the first and second sampling events, resistance in *E. faecium* increased for all antimicrobials tested except vancomycin (remained sensitive). The change observed for *E. faecalis* was much different as only the MIC_{50} for tetracycline changed from sensitive to resistant, however erythromycin changed from sensitive to intermediate resistance. While the MIC_{50} for quinupristin-dalfopristin remained resistant over time, those for rifampin changed from resistant to sensitive. CoNS were isolated from the scale in 7 of the 13 sampling events (Table 8). Although the numbers were too low to perform statistical tests, the overall resistance levels appeared to remain stable, with a few sporadic peaks.

Epidemiological Relatedness. The PFGE patterns for isolates of *E. faecium* fell into six clones, while the patterns for *E. faecalis* isolates comprised a single clone (Figure 2). *E. faecium* clone A was made up of two isolates from different sites (scale and floor) and areas from the VTH. Clone B consisted of two isolates from the same sample site (cage), but these isolates differed in their antimicrobial susceptibility pattern. Clones C, D and E consisted of one isolate of *E. faecium* each. *E. faecalis* clone F consisted of two isolates from the same sample site (scale), but differed in their antimicrobial susceptibility pattern.

Table 8: MIC_{50} for E. faecium, E. faecalis and SIG isolates obtained on repeated sampling of the scale between 2007 and 2009

Class	Antimicrobial	*E. faecium*		*E. faecalis*	
		10/07	04/08	10/07	01/09
Aminoglycocide	Gentamicin	≤	≤	≤	≤
Cephalosporin	Ceftriaxone	≤	≤	≤	≤
Fluoroquinolone	Ciprofoxacin	1	2	1	1
	Gatifloxacin	1	2	1	<1
	Levoflaxacin	2	2	2	1
Glycopeptide	Vancomycin	1	2	1	2
Lincosamide	Clindamycin	≤	≤	≤	≤
Lipopeptide	Daptomycin	4	2	1	1
Macrolide	Erythrotmycin	1	**>4**	0.25	1
Oxazolidinone	Linezolid	2	1	1.5	1
Penicillin	Ampicillin	0.25	**>16**	1	1
	Oxacillin	≤	≤	≤	≤
	Penicillin	0.5	**>8**	4	4
Rifampin	Rifampin	4	0.5	**4**	2
Streptogramin	Quinupristin-dalfopristin	1	**4**	**4**	**>4**
Sulfonomide	Trimethoprim-sulfamethoxazole	≤	≤	≤	≤
Tetracycline	Tetracycline	2	**>16**	2	**>16**

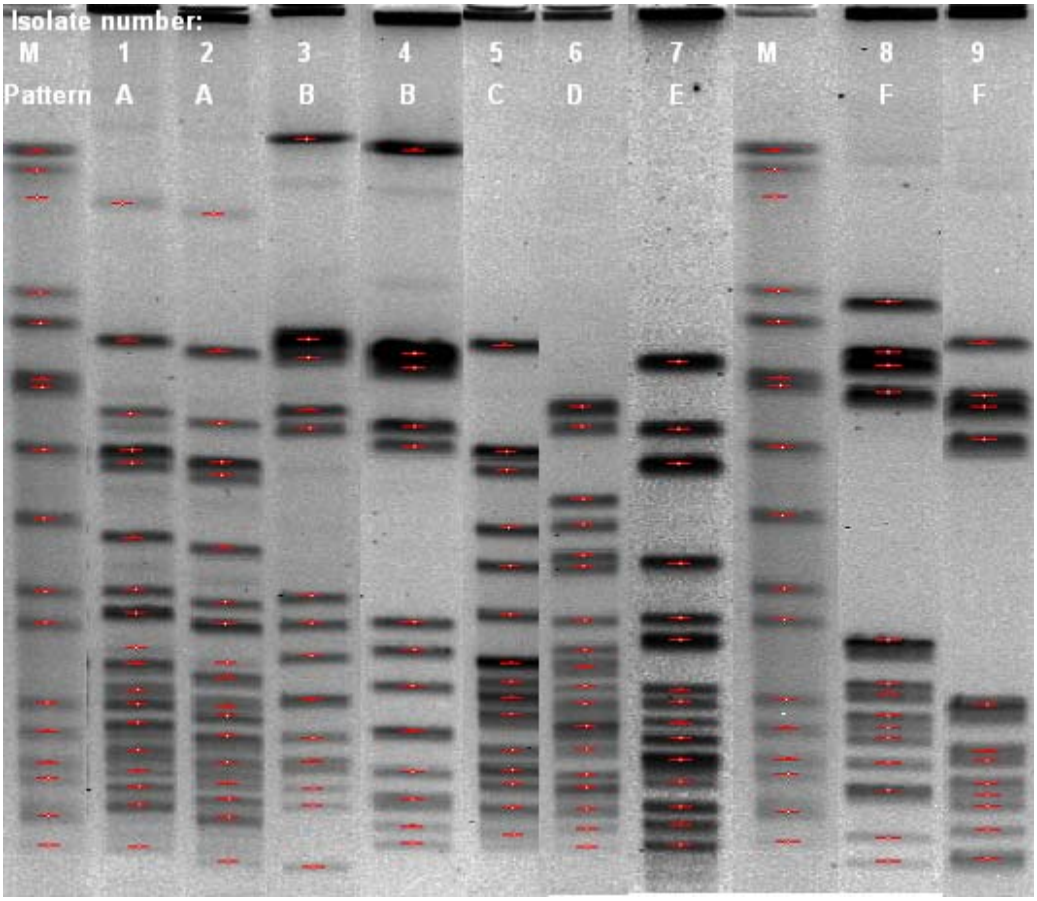
Table 8 (cont'd)

Class	Antimicrobial	SIG[a]						
		07/07	10/07	01/08	04/08	01/09	03/09	09/09
Aminoglycocide	Gentamicin	2	2	2	2	**16**	<2	<2
Cephalosporin	Ceftriaxone	8	8	8	8	<8	<8	<8
Fluoroquinolone	Ciprofoxacin	0.5	0.5	0.5	0.5	<0.5	<0.5	1
	Gatifloxacin	1	1	1	1	<1	<1	<1
	Levoflaxacin	0.25	0.25	0.25	0.25	<0.25	<0.25	0.5
Glycopeptide	Vancomycin	1	1	1	1	<1	<1	<1
Lincosamide	Clindamycin	<0.12	0.12	<0.12	<0.12	<0.12	<0.12	<0.12
Lipopeptide	Daptomycin	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25	<0.25
Macrolide	Erythrotmycin	0.25	1.5	0.25	0.25	<0.25	<0.25	<0.25
Oxazolidinone	Linezolid	1	1	1	1	1	1	1
Penicillin	Ampicillin	0.12	**4**	0.12	0.12	0.12	<0.12	<0.12
	Oxacillin	0.25	**4**	0.25	0.25	<0.25	<0.25	<0.25
	Penicillin	0.12	4	0.12	0.12	0.25	0.12	0.12
Rifampin	Rifampin	0.5	0.5	0.5	0.5	<0.5	<0.5	<0.5
Streptogramin	Quinupristin-dalfopristin	0.12	0.12	0.12	0.12	<0.12	<0.12	<0.12
Sulfonomide	Trimethoprim-sulfamethoxazole	0.5	0.5	0.5	0.5	<0.5	<0.5	<0.5
Tetracycline	Tetracycline	2	2	2	**16**	2	**>16**	2

[ς]MIC were not interpreted

[a]SIG: *Staphylococcus intermedius* Group (*S. pseudintermedius, S. intermedius, S. delphini*) and could also include other coagulase positive species: *S. schleiferi subsp. coagulans, S. hyicus, S. lutae*.

Figure 2: PFGE patterns for 7 isolates of *E. faecium* (# 1–7) and 2 isolates of *E. faecalis* (# 8–9).



The isolate numbers are listed at the top ('M' indicates the DNA marker pattern), and the pattern letters are listed at the bottom. For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this dissertation.

DISCUSSION

Bacteria are present in any hospital environment, however the presence of pathogenic and/or resistant bacteria can allow the cycle of transmission of resistant bacteria to persist. Various species of pathogenic and non-pathogenic enterococci and staphylococci were isolated throughout the MSU VTH, and the level of resistance measured in these isolates increased over the course of the study. Additionally, clinically important bacteria were identified, including MRSA.

Considering prevalence of either enterococci or staphylococci, the scale had the highest prevalence of bacteria and also had the highest likelihood of isolation. In this VTH, the scale is located within the ST/IM, which had the highest prevalence of isolation and was significantly associated with either bacterium during univariate analysis. While the cage did not have the highest prevalence of either bacterium, this site remained significantly associated with isolation of enterococci or staphylococci in multivariate logistic regression. The cages selected for this study were located in many areas, including ST/IM, ECC, Ortho, and Surgery Recovery. Univariate analysis revealed a significant likelihood of isolating enterococci from the ST/IM and ECC and staphylococci from the ST/IM; however these associations were not significant when site was included in the model. These results suggest that the cage, specifically, rather than the location of the cage within the VTH, has the largest impact on isolation of these organisms and should be a focus for targeted infection control.

It is interesting to note that the categorization of "area" within the VTH did not remain significant when site and area were analyzed in the same model, whereas many sites throughout the VTH did remain significant. This suggests that the association of the presence of enterococci or staphylococci are driven more by the specific site, rather than a general area within the VTH. This type of observation is important when considering infection control protocols, and would encourage the practice of uniform procedures for site-specific cleaning and disinfecting, regardless of what area of the VTH the site is located.

Staphylococci were found in 15.8 % of sites sampled and had significant likelihoods of isolation with most sites that were sampled. The most common species identified were CoNS, which also had more resistance and pentaresistance, compared with *S. aureus* and SIG. Previous studies which looked at staphylococci on environmental surfaces of a VTH found increased

prevalence in sites where healthcare worker hands were most likely to come in contact (Aksoy et al, 2010; Bartley et al, 2008). While this study showed increased likelihood of isolation of staphylococci from these types of sites (exam table and keyboard), the largest associations were with the floor area (OR = 26.70 [3.54, 201.61]) and the scale (OR = 30.798 [4.04, 235.70]). Healthcare provider hands are not as likely to come into direct contact with these two sites, as the floor area is where larger animals are bedded while in the ECC and the scale is used to weigh animals. While healthcare provider hand contact is not likely, there is ample opportunity for fecal (animal sitting or laying) and oral (animal lying with head down) contamination by animals. This finding illustrates inherent differences between human and veterinary hospitals. Infections control practices in veterinary hospitals must be tailored to the unique nature of the VTH environment.

Although *S. aureus* was not identified in high numbers, one isolate of MRSA was isolated from the cage. It was not documented if the dog housed in this cage was also positive for MRSA. Regardless, previous studies have shown the presence of MRSA in the housing unit of a VTH, independent of the inhabitant's MSRA status (Weese et al, 2004). Although the results presented by Weese et al. (2004) were based on large-animal housing facilities after an outbreak of MRSA, it is important to note that 6.9% of stalls housing MRSA-negative horses were positive for MRSA. Additionally, a study reported that MRSA of the same epidemic clone were found in dogs, staff and environmental surfaces of a VTH (Loeffler et al, 2005). These findings illustrates the ease with which pathogens can be transferred around a VTH, and how important the role that environmental surfaces can play.

This level of resistance observed in *E. faecium* should be of concern as it is an important pathogen in both human and animal medicine. While the likelihood of isolating enterococci

from sites throughout the VTH was much lower compared with staphylococci, the *E. faecium* isolated from those sites with significant associations had pentaresistance in over 50% of isolates. Additionally, the MIC_{90} and most of the MIC_{50} for *E. faecium* against tetracycline and ampicillin were the highest dilution tested against. PFGE analysis identified one clone of *E. faecium* which was isolated from samples that were collected from different sites on the same day; one from the scale and the other from the floor area. Upon entering the VTH, most animals are taken to the scale to record their weight before going on to their area of treatment. It is likely that an animal would have gotten weighed on the scale and then taken to be housed on a mat on the floor (as with many larger, more critical patients). In this VTH, the scale surface is a rubber material and underwent infrequent cleaning at the time these samples were collected.

Although no isolates of enterococci were resistant to vancomycin, three had intermediate resistance (VIE). Of note, two isolates of VI- *E. faecalis* were isolated from the cage and floor area. Duckro et al. (2005) reported that isolates of VRE were being transferred to 10.6% of previously disinfected sites after being touched by a nurse during routine tasks in a human hospital. Frequent healthcare provider contact is unlikely at the floor area, however, the cage is a site that has a high level of both animal and healthcare provider contact. Clearly, animals are housed in cages, where they lay, sit and put their head on the floor, and some may even lick or chew on the cage. Healthcare providers who open the cage without gloves would have the opportunity to deposit or pick up contaminants and continue the cycle of transmission.

Though many studies have reported isolation of pathogenic bacteria from either human or veterinary hospitals, the role of environmental surfaces in continuing the cycle of healthcare-associated transmission is unknown. Bartley et al (2008) stated that although the role of environmental surfaces as vectors in transmission of pathogens in both human and veterinary

clinics is gaining more attention, it is not the major contributing factor. Instead, healthcare worker hygiene should be the focus, over environmental surface disinfection. Considering sites sampled in our study where healthcare providers would interact with most, we identified increased likelihood of isolating either enterococci or staphylococci from the computer keyboard. Other studies reported isolation of various healthcare-associated pathogens from 24% to 31% of keyboards sampled (Fraser et al, 2009; Bures et al, 2000). Fraser et al (2009) specifically studied the bacteria present on computer keyboards within a VTH, in comparison to the cleaning schedules reported by the facilities being sampled. This group did not isolate *S. aureus*, although 92.7 % of samples were positive for the presence of bacteria, mostly commensal organisms found on dog and cat skin. Cleaning practices were very inconsistent, and the authors noted that while these practices used disinfectants to clean cages and tables, household cleaners were used to clean the keyboards, and were often neglected completely. Our study shows a higher likelihood for recovering enterococci and staphylococci from the computer keyboard (19.2%; OR = 6.9 [1.23-38.7] and 23.1%; OR = 7.07 [1.28, 39.1], respectively). Many computer users, no matter what their profession, tend to multi-task and may eat or drink while typing. Additionally, common use objects, such as keyboards, may be accessed by health care providers while caring for a patient, but are not a direct instrument used for routine patient care and may not be a focus of infection control or a cause for hand washing before or after use.

While this study has resulted in important findings, the limitations must be discussed. In order to collect samples of the surfaces, swabs were used, while some other investigators have used gauze or agar strips/slides. There are several absorptive media available, but either wipes or swabs are preferred (Teshale et al, 2002). Additionally, while the same surfaces were sampled repeatedly, steps were not taken to ensure the exact same amount of surface area was swabbed.

However, the same methods were used to collect these samples over three years. Additionally, we did not concurrently monitor things that could impact presence of organisms on the environmental surfaces, such as cleaning schedules or use of antimicrobials within the VTH prior to or during the study. Finally, although application of molecular methods was not all-encompassing, the presence of epidemiologically-linked isolates was investigated using PFGE. Given that PFGE on all isolates from all surfaces was not performed, we felt that by omitting these data from this analysis the links that were identified would have been missed.

This study has strengths which make it unique relative to prior research. Specifically, the longitudinal nature of the sample collection and the great lengths which were taken to identify all species of enterococci and staphylococci present, while other studies reported on genus level (Aksoy et al, 2010). Additionally, our antimicrobial susceptibility testing included antimicrobials from both human and veterinary medicine. This allowed for identification of resistance presumably originating from either host.

Aksoy et al (2010) performed a study that applied the infection control standards from human hospitals to VTHs. They reported on a link between environmental cleanliness and risk of healthcare associated infection (HAI) and the presence of staphylococci in the VTH environment in those areas with high hand contact. This highlights the importance of hand hygiene in infection control. This idea is supported by our study, where we report a significant association with staphylococci and many sites throughout the VTH. Additionally, Bartley et al (2008) concluded that while environmental surfaces can serve as reservoirs for HAI pathogens, it is the hands of the HCW that is a larger contributing factor.

Definitive standards for infection control in VTHs are lacking. A study of AVMA-accredited VTHs showed that although infection control is a stated priority, formalized training and education are lacking and staff are more likely to gravitate toward procedures for their convenience and not necessarily for their effect on infection control (Benedict et al, 2008). Additionally, an internal study of healthcare provider perception and practice of infection control practices shows that most healthcare providers acknowledge that infection control protocols would be beneficial for faculty, staff, students, patients and clients; however, less than half of all small animal faculty and staff have read protocols within the MSU VTH (Miller et al, 2010). Additionally, while protocols would be beneficial there were conflicting opinions about whether there are sufficient resources and administrative support to allow the implementation of infection control protocols. In the early months of this study, a preliminary set of these data were presented to staff at our VTH, illustrating that the scale had the highest prevalence of enterococci and staphylococci. Anecdotal reports indicated that a cleaning regime for the scale was initiated as a result of our presentation, followed by complete removal of the surface material (which was replaced with stainless steel). Although we are unable to document and assess these changes, this gives evidence that these data can be translated into enhanced infection control policies.

While it was not surprising to find pathogenic and resistant bacteria on environmental surfaces of a VTH, our study offers evidence that the presence of these bacteria could have an impact on infection control. Future studies should include documentation of not only prevalence over time, but also daily infection control practices. This is supported by the fact that the ST/IM scale showed such a high prevalence and likelihood of isolation of either enterococci or staphylococci. Considering that upon admission, the majority of animals are weighed on this scale before being taken to their final examination location and that identical clones of *E.*

faecium were isolated from the scale and floor area on the same day, sites such as this scale should be a primary focal point for infection control studies.

CHAPTER 3

Acquisition and Persistence of Antimicrobial Resistant Bacteria Isolated from Companion Animals Admitted to a Veterinary Teaching Hospital 2007 – 2009

STRUCTURED ABSTRACT

Objective – The objectives of this study were to describe the antimicrobial resistance profiles of bacteria isolated from animals upon admission to a VTH and determine the incidence of acquisition and frequency of persistent isolation of resistant organisms from these animals, as well as the association of epidemiological risk factors with acquisition and persistence.

Design – Longitudinal

Sample Population – Animals being admitted to the small animal hospital and expected to stay for >48 hours.

Procedures – Rectal and nasal/oropharyngeal swabs were collected at admission and discharge. Isolates of enterococci, staphylococci and *E. coli* were tested for antimicrobial resistance using microbroth dilution. A subset of isolates was analyzed using PFGE and MLST.

Results – From 2007-2009, resistance seen among staphylococci increased, whereas resistance among *E. coli* decreased. Two-thirds of dogs with persistent MDR *E. coli* acquired a new clone by discharge and thus, were considered to have acquired MDR *E. coli*. Dogs hospitalized for > 3 days had increased incidence of acquired MDR *E. coli* and MRSA, and the majority of acquired MRSA was ST 5.

Conclusion and Clinical Relevance – This study showed that extended hospitalization increased the risk of acquiring HAIs. It is unclear if these associations are confounded by the severity of patients' illnesses or non-uniform infection control being practiced throughout the

VTH. Additionally, the most commonly acquired MRSA clone (ST 5) is that which has been associated with small animal medicine. Further investigation is required in order to determine the source and transmission route of the MRSA ST 5 in this VTH.

INTRODUCTION

Antibiotics are used in both human and animal medicine and since the first use of penicillin in the 1940s, use of antibiotics is always followed by development of resistance, which leaves less options for treatment of bacterial infections. The occurrence of HAIs among companion animals is emerging as a public health threat (Murphy et al, 2010a), that not only impacts an infected animal's course of treatment and outcome, but may significantly impact the health of humans (owners or veterinary staff) or other animals. Like human patients in hospital settings, animals housed in VTHs are more susceptible to infection (Burke, 2003). However, unlike human medicine, there are a limited number of approved antimicrobials for use in companion animals (FDA, 2010). This leaves fewer options when resistance does emerge.

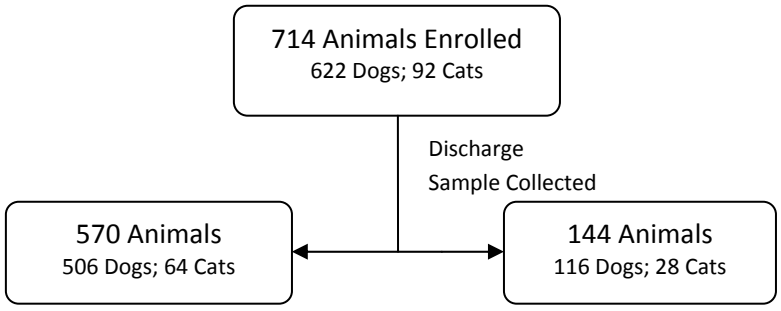
A qualitative risk assessment of acquisition of methicillin-resistant *staphylococcus aureus* (MRSA) in a VTH found that veterinary personnel pose the greatest risk, followed by environmental surfaces (Heller et al, 2010). Adherence to established infection control practices can help to prevent perpetuation of HAIs. However, other factors may confound this, such as the nature of the admission (Gibson et al, 2011; Berger et al, 2010), length of admission (Gibson et al, 2008; Ogeer-Gyles et al, 2006; Berger et al, 2010), antimicrobial usage (Ogeer-Gyles et al, 2006) or any combination of these. Ogeer-Gyles et al (2006) reported that for each day a dog is hospitalized, the odds of being colonized with resistant *E. coli* increased by a factor of 1.5, regardless of antimicrobial treatment while Gibson et al (2011) reported use of fluoroquinolones

to be associated with increased risk of colonization of MDR *E. coli* during a dog's hospitalization. The objectives of this study were to: (1) assess resistance profiles of bacterial isolates obtained at admission from animals hospitalized at a VTH, (ii) determine the incidence of acquisition and frequency of persistent colonization of MRSA, VRE and MDR *E. coli* and (iii) determine the epidemiologic risk factors associated with acquisition and persistent colonization MRSA, VRE and MDR *E. coli*.

MATERIALS AND METHODS

Study Design: A longitudinal study of dogs and cats admitted to the ECC, ST/IM or Ortho wards, in the Michigan State University (MSU) VTH was conducted from February 2007 through December 2009 resulting in 714 subjects (622 dogs and 92 cats) (Figure 3). In order to achieve a power of 80% and significant probability level of 5%, the required sample size was 280 animals. This study was approved by both the MSU Institutional Review Board for Research on Human Subjects and the MSU Institutional Animal Care and Use Committee.

Figure 3: Flow Chart of Enrolled Animals



Biological Sample Collection: An animal being admitted to one of the four areas aforementioned were considered for inclusion in our study, if the attending clinician anticipated a hospital stay of 48 hours or more. After owner consent was obtained, samples were collected

within 24 hours of admission and again at discharge, at least 48 hours after the admission sample. Sample collection consisted of one rectal swab and two nasal swabs (dogs only) or one rectal swab and one oropharyngeal swab (cats only). Initially, nasal samples were collected from both dogs and cats, however an assessment of recovery during the first year of this study showed very low recovery rates from feline nasal swabs (data not shown). Subsequently, only oropharyngeal samples were collected from cats to increase recovery, as supported by studies in humans (Mertz et al, 2007; Nilsson et al, 2006).

Rectal swabs were collected using a sterile swab and transport tube containing Stuart's transport medium (Becton, Dickinson (BD) and Company). Swabs were inserted into the colon 1-2 cm, just beyond the rectum, and rotated until feces adhered to the swab. Nasal and oropharyngeal samples were also collected using a sterile swab and transport tube containing Stuart's transport medium (BD and Co.). Nasal samples from dogs were collected with a sterile swab moistened with transport media and placed 2-3 mm into the nares and rotated. This process was performed in both nares using new swabs for each. Oropharyngeal samples from cats were collected with a sterile swab moistened with transport media and placed in the lateral oropharynx and rotated. Collected samples were then transported to the Center for Comparative Epidemiology-Microbial Epidemiology Laboratory at MSU for processing.

Laboratory Isolation and Identification: Nasal/oropharyngeal swabs were streaked onto a Columbia CNA plate. Rectal swabs were streaked onto one MacConkey plate and one CNA plate, using one side of the swab per plate. CNA plates were incubated for 48 hours at 37°C and MacConkey plates were incubated for 18-24 hours at 37°C. Up to five isolates demonstrating typical *Enterococcus* spp, *Staphyloccocus* spp and *E. coli* morphology were chosen for identification.

(i) Identification of enterococci and staphylococci were completed using methods previously described in Hamilton et al (2011).

(iii) Identification of *E. coli* was completed using the following biochemical tests. Isolates retrieved from the MacConkey agar were then streaked onto Urea and Triple Sugar Iron Agar (TSI) slants and incubated for 24 hours at 37°C. The slants were then inspected for typical growth: Urea – no color change and TSI (yellow throughout or yellow butt and red slant). Isolates showing typical results for *E. coli* were inoculated onto Simmons Citrate agar slant, indole medium and methyl red/vogues proskauer (MR/VP) medium and incubated for 24 hours at 37°C. If required, reagents were added and then assessed for typical growth or reactions: citrate – no color change, indole – development of a pink ring, MR – color change, and VP – no color change. Isolates with typical growth on all tests were considered to be *E. coli.*

Each positively identified isolate of *Enterococcus* spp, *Staphylococcus* spp or *E. coli* was suspended in Tryptic Soy Broth, 0.5 ml of the suspension was added to 0.5 ml 65% glycerol solution, and the mixture was frozen at -70°C.

Antimicrobial susceptibility testing. Of the five *Enterococcus* spp, *Staphylococcus* spp or *E. coli* isolated per sample, three isolates of each organism were randomly chosen for antimicrobial susceptibility testing. The Sensitire ® microdilution system (Trek Diagnostics, Inc.) was used to perform antimicrobial susceptibility testing on two commercially prepared plates (GPN3F and CMV1AGNF, Trek). Antimicrobials on the GPN3F plate were tested against enterococci and staphylococci and included ampicillin, ceftriaxone, ciprofloxacin, clindamycin, daptomycin, erythromycin, gatifloxacin, gentamicin and high-level gentamicin, levofloxacin, linezolid, oxacillin, penicillin, quinupristin/dalfopristin, rifampin, streptomycin, tetracycline,

trimethoprim/sulfamethoxazole and vancomycin. Antimicrobials on the CMV1AGNF plate were tested against *E. coli* and included amikacin, ampicillin, amoxicillin/clavulanic acid, ceftiofur, cefoxitin, ceftriaxone, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, nalidixic acid, sulfisoxazole, trimethoprim/sulfamethoxazole and tetracycline. These panels were chosen in order to ensure inclusion of antimicrobials used in both human and animal medicine. *E. faecalis* (ATCC 29212), *S. aureus* (ATCC 29213) and *E. coli* (ATCC 25922) were used as the quality control organisms. Quality control results were reviewed for each batch of tests, all of which were within acceptable limits. We did not address inducible clindamycin resistance.

The minimum inhibitory concentration (MIC) value at which no growth occurred was measured using the Trek AutoReader, which utilizes fluorescence technology, and an antimicrobial susceptibility/resistance profile was generated. Susceptibility, intermediate susceptibility and resistance (S, I, R) were determined by applying breakpoints as published by the Clinical and Laboratory Standards Institute (Performance Standards for Antimicrobial Susceptibility Testing: Informational Supplement M100, CLSI). Enterococcal resistance to gentamicin, ceftriaxone, clindamycin, trimethoprim-sulfamethoxazole and oxacillin were not interpreted, and high-level aminoglycoside resistance was not interpreted for staphylococci. Multi-drug resistance (MDR) was defined as an isolate being resistant to five or more antimicrobials.

Pulsed-field gel electrophoresis. In order to determine the epidemiological relatedness between different isolates of the same species, PFGE was performed on MDR *E. coli* isolated from both admission and discharge samples of the 17 animals with persistent MDR *E. coli* (34 isolates) based on Michigan State University, Diagnostic Center for Population and Animal Health's

Standard Operating Procedures # SPECIAL.001.02. Restriction enzyme, XbalI, was used and electrophoresis was performed using a CHEF unit (model CHEF-DRIII), achieved by ramping the switch times from 4 seconds to 35 seconds. The overall run time was 20 hours. PFGE clone groupings were determined according to the standard of Tenover et al (1995).

Multilocus Sequence Typing (MLST): In order to determine any genetic relatedness within each group of organisms, MLST was performed on the 34 persistent MDR *E. coli* isolates, as well as on 7 isolates of acquired MRSA. To isolate bacterial DNA, frozen stocks were subcultured onto trypticase soy agar (TSA) with 5% sheep blood and grown overnight in tryptic soy broth at 37ºC. DNA was extracted using the DNeasy kit (Qiagen, Inc.) per manufacturer's instructions. For MLST, PCR amplification of bacterial DNA, purification of PCR amplification products, and sequencing of 7 conserved housekeeping loci per species were performed at the MSU Genomic Research Support Technical Facility according to previously described methods (Qi et al, 2004; Enright et al, 2000). Briefly, internal fragments (400-500 bp) of uidA, mdh, lysP, idcA, fadD, clpX and aspC for *E. coli* and arcC, aroE, glpF, gmk, pta, tpi and yqiL for *S. aureus* were examined. The quality of the DNA sequence and base calling was validated in SeqMan (DNASTAR, Inc.) and consensus sequences were assembled and trimmed. Finally allele and sequence type (ST) assignments for MRSA were made using the MLST database (www.mlst.net) and for MDR *E. coli* were made using the Reference Center to Facilitate the Study of Shiga Toxin-Producing *Escherichia Coli* database[1] (www.shigatox.net). In instances where STs could not be assigned, due to previously incomplete gene amplification or unreported allele variation, the closest matching ST was assigned, followed by a 'v', for example, "ST 5v". Neighbor-joining trees were constructed with concatenated sequence data with the use of MEGA version 4 (Tamura et al, 2007).

Epidemiological Data Collection. Data on signalment (e.g. sex, age), housing and animal contact at the home, reason for visit, antimicrobial usage, length of visit, and locations visited while at the VTH were extracted from the medical record of each animal enrolled in the study (Table 9).

Table 9: Epidemiological data collected during medical record abstraction

Continuous
Weight at visit (kg)
Length of stay (days)
Age at visit (years)
Categorical
Gender (male/female)
Reason for visit (emergency/elective)
Contact with other animals at home
Type of housing at home (Indoor only/other)
Purpose of animal (pet/other)
Abnormal Physicial exam
Antimicrobials were prescribed at discharge
History of prolonged antimicrobial use
Antimicrobials were taken within 10 days of visit
Antimicrobials were being taken at time of visit
Antimicrobials were administered during stay at VTH
Antimicrobials were administered during surgical procedure at VTH
Animal admitted to ECC, Ortho, ST/IM, or other area
Animal was initially housed in ECC, NCU/ICU, Ortho, ST/IM or other area
Animal visited or had a consult from the following specialty areas:
Surgery
Radiology/Ultrasound
NCU/ICU
ST/IM
Ophthalmology
ECC
Oncology
Orthopedics
Cardiology
Physical Therapy
Dermatology

Statistical Analysis. Because up to 5 typical colonies of each *Enterococcus* spp, *Staphylococcus* spp and *E. coli* were chosen from each sample, we wanted to ensure that we were not unnecessarily over-counting the organisms isolated from each sample. In order to accomplish this, the susceptibility pattern produced by applying CLSI breakpoints to all antimicrobials tested were compared for each group of species isolated from each animal for each sampling event. Any species with identical susceptibility patterns, sample collection dates and animal were restricted, and one isolate was randomly chosen for inclusion in the analysis.

Not all enrolled animals were able to contribute a discharge sample. Reasons for a missing discharge sample included (i) an early discharge – less than 48 hour stay, (ii) euthanasia, (iii) mis-coordination of discharge (animal left and no sample was taken), or (iv) the animal's condition precluded collection a sample. Consequently, our study population was analyzed in two ways: those that had an admission sample only and those that had both admission and discharge samples (Figure 3).

Admission samples: All variables collected were described for the entire study population, stratified by animal species. Differences between species were assessed using 2-sample T-Test for continuous variables and Chi Square or Fisher's Exact for categorical variables (Table 10). Period prevalence of antimicrobial resistance among all admission isolates by organism was described. Additionally, significant increasing or decreasing trends in the proportion of resistance by organism, by year of the study were measured using the Cochran-Armitage test for trend.

Antimicrobial Resistance: Proportions of antimicrobial resistance was calculated for all isolates and was presented for those isolates obtained from admission samples. The results of

antimicrobial resistance testing were used to identify the three main organisms that were further analyzed in this study: MRSA, VRE and MDR *E. coli* (defined as *E. coli* resistant to five or more antimicrobials).

Incidence of Acquisition and Persistence: For the purposes of this study acquisition is defined as an admission sample being negative for an organism and the subsequent discharge sample being positive. Persistence is defined as both admission and discharge samples being positive for an organism. Instances of acquisition and persistence were analyzed for animals that had both admission and discharge samples collected. These data were presented for *E. coli,* MDR *E. coli, Enterococcus* spp, VRE, *Staphylococcus* spp, and MRSA. Additionally, multivariate logistic regression was performed using SAS 9.1.3 (SAS Institute, Cary, NC) to evaluate risk factors (Table 9) for outcomes of acquisition and persistence. Specifically, acquisition (yes or no) of MDR *E. coli* and MRSA, and persistence (yes or no) of MDR *E. coli* were modeled. The final model of each multivariate analysis was achieved using backwards stepwise elimination. Odds ratios and 95% confident intervals (CI) were reported for each and significance was determined by a 95% CI that did not cross the null of 1.0.

RESULTS

Characteristics of Admission Samples. Admission samples were collected from 716 animals (622 dogs and 92 cats) and paired admission and discharge samples were collected from 570 (80%) of these animals (506 dogs and 64 cats) (Figure 3 & Table 10). Cats enrolled in our study were significantly older (mean 7.5 years v 5.7 years, p=0.003) and stayed significantly longer at the VTH (mean 3.2 days v 2.6 days, p=0.030) compared with dogs (Table 10). Additionally, cats were more likely to have been admitted as an emergency, rather than an elective visit or referral (56.5% v 35.7%, p = 0.001) when compared to dogs. Dogs were admitted in fairly equal

proportions to the ECC, Ortho, and ST/IM wards, whereas cats were primarily admitted to ECC or ST/IM ($p < 0.0001$), and in accordance with this, there was a statistically significant difference in what area of the VTH cats and dogs were initially housed ($p < 0.0001$).

Areas visited in VTH as well as specialty consults were recorded for enrolled animals. Cats were significantly more likely to have visited the NCU/ICU, ST/IM, ECC, and Oncology areas, compared with dogs. Additionally, cats were more likely to have received antimicrobials during their stay compared to dogs (60.9% versus 45.8%, $p = 0.007$). However dogs were more likely than cats to have received antimicrobials during surgery (60.1% versus 32.6%, $p < 0.0001$), and there was no significant difference in having had a surgical procedure. *E. coli,* staphylococci, and enterococci were more likely to have been isolated from dog samples compared with cats.

Table 10: Characteristics** of animals enrolled in study by species (N = 714), 2007-2009

	Animal Species				
	Dog (N = 622)		Cat (N = 92)		P Value*
	Mean	**+/- SD**	**Mean**	**+/- SD**	
Weight at visit (kg, mean)	26.0	16.3	4.4	1.7	<0.0001
Length of stay (days, mean)	2.6	1.6	3.2	2.1	0.030
Age at visit (years, mean)	5.7	3.7	7.5	5.5	0.003
	No.	**Pct**	**No.**	**Pct**	
Gender					
Male	306	49.2	52	56.5	
Female	316	50.8	40	43.5	
Reason for visit					0.001
Emergency	222	35.7	49	53.3	
Elective/Referral	400	64.3	43	46.7	
Contact with other animals at home	407	65.4	65	70.7	
Housed					
Indoor only	411	66.1	60	65.2	
Other (Outdoor, In/Out, Unk)	211	33.9	32	34.8	
Abnormal Physical Exam	513	82.5	75	81.5	

Table 10 (cont'd)

Animal Purpose					
Pet	449	72.2	67	72.8	
Other	173	27.8	25	27.2	
History of Prolonged Ab use	237	38.1	33	35.9	
Ab taken w/in 10 days prior to visit	95	15.3	10	10.9	
Taking Ab at Admission	125	20.1	21	22.8	
Given Ab during Stay	285	45.8	56	60.9	0.007
Given Ab during Surgery	374	60.1	30	32.6	<0.0001
Prescribed Ab at Discharge	253	40.7	30	32.6	
Admitted To					<0.0001
ECC	214	34.4	48	52.2	
Ortho	225	36.2	4	4.3	
ST/IM	179	28.8	39	42.4	
Other (Derm., Onco., Opth.)	4	0.6	1	1.1	
Initially Housed					<0.0001
ECC	137	22.0	31	33.7	
NCU/ICU	140	22.5	31	33.7	
Ortho	217	34.9	4	4.3	
ST/IM	118	19.0	24	26.1	
Isolation	1	0.2	0	0.0	
Visited or Consult. during Stay					
Surgery	373	60.0	48	52.2	
Radiology/Ultrasound	451	72.5	73	79.3	
NCU/ICU	218	35.0	45	48.9	0.010
ST/IM	228	36.7	58	63.0	<0.0001
Ophthalmology	20	3.2	0	0.0	
ECC	215	34.6	48	52.2	0.009
Oncology	13	2.1	7	7.6	<0.0001
Orthopedics	252	40.5	10	10.9	
Cardiology	45	7.2	12	13.0	
Physical Therapy	10	1.6	0	0.0	
Neruology	11	1.8	0	0.0	
Dermatology	8	1.3	3	3.3	
Admission Sample:					
E. coli Isolated	554	89.1	74	80.4	0.009
Staphylococcus Isolated	231	37.1	10	10.9	<0.0001
Enterococcus Isolated	529	85.0	52	56.5	<0.0001

Table 10 (cont'd)

*Only significant P values are displayed. Significance was assessed via 2-Sample T-test for continuous variables and Chi Square for categorical variables. Fisher's exact was used for categorical variables with cell counts of < 5.
**'Ab' = 'Antimicrobial'

Antimicrobial susceptibility testing of isolates obtained from admission samples revealed that resistance among enterococci (N = 1,111 isolates) was most commonly seen against quinupristin-dalfopristin (52%), tetracycline (39%) and rifampin (38%; Table 11a). VRE was seen in 0.4% of isolates (n = 4). Resistance among staphylococci (N = 325 isolates) was most commonly seen against penicillin (31%), tetracycline (27%) and erythromycin (26%; Table 11a). MRSA was seen in 1.5% of isolates (n = 5). Resistance among *E. coli* (N = 766 isolates) was most commonly seen against ampicillin (30%). Considering those isolates of MDR *E. coli* (n=95), 80% were resistant to ampicillin, a sulfanomide and tetracycline, and 60% of these were also resistant to nalidixic acid.

Overtime, significant trends were seen among the proportions of resistant isolates for all organisms obtained from admission samples ($p<0.05$; Table 11b). Notably, resistance of enterococci to all penicillins tested and most fluoroquinolones decreased during the study period. Resistance to rifampin and quinupristin-dalfopristin increased by 22% and 27%, respectively. Resistance among staphylococci also showed significant trends for all penicillins tested for and most fluoroquinolones, but the most notable increasing trend was that to penicillin, which increased 225% from 2007 to 2009. Significant trends were most common among *E. coli*, however all trends were decreasing.

Table 11a: List of antimicrobials assessed for *Enterococcus* spp., *Staphylococcus* spp. and *E. coli* isolates from admission samples and results of antimicrobial susceptibility testing

Class	Antimicrobial	*Enterococcus* spp. (N = 1,111)			
		S	I	R (%)	
Aminoglycocide	Gentamicin	ç	ç	ç	
	Amikacin	ŧ	ŧ	ŧ	
	Kanamycin	ŧ	ŧ	ŧ	
	Gentamicin 500[a]	1058	--	53	(5)
	Streptomycin 1000[a]	1027	--	84	(8)
Cephalosporin	Ceftriaxone	ç	ç	ç	
	Cefitoxin	ŧ	ŧ	ŧ	
	Ceftiofur	ŧ	ŧ	ŧ	
Fluoroquinolone	Ciprofoxacin	840	169	102	(9)
	Gatifloxacin	1046	20	45	(4)
	Nalidixic Acid	ŧ	ŧ	ŧ	
	Levoflaxacin	1024	32	55	(5)
Glycopeptide	Vancomycin	1079	28	4	(<1)
Lincosamide	Clindamycin	ç	ç	ç	
Lipopeptide	Daptomycin	1111	--	--	
Macrolide	Erythromycin	668	305	138	(12)
Oxazolidinone	Linezolid	1110	--	1	(<1)
Penicillin	Ampicillin	953	--	158	(14)
	Amoxicillin/ Clavulanic Acid	ŧ	ŧ	ŧ	
	Oxacillin	ç	ç	ç	
	Penicillin	946	--	165	(15)
Rifampin	Rifampin	510	184	417	(38)
Streptogramin	Quinupristin-dalfopristin	322	207	582	(52)
Sulfonamide	Trimethoprim-sulfamethoxazole	ç	ç	ç	
	Sulfisoxazole	ŧ	ŧ	ŧ	
Tetracycline	Chloramphenicol	ŧ	ŧ	ŧ	
	Tetracycline	661	17	433	(39)

Table 11a (cont'd)

Class	Antimicrobial	*Staphylococcus* spp. (N = 325)		
		S	I	R (%)
Aminoglycocide	Gentamicin	302	15	8 (2)
	Amikacin	ŧ	ŧ	ŧ
	Kanamycin	ŧ	ŧ	ŧ
	Gentamicin 500[a]	ς	ς	ς
	Streptomycin 1000[a]	ς	ς	ς
Cephalosporin	Ceftriaxone	314	5	6 (2)
	Cefitoxin	ŧ	ŧ	ŧ
	Ceftiofur	ŧ	ŧ	ŧ
Fluoroquinolone	Ciprofoxacin	298	6	21 (6)
	Gatifloxacin	--	304	21 (6)
	Nalidixic Acid	ŧ	ŧ	ŧ
	Levoflaxacin	301	7	17 (5)
Glycopeptide	Vancomycin	324	--	1 (<1)
Lincosamide	Clindamycin	251	5	69 (21)
Lipopeptide	Daptomycin	1111	--	--
Macrolide	Erythromycin	237	5	83 (26)
Oxazolidinone	Linezolid	324	--	1 (<1)
Penicillin	Ampicillin	291	--	34 (10)
	Amoxicillin/ Clavulanic Acid	ŧ	ŧ	ŧ
	Oxacillin	296	--	29 (9)
	Penicillin	225	--	100 (31)
Rifampin	Rifampin	321	1	3 (1)
Streptogramin	Quinupristin-dalfopristin	320	1	4 (1)
Sulfonamide	Trimethoprim-sulfamethoxazole	292	--	33 (10)
	Sulfisoxazole	ŧ	ŧ	ŧ
Tetracycline	Chloramphenicol	ŧ	ŧ	ŧ
	Tetracycline	235	1	89 (27)

Table 11a (cont'd)

Class	Antimicrobial	*E. coli* (N = 766)			
		S	I	R	(%)
Aminoglycocide	Gentamicin	721	9	36	(5)
	Amikacin	747	18	1	(<1)
	Kanamycin	697	20	49	(6)
	Gentamicin 500[a]	ŧ	ŧ	ŧ	
	Streptomycin 1000[a]	ŧ	ŧ	ŧ	
Cephalosporin	Ceftriaxone	699	22	45	(6)
	Cefitoxin	655	21	90	(12)
	Ceftiofur	691	2	73	(10)
Fluoroquinolone	Ciprofoxacin	700	--	66	(9)
	Gatifloxacin	ŧ	ŧ	ŧ	
	Nalidixic Acid	684	--	82	(11)
	Levoflaxacin	ŧ	ŧ	ŧ	
Glycopeptide	Vancomycin	ŧ	ŧ	ŧ	
Lincosamide	Clindamycin	ŧ	ŧ	ŧ	
Lipopeptide	Daptomycin	ŧ	ŧ	ŧ	
Macrolide	Erythromycin	ŧ	ŧ	ŧ	
Oxazolidinone	Linezolid	ŧ	ŧ	ŧ	
Penicillin	Ampicillin	536	2	228	(30)
	Amoxicillin/ Clavulanic Acid	629	38	99	(13)
	Oxacillin	ŧ	ŧ	ŧ	
	Penicillin	ŧ	ŧ	ŧ	
Rifampin	Rifampin	ŧ	ŧ	ŧ	
Streptogramin	Quinupristin-dalfopristin	ŧ	ŧ	ŧ	
Sulfonamide	Trimethoprim-sulfamethoxazole	666	--	98	(13)
	Sulfisoxazole	639	--	127	(17)
Tetracycline	Chloramphenicol	678	37	51	(7)
	Tetracycline	642	5	119	(16)

[a]S: Synergy and R: No Synergy

ςMIC were not interpreted

ŧAntimicrobial was not tested against this organism

Table 11b: Percent resistance against select antimicrobials over time for *Enterococcus spp., Staphylococcus spp.* and *E. coli* isolates from admission samples

Class	Antimicrobial	*Enterococcus* spp.			*Staphylococcus* spp.		
		2007 (n=414)	2008 (n=350)	2009 (n=347)	2007 (n=74)	2008 (n=129)	2009 (n=122)
Fluoroquinolone	Ciprofoxacin	10	10	7	**1**	**6**	**10**
	Gatifloxacin	**6**	**3**	**3**	**1**	**7**	**9**
	Levoflaxacin	**7**	**5**	**3**	1	6	7
Glycopeptide	Vancomycin	<1	1	<1	0	1	0
Macrolide	Erythromycin	13	12	12	23	24	29
Penicillin	Ampicillin	**16**	**15**	**11**	**1**	**14**	**12**
	Oxacillin	ς	ς	ς	1	13	9
	Penicillin	**17**	**16**	**11**	**12**	**34**	**39**
Rifampin	Rifampin	**33**	**39**	**42**	0	2	1
Streptogramin	Quinupristin-dalfopristin	**50**	**47**	**61**	0	2	2
Tetracycline	Tetracycline	40	41	36	30	25	29

ςMIC were not interpreted

Bolded proportions were significant for that antimicrobial/organism using the Cochran-Armitage test for trend (one-sided p value).

Table 11b (cont'd)

Class	Antimicrobial	*E. coli* 2007 (n=294)	2008 (n=244)	2009 (n=228)
Aminoglycocide	Gentamicin	3	5	6
	Kanamycin	**9**	**6**	**4**
Cephalosporin	Ceftriaxone	**9**	**6**	**1**
	Cefitoxin	**16**	**11**	**7**
	Ceftiofur	**14**	**9**	**4**
Fluoroquinolone	Ciprofoxacin	**11**	**9**	**5**
	Nalidixic Acid	**13**	**11**	**8**
Penicillin	Ampicillin	**36**	**32**	**19**
	Amoxicillin/ Clavulanic Acid	**16**	**14**	**8**
Sulfonamide	Trimethoprim-sulfamethoxazole	**16**	**14**	**7**
	Sulfisoxazole	**20**	**17**	**11**
	Chloramphenicol	**8**	**8**	**4**
Tetracycline	Tetracycline	**19**	**15**	**11**

Changes during stay at the VTH. Among animals from whom paired admission and discharge samples were collected (N=570), the highest proportion of isolation occurred among animals with persistent *E. coli,* staphylococci, or enterococci (77.4%, 20.5%, and 73.2%, respectively; Table 12a). MDR *E. coli* was acquired by 6.8% of animals, and 3.0% maintained persistent isolation of MDR *E. coli* during their stay. Considering the findings from molecular analysis of persistent MDR *E. coli* isolates, these figures change to 8.6% acquisition and 1.5% persistence (data not in tables). An additional 1.2% of animals acquired MRSA during their stay at the VTH. Occurrence of persistence and acquisition of MDR *E. coli*, MRSA or VRE were too low among feline patients to warrant further analysis (Table 12b), thus multivariable analyses were performed using data from dogs only.

Table 12a: Comparison of bacteria isolated at admission and at discharge for those animals with samples collected at both events (N = 570)

	Admission only		Admission & Discharge *(Persistance)*		Discharge Only *(Acquisition)*		Neither Admission nor Discharge	
	No.	Pct.	No.	Pct.	No.	Pct.	No.	Pct.
E. coli	68	11.9%	441	77.4%	27	4.7%	34	6.0%
MDR *E. coli*	50	8.8%	7	1.2%	49	8.6%	464	81.4%
Staphylococcus	73	12.8%	117	20.5%	51	8.9%	329	57.7%
MRSA	4	0.7%	0	--	7	1.2%	559	98.1%
Enterococcus	50	8.8%	417	73.2%	67	11.8%	36	6.3%
VRE	2	<1%	1	<1%	3	0.5%	564	98.9%

Table 12b: Summary of acquisition and persistance of pathogens by animal speceis

	Dog		**Cat**		Total
	No.	Row Pct.	No.	Row Pct.	
Persistent MDR *E. coli*	15	88.2%	2	11.8%	17
Acquired MDR *E. coli*	35	89.7%	4	10.3%	39
Acquired MRSA	7	100%	0	--	7
Persistent VRE	1	100%	0	--	1
Acquired VRE	3	100%	0	--	3

Multivariable analysis of the incidence of acquired MDR *E. coli* (n=40) and MRSA (n=7) among dogs show that one risk factor was common for both organisms: length of stay at the VTH (Table 13). Those dogs staying for 3 or more days were 2.51 (95% CI: 1.24, 5.08) times more likely to acquire MDR *E. coli* and 15.13 (95% CI: 1.12, 205.10) times more likely to acquire MRSA. Having been housed solely indoors while at home reduced the risk (OR=0.11, [0.01, 0.91]) of acquiring MRSA. There were not enough isolates of VRE or persistent MDR *E. coli* to warrant multivariable analysis.

Table 13: Multivariate logistic regression model of risk factors associated with recovery of MDR *E. coli* or MRSA from dogs that acquired either organism or had persistant MDR E. coli during their stay at the VTH

T 13a: Acquired MDR *E. coli* between admission and discharge

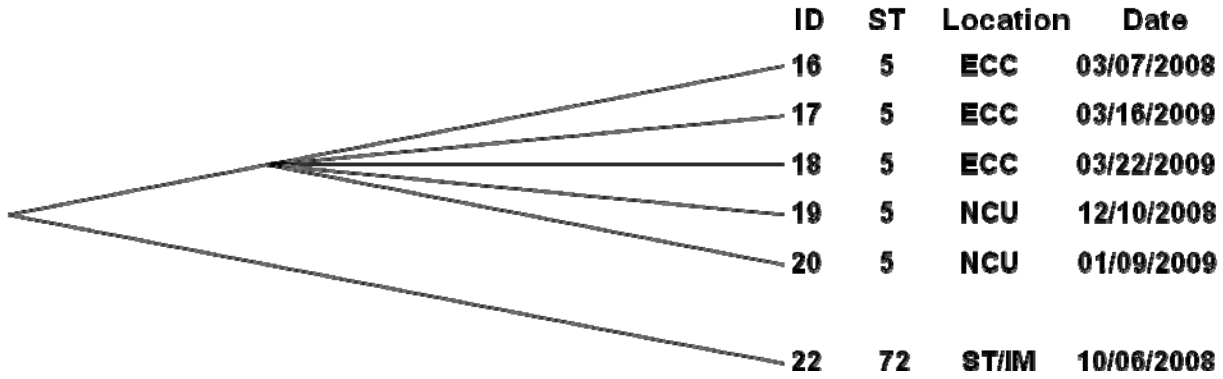
	OR	95% CI	
Length of stay at VTH (< 3 v 3+ days)	**2.507**	**1.238**	**5.077**
Initially housed in: ECC Ward	0.526	0.122	2.263
Initially housed in: Ortho Ward	1.484	0.437	5.035
Initially housed in: NCU/ICU	2.525	0.928	6.87
Initially housed elsewhere	--- referent ---		
Nature of visit (emergency v elective)	2.42	0.904	6.475
Received antimicrobials during stay	1.45	0.703	2.988
Visited the Oncology Ward	3.099	0.654	14.675
Had a Cardiology consult	2.066	0.767	5.562

T 13b: Acquired MRSA between admission and discharge

	OR	95% CI	
Length of stay at VTH (< 3 v 3+ days)	**15.13**	**1.12**	**205.10**
Nature of visit (emergency v elective)	12.74	0.97	166.77
Was animal housed solely 'indoors' at home	**0.11**	**0.01**	**0.91**
History of prolonged antimicrobial use	1.40	0.11	18.63
Was taking antimicrobials at admission	3.61	0.40	32.14
Had taken antimicrobials in the past 10 days	1.53	0.20	11.71
Visited the ECC	0.44	0.07	2.75
Received antimicrobials during surgery	0.14	0.02	1.19
Received antimicrobials during stay	0.60	0.08	4.81

Epidemiological Relatedness. A dendrogram of MLST sequence types on the 7 isolates of acquired MRSA reveal the majority of these belong to MLST ST 5 (Figure 4). The majority of these dogs were housed in the ECC, however low numbers prevented any analysis for associations.

Figure 4: Distribution of MRSA isolates acquired by dogs during admission to the VTH, representing 3 distinct multilocus sequence types (STs), by location of admission and collection date. Dendrogram is a consensus of 1000 bootstrap trees generated with the neighbor-joining algorithm with use of sequence data for 7 genes.



Molecular analysis of the 30 isolates obtained from the admission and discharge samples of the 15 dogs with persistent MDR *E. coli* shows the most prevalent PFGE clone to be clone E (Table 14), however presence of this clone was not significantly associated with any epidemiological factors (data not shown). Notably, three of the five dogs with PFGE clone E isolated at either admission or discharge were initially housed in the NCU. Additionally, every instance of isolation of identical PFGE clones from admission and discharge also had identical ST (Table 14, subjects 1-5). In addition to ST 288, which was the most prevalent, ST 171 was isolated frequently.

Of these 15 dogs with persistent MDR *E. coli,* five retained the identical PFGE clones and STs during their stay and were truly persistent. The remaining 10 dogs actually acquired

new clones of MDR *E. coli* and were considered to be instances of acquisition, and not persistence, in the multivariable analysis.

Table 14: Listing of PFGE and ST groupings of those dogs with persistent MDR *E. coli.*

	PFGE group[a]		ST[b]			
Animal	Admission	Discharge	Admission	Discharge	Admission Date	Initially Housed
1	A	A	644v	644v	3/21/2007	NCU/ICU
2	M	M	83	83	7/24/2007	ST/IM
3	E	E	288	288	7/23/2007	NCU/ICU
4	C	C	855	855	10/29/2007	ST/IM
5	B	B	722	722	1/16/2008	Ortho
6	--	--	653	171	4/30/2007	ST/IM
7	--	E	692	288	8/8/2007	ST/IM
8	--	--	x	86	8/13/2007	ECC
9	E	J	288	171	7/12/2007	NCU/ICU
10	--	J	171	171v	8/29/2007	Ortho
11	--	E	302v	288	10/5/2007	NCU/ICU
12	E	--	288	604v	10/29/2007	Ortho
13	--	--	657v	160	1/9/2008	ECC
14	--	--	287	13v	4/30/2008	Ortho
15	B	J	392v	171	10/6/2008	NCU/ICU

[a]The PFGE grouping " -- " indicates this isolate was not part of any identified groups

[b]The ' v ' following an ST indicates that there was no available match in the database, but the ST shown was the closest match available.

The 'x' listed under ST indicates bad sequence data for housekeeping genes. Thus, we were not able to assign an ST.

DISCUSSION

This study accomplished the major objectives: (i) to describe the level of antimicrobial resistance seen in admission samples, which may be reflective of the companion animal community, (ii) to determine the frequency of and (iii) to assess risk factors for the acquisition of

MRSA and MDR *E. coli* while at a VTH. Additionally, common clones were identified from those isolates that were acquired and persisted.

The differences seen between cats and dogs enrolled in our study is interesting. Cats were more likely to be admitted as an emergency and more likely to have visited multiple specialty areas/received consults than dogs; however dogs were more likely to have bacteria of interest isolated at admission (Table 10). With the absence of a measurement of illness-severity upon admission, these findings could be interpreted as cats enrolled in this study having more severe illness, compared with admitted dogs, which appeared to have been largely admitted due to scheduled appointments. Gibson et al (2011) found that severity of illness was associated with colonization of MDR *E. coli.* This contradicts our findings that cats, which we are inferring to have had more severe illness than dogs, were less likely than dogs to have been colonized.

Other studies have described resistance seen in the general dog population by assessing isolates obtained from admission samples (Hanselman et al, 2008; Ogeer-Gyles et al, 2006). Although a convenience sample, this method provides a good opportunity to assess what is occurring in the community. While our admission samples had less than 1% of VRE, it is notable that the highest level of resistance observed among enterococci, which also increased over time, was to quinupristin-dalfopristin. Although not optimal, quinupristin-dalfopristin has been used to treat VRE bloodstream infections in humans (Crank et al, 2010). Daptomycin and linezolid have also been shown to be an effective treatment for VRE, to which <1% of all enterococci isolates in our study were resistant. MRSA was isolated in very low frequency from admission samples, which is similar to what has been reported in the companion animal community (Hanselman et al, 2008).

During the course of the study, the period prevalence of staphylococcal resistance to penicillin significantly increased by 225%. While not unexpected, this finding is noteworthy as the greatest prevalence of staphylococcal resistance to penicillin was 39%, reported in the final year of our study. Studies on human staphylococcal isolates show that in 2011, less than 5% remain sensitive to penicillin (Tolan et al, 2001), a stark contradiction to our findings. However Delgado et al (2011) reported increased staphylococcal resistance to penicillin from human isolates compared with bovine isolates in a study on mastitis, a finding which is also supported results on human isolates from our study (data not shown).

A previous study that looked at resistance to *E. coli* isolated from admission samples to a VTH reported high levels of resistance to ampicillin and amoxicillin-clavulanic acid (Ogeer-Gyles et al, 2006). We also saw the largest proportion of resistance among *E. coli* isolates to ampicillin (Table 11b). However, the proportion of *E. coli* isolates resistant to cephalosporins, fluoroquinolones, penicillin, sulfonomides, chloramphenicol and tetracycline experienced a significant downward trend during our study period. Additionally, the most common antimicrobials making up our isolates of MDR *E. coli* showed decreasing trends, except for naladixic acid (which decreased, but was not significant). These opposing trends present an interesting finding that could have been driven by a number of factors, including the types of antimicrobials used or disinfection procedures used within the VTH during the course of our study, however our study design did not include collection of these types of data.

Length of hospital stay was significantly associated with the incidence of dogs acquiring pathogens in this study. Whether in a human or animal hospital, the longer a patient is admitted to a hospital, the more opportunity there is to acquire a healthcare associated infection (Ogeer-Gyles et al, 2006; Gibson et al, 2008; Berger et al, 2010). Likewise in our study, staying in the

VTH >3 days increased the incidence of acquired MDR *E. coli* or MRSA in dogs. Although additional associations were observed for acquisition of a HAI, these must be interpreted with caution, as our low numbers of acquired MRSA resulted in extremely wide CI.

We expected antimicrobial usage to be associated with either acquisition or persistence of resistant organisms, but this was not observed. Studies at both animal and human hospitals show conflicting results in this area (Ogeer-Gyles et al, 2006 and Nseir et al, 2010). Ogeer et al (2006) reported that administration of and the type of antimicrobials administered during a dog's stay at a VTH was not significant for acquisition of *E. coli*. In contrast, Nseir et al, 2010 showed that antimicrobial treatment of hospitalized humans increased their risk of acquisition of resistant-gram negative bacteria by 4.6 to 9.9 times. Our study did not assess how long an animal had previously been on antibiotics and the types of antibiotics, rather these data were collected binomially: had been treated or not. This omission may have diluted the effect of history of prolonged antimicrobial use and needs to be further investigated.

Although published data show that antimicrobial usage has a role in the ability for MDR *E. coli* to persist, we were unable to show any significant associations with persistence of MDR *E. coli*. A study by Trott et al (2004) reported that canine MDR *E. coli* do not compete well with normal flora in the absence of selection pressure caused by use of antimicrobials, but once given the opportunity to thrive (via treatment with antimicrobials), the MDR *E.coli* will persist, despite stopping treatment and the return of normal flora. As previously stated, they manner of our data collection may have diluted this effect.

We did identify a predominant clone among those dogs from which MDR *E. coli* was persistently recovered. While the majority of those dogs carrying PFGE clone E did so at

admission, dogs 7 and 11 apparently acquired PFGE clone E while at the VTH. This suggests that clone E could represent a common strain circulating among the general population of companion animals, and its acquisition while at the VTH may have been transmitted by another patient. Sanchez et al (2002) presented findings of multiple PFGE clones of *E. coli* isolated from animals and the environment of a VTH. The lack of a prevalent clone was explained as the effects of circulating genetic elements conferring resistance, rather than specific bacteria. Our study identified a prevalent clone (PFGE clone E), however, despite our efforts, the large time gaps between admission of the dogs from which clone E was isolated leaves us unable to make a firm conclusion.

Not surprising, the most commonly acquired MRSA sequence type has been studied previously (Lin et al, 2010). MRSA ST5 has previously been reported as being isolated from veterinary personnel who work with small animals and has been distinctly differentiated from isolates of large animal healthcare providers (Moodley et al, 2006). Additionally, Lin et al (2010) reported HA-MRSA ST5 to represent companion animal isolates of MRSA whereas ST8 represented equine MRSA isolates. Based on this, further study within our VTH is warranted in order to determine if MRSA ST5 is prevalent among our VTH healthcare providers.

While infection control practices can help to prevent perpetuation of HAIs, other factors, unique to a patient's experience while admitted, may confound this. While we confirmed previously reported associations with the acquisition of HAIs, such as length of stay, our numbers were not able to produce more significant associations. Additionally, this study reports that the most commonly acquired MRSA clone (ST 5) is that which has been previously reported to be associated with small (vs. large) animal medicine. Further investigation is required in order to determine the source and transmission route of the MRSA ST 5 in this VTH. However, less

can be concluded about the most common clone/strain of persistently isolated MDR *E. coli* from the patients in our study. The epidemiological and molecular data overlay reported in this study provides insight about the occurrences of HAIs in a VTH. Although the identified associations do not necessarily imply causality, they do serve as a provisional template for additional studies.

CHAPTER 4

Longitudinal Study of Resistant Bacteria Isolated from Students, Faculty and Staff of a Veterinary Teaching Hospital

ABSTRACT

The objectives of this study were (i) to describe the risk of acquisition and carriage of antimicrobial resistant bacteria by veterinary students going through their clinical rotations and faculty and staff working in specific clinical areas within a veterinary teaching hospital (VTH), and (ii) to determine the epidemiological and genetic relatedness of isolates obtained from human and animal subjects, as well as environmental surfaces from a VTH. This was achieved through a longitudinal study design with a sample population of veterinary students, faculty, and staff working in or participating in clinical rotations within the Emergency Critical Care (ECC), Orthopedic Surgery (Ortho), Soft Tissue Surgery (ST), or Internal Medicine (IM) wards.

The period prevalence of resistant bacteria was greater among faculty and staff compared with students and certain clinical procedures increased the risk of carrying methicillin-resistant *S. aureus* (MRSA). Two distinct lineages of MRSA were isolated from the students and from faculty/staff. Certain clinical procedures were associated with increased risk of having had resistant bacteria isolated veterinary personnel. Thus, attention must be given to infection control techniques for clinical procedures where the technician is exposed to potentially infectious materials. MRSA ST 5, commonly known as hospital acquired (HA)-MRSA, was recovered exclusively from faculty/staff and VTH patients, while MRSA ST 8, commonly identified as community acquired (CA)-MRSA, was recovered exclusively from students and

environmental surfaces. Being that students were not colonized with the MRSA that seemed to originate from within the VTH is reflective of adherence to infection control.

INTRODUCTION

Development and transmission of antimicrobial resistance among the human population is a major public health threat and has been compounded by our interaction with animals. Companion animals are a deeply rooted part of human society as it is estimated that by the end of 2009 there were approximately 77.5 million dogs residing in two-thirds of American households (American Pet Products Association, 2009). Research has established that animals can act as vehicles for passage of potentially devastating pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus* (VRE) (Simjee et al, 2002; van Duijkeren et al, 2005; Weese et al, 2006). Many studies have explored the capacity for bacteria to be transmitted between living beings and inanimate objects (Bures et al, 2000; Guardabassi et al, 2004). This is particularly important to understand in a hospital setting (Cohen et al, 2008), as the occurrence of healthcare associated infections due to antimicrobial-resistant organisms has been increasing (Eliopoulos et al, 2006; Klevens et al, 2008).

Companion animals, such as cats and dogs, represent a realistic source of spread of antimicrobial resistance due to their close contact with humans and extensive use of antimicrobials in veterinary medicine. This role is increased within veterinary hospitals, where antimicrobial exposure to both patients and veterinary personnel is more direct and contact between the patient and healthcare provider is more intimate than in human hospitals. There have been conflicting opinions on whether the patients or veterinary personnel are at higher risk of exposure to antimicrobial resistance from each other (Guardabassi et al, 2004; Lin et al, 2010). However a qualitative risk assessment of acquisition of MRSA in patients of VTHs found that

veterinary personnel pose the greatest risk, followed by environmental surfaces (Heller et al, 2010).

The types of exposure that veterinary personnel have to companion animals vary. Compared to veterinary students, veterinary faculty and staff have much higher frequency of contact with animal patients, increased closeness of contact during treatment, and longer durations of their experience in terms of contact with animal patients (Ishihara et al, 2010). This difference may contribute to increased risk of carrying resistant organisms, especially MRSA. Studies in human hospitals have documented medical equipment and healthcare providers as vehicles for transfer of many bacteria, such as VRE (Boyce et al, 1997; Duckro et al, 2005). Research focusing on the transmission of resistant bacteria in veterinary hospitals is sparse, although there is evidence that exam tables, cages, and surgical tables can harbor pathogenic bacteria (Loeffler et al, 2005; Sidhu et al, 2007). The interaction between healthcare providers and patients at veterinary hospitals is extremely different than in human hospitals, thus, interactions between caregivers, animals, and inanimate objects within veterinary hospitals need to be further investigated to assess the occurrence of transmission. The hypothesis being tested in this study is that the interactions that veterinary personnel have with their patients and environment will affect their likelihood of being colonized with resistant bacteria. The objectives of our study were: (i) to describe the risk of acquisition and carriage of antimicrobial resistant bacteria by veterinary students going through their clinical rotations and faculty and staff working in specific clinical areas within the VTH, and (ii) to determine the epidemiological and genetic relatedness of isolates obtained from human and animal subjects, as well as environmental surfaces from a VTH.

MATERIALS AND METHODS

Study Design: A longitudinal study focusing on certain areas of the Michigan State University (MSU) VTH was conducted from February 2007 through December 2009. Participants were veterinary students rotating through the Emergency Critical Care (ECC), Orthopedic Surgery (Ortho), Soft Tissue Surgery (ST), or Internal Medicine (IM) wards during their 3-week clinical rotations and VTH faculty and staff who worked in these areas. In order to detect an expected prevalence of 4% (± 5%) resistant bacteria at a significance level of 5% required a sample size of 70 people to achieve a power of 80%. This study was approved by the Institutional Review Board for Research on Human Subjects and written informed consent was required.

Concurrent to human sample collection, samples were also collected from animals and environmental surfaces. These sampling procedures have been described elsewhere (Hamilton et al, 2011). Briefly, at admission and again at discharge, rectal and nasal samples were collected from animals admitted to any of the aforementioned wards and were expected to stay for at least 48 hours. Additionally, specific environmental surfaces within the VTH areas of focus were sampled at every 4th clinical rotation.

Students: Participation differed for students and faculty/staff. Students were invited to enroll during their rotation orientation. Those who enrolled in the study and gave their consent were given a study package which contained materials with which to collect a nasal and fecal sample within 5 days of the start of their rotation, as well as a questionnaire. They were then given another study package two weeks later and were instructed to complete a second questionnaire and provide a second nasal and fecal sample within 5 days of the end of their rotation. Students were encouraged to participate during every clinical rotation through our areas of interest and were given a $25 incentive for complete participation, which was defined as (1) completed

consent form, (2) “pre-rotation” fecal and nasal samples and completed questionnaire, and (3) “post-rotation” fecal and nasal samples and completed questionnaire.

Faculty and Staff: Faculty and staff who worked in any of the aforementioned areas of the VTH were invited to enroll in the study as well. Those who chose to enroll and gave their consent were asked to provide fecal and nasal samples within 5 days of the start of every 4th clinical rotation, as well as complete a questionnaire. Study packages were distributed to participants a week prior to the expected sample collection time frame. Faculty and staff were able to enroll at any point during the three-year study, but could only start their participation at a designated sample collection time. Additionally, faculty and staff were encouraged to participate during the entire study and were given a $25 incentive for completion of each sampling period. Complete participation was different for faculty and staff and was defined as (1) completed consent form, (2) fecal and nasal samples and completed questionnaire.

Epidemiological Data Collection. With every sample collection, students, faculty and staff were asked to complete a questionnaire about the 3 weeks prior to sample collection. Information collected focused on antibiotics taken, clinical procedures they performed while at the VTH, small and large animals in their home and antibiotic usage of those animals, exposure to human hospital, physician’s office or nursing home, and the same information as it pertained to their roommate or household contact.

Biological Sample Collection. Fecal samples were collected in a specimen collection tube with Cary Blair Medium. Nasal samples were collected via a sterile swab eSwab Transport System. The swab was first moistened with sterile saline then rotated in both nares. Collected samples were then sent to the Michigan Department of Community Health’s Bureau of Laboratories for

bacterial isolation. Presumptive isolates of *E. coli*, staphylococci or enterococci were transported via courier back to the MSU Center for Comparative Epidemiology-Microbial Epidemiology Laboratory to be further processed.

Laboratory Isolation and Identification: Presumptive isolates of staphylococci or enterococci were streaked onto a Columbia CNA plate and presumptive isolates of *E. coli* were streaked onto a MacConkey plate. CNA plates were incubated for 48 hours at 37°C and MacConkey plates were incubated for 18-24 hours at 37°C. Up to five isolates demonstrating typical *Enterococcus* spp, *Staphyloccocus* spp and *E. coli* morphology were chosen for identification.

(i) Identification of enterococci and staphylococci was completed using methods described in Hamilton et al (2011).

(ii) Identification of *E. coli* was completed using the methods described in Chapter 3.

Antimicrobial susceptibility testing. Of the five *Enterococcus* spp, *Staphylococcus* spp or *E. coli* isolates identified per sample, three of each organism were randomly chosen for antimicrobial susceptibility testing. The Sensitire ® microdilution system (Trek Diagnostics, Inc.) was used to perform antimicrobial susceptibility testing as previously described: staphylococci and enterococci (Hamilton et al, 2011); *E. coli* (Chapter 3). *E. faecalis* (ATCC 29212), *S. aureus* (ATCC 29213) and *E. coli* (ATCC 25922) were used as the quality control organisms. Quality control results were reviewed for each batch of tests, all of which were within acceptable limits. Susceptibility, intermediate susceptibility and resistance (S, I, R) were determined by applying breakpoints as published by the Clinical and Laboratory Standards Institute (Performance Standards for Antimicrobial Susceptibility Testing: Informational Supplement M100, CLSI).

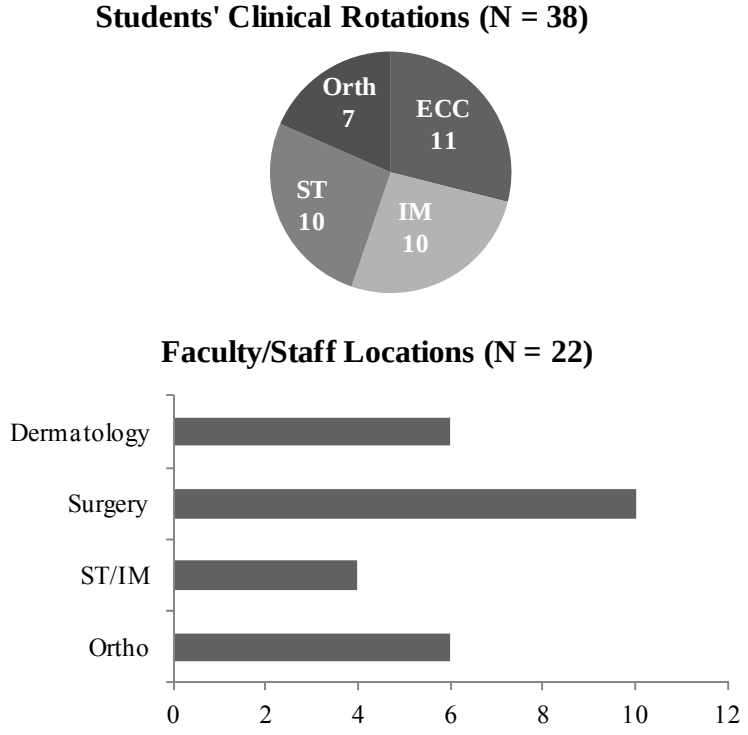
Pulsed-field gel electrophoresis (PFGE). In order to determine the epidemiological relatedness between isolates of *S. aureus*, PFGE was performed on 12 isolates, of which six were MRSA. PFGE was conducted based on Michigan State University, Diagnostic Center for Population and Animal Health's Standard Operating Procedures # SPECIAL.001.02. The restriction enzyme *SmaI* was used and electrophoresis was performed as described in Chapter 3.

Multilocus Sequence Typing (MLST). In order to determine any genetic relatedness among MRSA isolated across all subject types (human, animals, environmental surfaces), MLST was performed on all MRSA identified during the study (16 isolates: 6 from humans, 9 from animals and 1 from an environmental surface). To isolate bacterial DNA, frozen stocks were subcultured onto trypticase soy agar with 5% sheep blood and grown overnight in tryptic soy broth at 37ºC. DNA was extracted using the DNeasy kit (Qiagen GmbH) per manufacturer's instructions. For MLST, PCR, purification, and sequencing of 7 conserved housekeeping loci per species was performed at the MSU Genomic Research Support Technical Facility according to previously described methods (Enright, et al, 2000; Qi et al, 2004). Briefly, internal fragments (400-500 bp) of arcC, aroE, glpF, gmk, pta, tpi and yqiL were examined. The quality of the DNA sequence and base calling was validated in SeqMan (DNASTAR) and consensus sequences were assembled and trimmed. Final allele and ST assignments were made using the MLST database (www.mlst.net). In instances where STs could not be assigned, due to previously incomplete gene amplification or unreported allele variation, the closest matching ST was assigned, followed by a 'v', for example, "ST 5v". A neighbor-joining tree was constructed with concatenated sequence data with the use of MEGA version 4 (Tamura et al, 2007).

Statistical Analysis and Data Presentation. Frequencies of epidemiological data collected from the questionnaires completed at each sample submission are presented in Table 15. These

data are stratified by student at the beginning of a rotation, student at the end of a rotation, and faculty/staff. Chi-square tests, or Fisher's exact tests when counts were <5, were used to assess differences between (i) students beginning and end of rotation and (ii) faculty/staff and students. A p value of < 0.05 was considered a significant difference. Additionally, the location of either the rotation (students) or work place (faculty/staff) is listed in Figure 5. As seen in Figure 6, some subjects provided samples up to 5 times throughout the study, thus data in Table 15 represent characteristics at each sample submission and not for each subject.

Figure 5: Chart and graph of students' clinical rotations and faculty and staff work site locations while participating in the study.

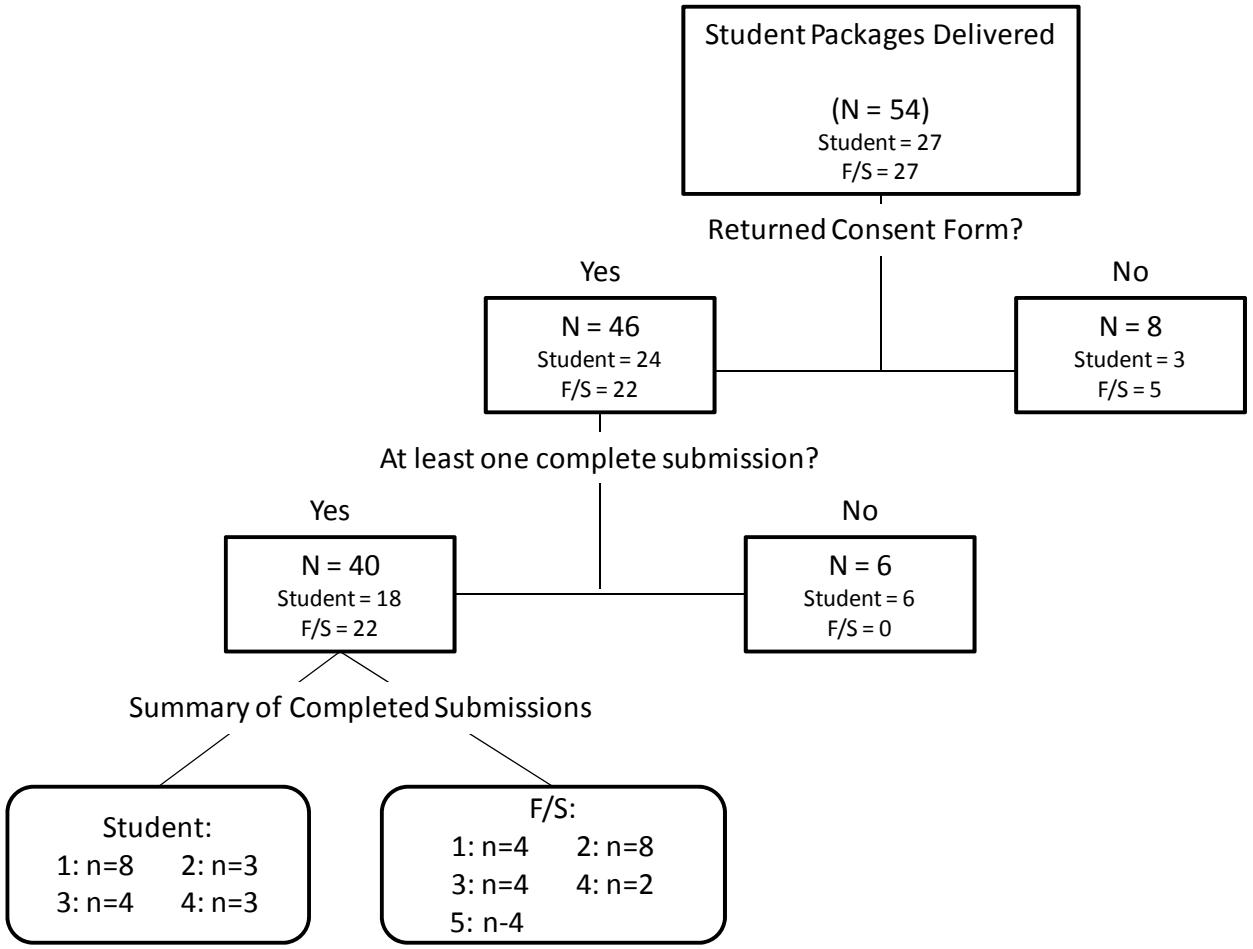


The period prevalence of resistance for each antimicrobial against enterococci, staphylococci and *E. coli* isolates is presented in Table 16, stratified by samples provided by (i) students at beginning of a rotation, (ii) students at the end of a rotation and (iii) faculty and staff. Differences in prevalence of resistance was assessed using chi-squared or Fisher's exact tests

between (i) students beginning and end of rotation and (ii) faculty/staff and students. Instances where all isolates were sensitive to a given antimicrobial are omitted from Table 16, including linezolid for enterococci, gentamicin, clindamycin, trimethoprim-sulfamethoxazole, and linezolid for staphylococci, and ceftriaxone, cefoxitin and ceftiofur for *E. coli.*

The generalized estimating equations (GEE) with an exchangeable correlation structure was used to analyze the effects of each characteristic listed in Table 15 on the probability of a student or faculty/staff having resistant enterococci, staphylococci, *E. coli*, as well as MDR *E. coli,* VRE or MRSA using a repeated measures approach. Univariable analyses were carried out using study ID within each rotation as the unit of analysis (subject variable) for students, and study ID within each sampling event as the unit of analysis (subject variable) for faculty/staff. The results were expressed as odds ratios (ORs) with 95% confidence intervals (CIs). Probability of having resistance to each organism was modeled separately. For students, only the isolation of resistant staphylococci and MRSA are included in the results table, as isolation of resistant enterococci, VRE, *E. coli*, or MDR *E. coli* did not produce any significant associations. For faculty and staff, only the isolation of resistance enterococci is included in the results table. Multivariable modeling was not conducted. All statistical analyses were performed using SAS 9.1.3 (SAS Institute Inc, Cary, NC).

Figure 6: Flow chart of participation, including those subjects removed due to lack of consent and failure to provide at least one complete submission. Under the Summary of completed Submissions, the number indicates the number of completed submissions, while the "n=#' indicates the number of subjects having done so. For example "3: n=4" should be interpreted as 4 subjects completed 3 submissions.



RESULTS

The intended sample size for this study was 70 participants; however, 40 subjects (18 students and 22 faculty/staff) completed consent forms and had at least one complete submission (Figure 6). Thus, the ability to perform multivariable statistical analyses was limited. Table 15 displays each subject's characteristics during the 3 weeks prior to sample submission. Data presented for students are stratified by whether they were beginning or ending a rotation, thus the data presented for 'end of rotation' represents experiences throughout the most recent rotation. The majority of students with completed submissions did so during their rotation through the ECC (30.6%, Figure 5).

The frequency of epidemiological data reported at the beginning compared with the end of the students' rotations were generally stable, however, significantly more students reported contact with a large animal outside of the VTH environment at the beginning of a study rotation, compared to the end. Additionally, students performed significantly more urinary catheter placement and cleaning soiled cages at the end of a rotation compared to the beginning. Data presented for faculty and staff showed that the majority of participants worked in surgery, however many faculty and staff are not assigned to one location (Figure 5). Significantly more faculty and staff had contact with small animals outside of the VTH as well as human hospitals, compared with students. Whereas significantly more students had contact with cats outside the VTH compared to faculty and staff.

Table 15: Epidemiological Data: Characteristics of students and faculty/staff during the 3 weeks prior to sample collection

	Student				Faculty/Staff (N = 60)	
	Beginning of Rotation (N = 36)		End of Rotation (N = 36)			
	No.	Pct	No.	Pct	No.	Pct
Had taken any antimicrobials	0	--	3	8.3	5	8.3
Had a household contact taken antimicrobials	5	13.9	2	5.6	4	6.7
Performed the following procedures on VTH patients:						
Urinary catheter[¥£]	5	13.9	20	55.6	31	51.7
Venous catheter	15	41.7	28	77.8	42	70.0
Wound cleaning or bandage change	18	50.0	28	77.8	39	65.0
Nasogastric Tube[¥]	4	11.1	7	19.4	16	26.7
Cleaning soiled cages[£]	23	63.9	32	88.9	43	71.7
Dental	3	8.3	0	--	2	3.3
Endoscopy[¥]	6	16.7	10	27.8	7	11.7
Ultrasound	10	27.8	14	38.9	13	21.7
Surgery[¥]	6	16.7	5	13.9	2	3.3
Rectal Exam	8	22.2	6	16.7	6	10.0
Blood Draw	0	--	1	2.8	5	8.3
Bathe	0	--	0	--	4	6.7
Surgery Prep	1	2.8	3	8.3	5	8.3

Table 15 (cont'd)

Contact outside of the VTH with the following:						
Dogs	19	52.8	17	47.2	42	70.0
Cats[¥]	23	63.9	22	61.1	44	73.3
Other small animals[¥]	15	41.7	16	44.4	11	18.3
Large Animals[£]	11	30.6	2	5.6	10	16.7
Human Hospial Urgent Care[¥]	2	5.6	2	5.6	12	20.0
Human Physician's Office[¥]	5	13.9	4	11.1	15	25.0
Other Human Healthcare Facility	0	--	0	--	8	13.3
A household contact have contact with the following:						
Veterinary Hospital	8	22.2	6	16.7	12	20.0
Human Hospial Urgent Care	2	5.6	2	5.6	3	5.0
Human Physician's Office	0	--	0	--	3	5.0
Nursing Home	0	--	0	--	1	1.7

[£]Signigicant difference between students beginning and end of rotation ($p<0.05$)

[¥]Significant difference between students and faculty/staff ($p<0.05$)

There were no statistically significant differences in the proportion of resistance observed from isolates submitted from students at the beginning compared to the end of their rotations. However, it is notable that staphylococcal resistance to penicillin increased by 60% during students' rotations, which was just shy of significance (p=0.053). In general, the resistance seen in staphylococci and enterococci isolates from students and faculty/staff was similar, however, differences in resistance among *E. coli* isolates were noted between students and faculty/staff (Table 16). *E. coli* resistance to gentamicin, ciprofloxacin, nalidixic acid, and ampicillin was significantly higher among faculty/staff. Although not presented in Table 16, MRSA was isolated from one student at three different sampling events, as well as from two faculty/staff members. Additionally, VRE was isolated from one faculty/staff member.

Although the target sample size was not met, univariable analysis of student and faculty/staff data provided some significant results (Table 17). For students, contact with a dog or large animal outside the VTH was associated with increased risk of carriage of resistant staphylococci (OR = 5.18 [95% CI, 1.65, 16.23] and OR = 4.08 [95% CI, 1.18, 14.13], respectively). Additionally, performing a rectal exam in the VTH increased the risk of isolating resistant staphylococci (OR = 4.22 [95% CI, 1.29, 13.80]). When assessing the risk for MRSA, different characteristics were significant, however, these data were from one student from whom MRSA was isolated on three separate sampling events (Table 17). For faculty and staff, only isolation of resistant enterococci was significantly associated with the epidemiological data listed on Table 15. Those who performed either urinary or venous catheter placement (OR = 4.08 [95% CI, 1.27, 13.13] and OR = 4.40 [95% CI, 1.24, 15.57], respectively) or cleaned a soiled cage (OR = 3.69 [95% CI, 1.04, 13.12]) had increased risk of carriage of resistant enterococci.

Table 16: Percent resistance against select antimicrobials for *Enterococcus spp., Staphylococcus spp.* and *E. coli* isolates obtained from beginning and end of rotation samples (students) and from faculty/staff samples

Antimicrobial	*Enterococcus* spp.			*Staphylococcus* spp.		
	Student		Faculty/	Student		Faculty/
	Beginning (n=37)	End (n=54)	Staff (n=77)	Beginning (n=15)	End (n=16)	Staff (n=22)
Ceftriaxone	ς	ς	ς	7	13	14
Ciprofoxacin	8	20	13	13	25	14
Gatifloxacin	0	0	3	100	100	100
Levoflaxacin	3	7	3	13	25	14
Vancomycin	0	4	8	0	0	0
Erythromycin	65	57	52	33	31	18
Ampicillin	0	0	3	73	81	64
Oxacillin	ς	ς	ς	20	38	14
Penicillin	0	0	3	53	88	64
Rifampin	51	65	53	0	0	0
Quinupristin-dalfopristin	76	70	77	0	0	0
Tetracycline	35	24	30	0	0	14

Table 16 (cont'd)

Antimicrobial	*E. coli*		
	Student		Faculty/
	Beginning (n=34)	End (n=38)	Staff (n=56)
Gentamicin	0	0	23¥
Amikacin	0	8	0
Kanamycin	3	8	0
Ciprofoxacin	3	5	32¥
Nalidixic Acid	3	5	36¥
Ampicillin	26	24	46¥
Amoxicillin/ Clavulanic Acid	5	9	11
Trimethoprim-sulfamethoxazole	9	13	5
Sulfisoxazole	26	21	14
Chloramphenicol	6	13	4
Tetracycline	12	24	14

ᶜMIC were not interpreted

¥Significanly higher prevalence among faculty/staff (chi-square $p<0.05$)

Genotypic Analysis of *S. aureus*

PFGE analysis was performed on 12 isolates of *S. aureus*, six of which were MRSA, in order to identify any epidemiological grouping. This resulted in four distinct clones (Table 18). Two groupings were of isolates submitted from the same subjects at different sampling events; Group B consisted of samples obtained from a student at the beginning and end of an ECC and isolates in Group D were submitted by a surgical staff member three months apart. Additionally, MRSA isolated from a single student grouped into clone C. The MRSA isolated from two faculty/staff grouped into a clone A; two isolates were collected from the same subject one month apart and 18 months after MRSA was isolated from a different subject.

Table 17. Results of univariate GEE regression on isolation of resistant staphylococci and MRSA from students and faculty/staff

	OR	95% Confidence Limits	
STUDENTS			
Isolation of resistant staphylococci¥			
Contact with a dog outside the VTH	5.18	1.65	16.23
Contact with a large animal outside the VTH	4.08	1.18	14.13
Performed a rectal exam procedure	4.22	1.29	13.80
Isolation of MRSA£			
Performed a nasogastric tube procedure	12.60	1.04	152.19
Performed a dental procedure	17.75	1.10	286.56
FACULTY & STAFF			
Isolation of resistant enterococci€			
Cleaned a soiled cage	3.69	1.04	13.12
Performed a urniary catheter procedure	4.08	1.27	13.13
Performed a venous catheter procedure	4.40	1.24	15.57

¥21 of 72 staphylococcal isolates obtained from student samples were resistant

£MRSA was identified from 3 of 72 staphylococci isolates obtained from student samples.

Note: These 3 samples were provided from a single student at three separate sampling events.

€33 of 60 enterococcal isolates obtained from faculty/staff samples were resistant

Table 18. Summary of resistance profiles and clonal grouping for *S. aureus* isolates selected for PFGE

Date of Sample Collection	Subject Type	Location	Antimicrobial Resistance[1]	PFGE Group[2]
10/31/2007	FS-1	ST	AMP, CEF, CIP, ERY, GAT, LEV, OX, PEN	A
1/22/2009	FS-2	ECC	AMP, *GAT*, PEN	~
1/22/2009	S-1	ECC	AMP, *GAT*	B
1/28/2009	S-2	ECC	AMP, *CEF*, CIP, ERY, GAT, LEV, OX, PEN	C
2/2/2009	S-1	ECC	AMP, *GAT*, PEN	B
2/20/2009	S-2	ECC	AMP, *CEF*, CIP, ERY, GAT, LEV, OX, PEN	C
3/24/2009	FS-3	Surg	*GAT*	D
3/29/2009	FS-4	Derm	AMP, CEF, CIP, CLIN, ERY, GAT, LEV, OX, PEN	A
4/29/2009	FS-4	Derm	AMP, CEF, CIP, CLIN, ERY, GAT, LEV, OX, PEN	A
6/9/2009	S-2	Ortho	AMP, *CEF*, CIP, ERY, GAT, LEV, OX, PEN	C
6/25/2009	FS-3	Surg	*GAT*	D
6/30/2009	FS-2	ECC	AMP, ERY, *GAT*, PEN	~

[1]: Those in italics had intermediate resistance. AMP - ampicillin; CEF - ceftriaxone; CLIN - clindamycin; CIP - ciprofloxacin; ERY - erythromycin; GAT - gatifloxacin; LEV - levofloxacin; OX - oxacillin; PEN - penicillin

[2]: The symbol " ~ " indicates that PFGE pattern of this isolate did not fall into any grouping

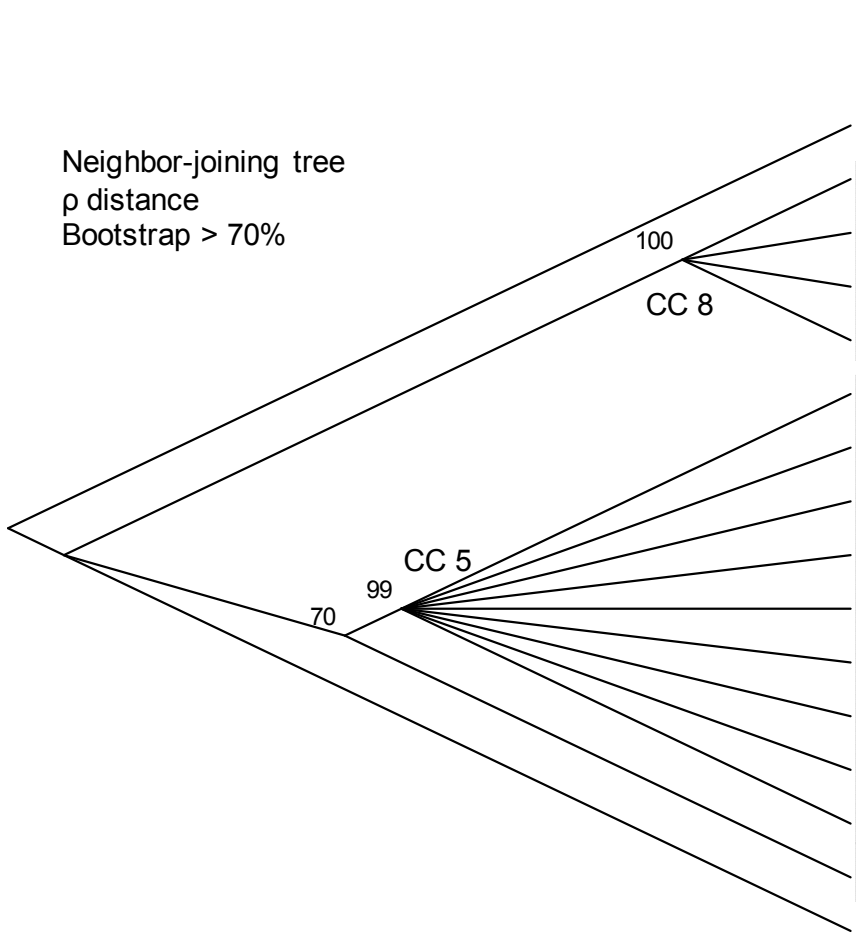
In order to understand the relationship between MRSA isolates collected from humans, animals and environmental surfaces (Hamilton et al, 2011), a phylogenetic tree was constructed from MLST data on 16 MRSA isolates collected from these three sources (Figure 7). Two distinct STs were observed with two additional singletons. Isolates in ST 8 were further grouped into clonal complex (CC) 8, which included those MRSA isolated from a single student (PFGE Group C; Table 18) and an environmental surface (a cage in the ECC). Additionally ST 5 appeared to be a closely related genotype that grouped with ST 221 with 70% bootstrap support. This grouping, which was further identified as CC 5, consisted of the MRSA isolated from faculty and staff (PFGE Group A; Table 18) as well as six dogs.

DISCUSSION

Despite a limited sample size, our analyses identified important risk factors associated with veterinary students going through their clinical rotations or faculty and staff working in specific clinical areas within the VTH. Additionally, this study presents two distinct MRSA strains present among students and faculty and staff of a VTH.

We did not identify any significant differences in the proportion of resistance seen over the course of a student's rotation, likely due to the small sample size. For example, the 60% increase in staphylococcal resistance to penicillin was very close to significance ($p=0.053$). This trend was also observed among our animal subjects. However, we expected to see differences between the student and faculty/staff due the duration of exposure that the faculty and staff have and we did identify a few instances of faculty having more resistance to *E. coli* than students. However, the level of resistance to penicillin among staphylococci isolated from faculty/staff was lower than that of the level observed from students' isolates collected after their rotations.

Figure 7: Distribution of MRSA isolated from humans (Hum), animals (An) and environmental surfaces (Env) of a VTH, representing five distinct multilocus sequence types (STs) and two clonal complexes (CC), by collection date, isolate source and location. Dendrogram is a consensus of 1000 bootstrap trees generated with the neighbor-joining algorithm with use of sequence data for 7 genes.



ID	ST	Collection Date	Isolate Source	Location
1-27	72	10/09/2008	An	ST/IM-Dis
1-11	8	02/15/2009	Hum	ECC-End Rot.
1-11	8	05/31/2009	Hum	Ortho-Beg Rot.
1-11	8	01/27/2009	Hum	ECC-End Rot.
1-66	8	09/14/2009	Env	ECC-Cage
1-42	5	03/27/2008	An	ECC-Dis
1-41	5	03/24/2009	An	ECC-Dis
1-32	5	01/22/2009	An	NCU-Dis
1-44	5	03/29/2009	Hum	Derm
1-25	5	10/23/2007	Hum	Gen
1-44	5	04/29/2009	Hum	Derm
1-30	5	12/17/2008	An	NCU-Dis
1-22	5	03/30/2008	An	ECC-Dis
1-19	5	02/20/2008	An	ST/IM-Adm
1-05	221	04/16/2008	An	ST/IM-Adm
1-49	45v	04/22/2009	An	NCU-Adm

We did not observe any overlapping risk factors for students and faculty or staff. However, overlaying the GEE results with the significant differences observed in epidemiological characteristics compared between students beginning and ending their rotations and also between students and faculty and staff, provides some interesting patterns. Faculty and staff who reported cleaning soiled cages were more likely to have resistant enterococci isolated, compared to those who did not (OR= 3.69 [95% CI, 1.04, 13.12]). However, students as a whole were more likely to report cleaning soiled cages during their rotations, compared to faculty and staff (76.3% compared to 71.7%). This discrepancy may be a result of our low numbers. However the National Association of State Public Health Veterinarians Veterinary Infection Control Committee of the American Veterinary Medical Association (AVMA) released an update to the 2008 compendium on routine infection control practices designed to minimize transmission of zoonotic pathogens from animal patients to veterinary personnel in private practice (Scheftel, J.M, et al, 2010). This report mentions the risk that exposure to cages may pose to veterinary personnel and offers guidelines to minimize the risk of exposure to bacteria.

A study from Ishihara, et al (2010), reported that being an employee of the VTH, compared with being a student, carried an increased the risk of colonization, which agrees with our findings in this study. Due to the smaller sample size of our study, we may not have been able to fully describe risks and associations for colonization. Also, students who had contact with large animals outside the VTH were more likely to have resistant staphylococci compared to those who did not (OR = 4.08 [95% CI, 1.27, 13.13]). At the same time, students reported this behavior at the beginning of their rotations more frequently then at the end (30% compared to 5%). Given that this risk factor is specifically measuring an exposure outside the VTH, the

relevance that most students reported this behavior prior to starting a rotation in which we were studying is not obvious.

MRSA has been reported to be a commensal organism in around 1 to 1.5 percent of human beings in the general population (Abudu et al, 2001; Sahm et al, 2008), and even up to 59 percent among healthcare personnel (Albrich et al, 2008). Thus it was not surprising to find MRSA among the human subjects in our study. We observed two distinct epidemiologically-linked clones isolated from a student and from faculty/staff. The student participated throughout 2009 and did not have MRSA isolated from any of the four samples collected after May 31. Without further investigation in this specific instance, we cannot conclude if this student's MRSA had cleared or was just not detected by our microbiological techniques. The second distinct MRSA clone was isolated from different faculty members, about 18 months apart. As mentioned previously, the consistent nature of faculty/staff's daily exposure to the VTH may contribute to acquisition to this distinct clone (Ishihara et al, 2010). Despite the limited data points, this would lead to a conclusion that a distinct strain of MRSA is circulating among the faculty and staff of the VTH.

In order to gain a more complete picture of MRSA within our VTH, we combined the MLST results from all MRSA isolated during our entire study (2007 to 2009) from all sources (students, faculty/staff, animals, and environmental surfaces [Hamilton et al, 2001]). The differences observed in resistance between students and faculty/staff, although not surprising, should be highlighted. Overall, the period prevalence of MRSA among all veterinary personnel was low (3 of 40 subjects); and the lineage of MRSA isolated from students was distinct from that which was isolated from faculty and staff, in concordance with lack of overlapping risks from the univariable analysis. The MRSA lineage which was consistently isolated from a

student was also isolated from a cage within the ECC. CC 8 (ST 8) is considered to be community associated (CA)-MRSA (David et al, 2008), but has also been isolated from equines (Lin et al, 2010; Loeffler et al, 2010). Without further molecular testing, the ST 8 identified in this study cannot be further categorized.

The second distinct MRSA lineage, CC 5 (ST 5 and ST 221), was isolated from faculty/staff and dogs. MRSA ST 5 has been reported as healthcare associated (HA)-MRSA (Peterson et al, 2010), and has been reported as being isolated from veterinary faculty and staff who work with small animals (Moodley et al, 2006; Hanselman et al, 2006; Lin et al, 2010). Presence of MRSA ST 5 among VTH patients provides evidence that it is being transmitted between the patients and faculty/staff, however, our data are unable to identify possible routes of this transmission. This documentation of two distinct lineages of MRSA circulating among (i) faculty/staff and patients and (ii) students and environmental surfaces warrants more in-depth study.

Considering our student subjects, because we were unable to perform multivariable analysis, we cannot focus in on a major risk, but it is fair to conclude that risks to students are not solely a result of their exposures within the VTH. We expected to capture some level of change in prevalence of resistance over the course of clinical rotations, but did not. These findings should be interpreted with caution as our minimal sample size was not met, however, considering the intensity of the clinical rotations, enhancement of reminders on application of proper infection control, not only within their clinics, but also outside of the VTH is suggested.

Our finding of a distinct strain of MRSA being shared between the faculty/staff and dogs isolates coincides with previous reports of MRSA ST 5 being commonly isolated from companion animals as well as small animal veterinary personnel. However, in the absence of

identifying vehicles for transmission, we can only suggest that transmission is occurring between faculty/staff and dogs. Other findings from this study provide direction as potential vehicles for transmission were identified, such as the cage, scale, phone keyboard. Regardless, further study into the relationship between patients and healthcare providers is necessary to tease out the implication of this finding.

OVERALL CONCLUSIONS

Healthcare associated infections, such as MRSA and VRE, were present among patients, healthcare providers and environmental surfaces of this VTH, however no cluster or outbreak was reported during this study period. This suggests that either the pathogens isolated were colonizing, rather than infecting, their hosts, or, that infection control guidelines were consistently practiced, preventing the spread. While molecularly identical strains were identified from multiple sources across the course of this study, we were unable to make any definite conclusions regarding direction of transmission.

Conclusions to this dissertation research include the following:

1. Potentially pathogenic bacteria were identified in patients, healthcare providers and environmental surfaces within a veterinary teaching hospital

2. We have identified sites in the VTH (such as cages, scales, keyboards and phones) that have increased risk of harboring resistant organisms. Additionally, our molecular testing revealed identical clones of *Enterococcus* on different environmental surfaces on the same day, but also molecularly-related strains of MRSA from a student and cage.

3. Even though the univariable analysis was not conducted, descriptive analysis results showed that the risks of these infections to students may not be solely a result of their exposures within the VTH. Additionally, we expected to capture some level of change in incidence of resistance over the course of clinical rotations, but did not.

4. We observed more resistant organisms isolated from the animals compared to veterinary personnel. Hospitalization of animals for 3 or more days was associated with higher risk for acquiring an HAI.

5. Prior use of antimicrobials by patients was not found to be a significant risk for acquisition nor persistence of resistance. This finding could be due to weakness in documentation of previous drug use or longer period from previous antimicrobial use and admission to the study in the VTH.

6. If we overlay the findings of increased pentaresistance from environmental enterococci isolates with those veterinary personnel who cleaned soiled cages being at increased risk for resistant enterococci, the magnitude of these findings increases and provides a basis for stressing the utilization of proper infection control.

7. Our finding of a distinct strain of MRSA being shared between the faculty/staff and dogs isolates agrees with previous reports of MRSA ST 5 being commonly isolated from companion animals as well as small animal veterinary personnel. However, in the absence of identifying vehicles for transmission, we can only suggest that transmission may be occurring between faculty/staff and dogs.

RECCOMMENDATIONS

Veterinary hospital administrators need to consider areas and surfaces within their healthcare settings that are most likely to (i) retain bacteria and (ii) come into contact with multiple patients and healthcare providers. The flow of a patient being admitted will vary between VTHs, however, special attention should be paid to areas that are not a focus of routine

cleaning and are likely to contact patient's mucous membranes and /or backsides, such as the scale. While finding of bacteria on the VTH environmental surfaces is not surprising, these findings have implications for the MSU VTH and suggest that infection control efforts on these surface types would benefit from review to identify any gaps in protocols.

Our findings on veterinary students should be interpreted with caution as our minimal sample size was not met, however, considering the intensity of the clinical rotations, enhancement of reminders on application of proper infection control, not only within their clinics, but also outside of the VTH is suggested.

Other findings from this dissertation provide direction as potential vehicles for transmission were identified, such as the cage, scale, phone keyboard. Regardless, further study into the relationship between patients and healthcare providers is necessary to tease out the implication of this finding. In conclusion, these findings suggest that a review of current infection control practices would be practical for students as well as faculty and staff, stressing the continued application outside of the VTH. Additionally, our study has identified sites within the VTH that could benefit from more focused infection control procedures.

AREAS OF FUTURE RESEARCH

1. One of the goals of this dissertation was to establish clear indications for direction of transmission of resistant bacteria between humans and animals. This particular goal was not met, however, future adjustments to the study presented may allow for documentation of directionality of transmission.
    a. Environmental sampling was performed every 4th rotation, which equated to about every 3 to 4 months. Increasing sampling of environmental surfaces to weekly

b. The discordant findings of increased resistance among isolates from animal subjects but lack of significant associations with risk factors would benefit from further study. More detailed data collection on previous antimicrobials use by patients with concurrent documentation of antimicrobial use within the VTH, as well as documentation of areas visited by patients within the VTH and types of services received is suggested. Such detail may have been missed during our medical chart abstraction.

c. Finally, due to small sample size, multivariate analysis was not performed on isolates from healthcare providers. Participation from human subjects would need to be increased in order to identify procedures or characteristics associated with isolation of MRSA, VRE or MDR *E. coli*.

2. Further study on the two distinct strains of MRSA identified from students and environmental surfaces (CA-MRSA, ST 5) and faculty/staff and patients (HA-MRSA, ST 8) within the MSU VTH. Although the sources of these two strains were segregated, use of the terms 'community-acquired' and 'healthcare associated' carry less meaning as a descriptor of the actual source of the bacteria. A more focused study, including sampling of the VTH healthcare provider and animal patients home environment, may offer a more complete picture of the source of these two strains.

REFERENCES

Abudu L, Blair I, Fraise A, et al. Methicillin-resistant *Staphylococcus aureus* (MRSA): a community-based prevalence survey. *Epidemiol. Infect.* 2001;126:351-356.

Aksoy E, Boag A, Brodbelt D, et al. Evaluation of surface contamination with staphylococci in a veterinary hospital using a quantitative microbiological method. *J Small Anim Pract* 2010; 51: 574-580.

Albrich WC and Harbarth S. Health-care workers: source, vector, or victim of MRSA? *Lancet Infect. Dis.* 2008;8:289-301.

American Pet Products Association. 2009. 2009/2010 APPA National Pet Owners Survey. American Pet Products Association, http://www.americanpetproducts.org/pubs_survey.asp, as quoted from the following article: U.S. Pet Ownership, by The Humane Society of the United States, December 30, 2009, http://www.humanesociety.org/issues/pet_overpopulation/facts/pet_ownership_statistics.html

American Veterinary Medical Association (AVMA). 1998. Extralabel Drug Use (ELDU): An Informational Outline of the Animal Medicinal Drug Use Clarification Act (AMDUCA). http://www.avma.org/reference/amduca/amduca1.asp Accessed on July 14, 2010.

Bartley JM and Olmsted RN. Reservoirs of Pathogens Causing Health Care-Assocaited Infections in the 21st Century: Is Renewed Attention to Inanimate Surfaces Warranted? *Clin Microbiol News* 2008;30:113-116.

Bates J, Jordens JZ & Griffiths DT. Farm Animals as a Putative Reservoir for Vancomycin-Resistant Enterococcal Infection in Man. *J Antimicrob Chemother* 1994;34:507-514.

Benedict KM, Morley PS and Van Metre DC. Characteristics of biosecurity and infection control programs at veterinary teaching hospitals. *JAVMA* 2008;233:767-773.

Berger J, Diab-Elschahawi M, Blacky A, et al. A matched prospective cohort study on *Staphylococus aureus* and *Escherichia coli* bloodstream infections: Extended perspectives beyond resistance. *Am J Infect. Control* 2010;38:839-845.

Boerlin P, Eugster S, Gaschen F, et al. Transmission of Opportunistic Pathogens in a Veterinary Teaching Hospital. *Vet Microbiol* 2001;82:347-359

Boyce JM, Potter-Bynoe G, Chenevert C, et al. Environmental Contamination due to Methicillin-Resistant *Staphylococcus aureus:* Possible Infection Control Implications. *Infect. Control Hosp. Epidemiol.* 1997;18:622-627.

Bures S, Fishbain JT, Uyehara CFT, et al. Computer Keyboards and Faucet Handles as Reservoirs of Nosocomial Pathogens in the Intensive Care Unit. *Am J Infect Control* 2000;28:465-471.

Burgess BA, Morley PS, Hyatt DR. Environmental Surveillance for *Salmonella enterica* in a Veterinary Teaching Hospital. *JAVMA* 2004;229:1344-1348.

Burke JP. Infection Control – A Problem for Patient Safety. *NEJM* 2003, 348(7): 651-656.

Cohen A L, Calfee D, Fridkin SK, et al. Recommendations for Metrics for Multidrug-Resistant Organisms in Healthcare Settings: SHEA/HICPAC Position Paper. *Infect. Cont. Hosp. Ep*. 2008;29:901-13.

Crank CW, Scheetz MH, Brieknauer B, et al. Comparison of Outcomes From Daptomycin or Linezolid Treatment for Vancomycin-Resistant Enterococcal Bloodstream Infections: A Retrospective, Multicenter, Cohort Study. *Clin Ther* 2010;32:1713-1719.

David MA, Glikman D, Crawford SE, et al. What is Community-Associated Methicillin-Resistant *Staphylococcus aureus*? *JID* 2008;197:1235-1243.

Delgado S, Garcia P, Fernandez L, et al. Characterization of *Staphylococcus aureus* strains involved in human and bovine mastitis. *FEMS Imm Med Microbiol* 2011; 62:225-235.

DeVincent SJ, Reid-Smith R. Stakeholder position paper: Companion animal veterinarian. *Prev Vet Med* 2005, 73: 181-189.

Duckro AN, Blom DW, Lyle EA, et al. Transfer of Vancomycin-Resistant Enterococci via Health Care Worker Hands. *Arch. Intern. Med.* 2005;165:302-307.

Duquette RA & Nuttall TJ. Methicillin-resistant *Staphylococcus aureus* in dogs and cats: an emerging problem? *J Small Anim Pract* 2004;45:591-597.

Eliopoulos GM. Enterococci: On the back burner but still simmering. *Cur. Infect. Dis. Rep.* 2006;8:333-336.

Enright MC, Day NPJ, Davies CE, et al. Multilocus Sequence Typing for Characterization of Methicillin-Resistant and Methicillin-Susceptible Clones of *Staphylococcus aureus*. *J Clin Microbiol* 2000;38:1008-1015.

Food and Drug Administration (FDA). 2009. Extra-Label Use of FDA Approved Drugs in Animals.http://www.fda.gov/AnimalVeterinary/ResourcesforYou/FDAandtheVeterinarian/ucm077390.htm Accessed on July 14, 2010.

Food and Drug Administration (FDA). 2010 FDA APPROVED ANIMAL DRUG PRODUCTS http://www.fda.gov/AnimalVeterinary/Products/ApprovedAnimalDrugProducts/ucm042847.htm

Frank LA, Kania SA, Kirzeder EM, et al. Risk of colonization or gene transfer to owners of dogs with Methicillin-resistant *Staphylococcus pseudintermedius*. *Vet Derm* 2009;20:496-501.

Fraser MA and Girling SJ. Bacterial carriage of computer keyboards in veterinary practices in Scotland. *Vet Rec* 2009;165:26-27.

Gibson JS, Morton JM, Cobbold RN, et al. Multidrug-Resistant *E. coli* and *Enterobacter* Extraintestinal Infection in 37 dogs. *J Vet Intern Med* 2008;22:844-850.

Gibson JS, Morton JM, Cobbold RN, et al. Risk factors for multidrug-resistant *Escherichia coli* rectal colonization of dogs on admission to a veterinary hospital. *Epidemiol. Infect*. 2011;39:197-205.

Guardabassi L, Schwarz S, and Lloyd DH. Pet animals as reservoirs of antimicrobial-resistant bacteria. *J. Antimicrob. Chemother*. 2004;54:321-332.

Hamilton E, Kaneene JB, May K, et al. Prevalence and Antimicrobial Resistance of *Enterococcus* spp and *Staphylococcus* spp isolated from repeated sampling of surfaces within a Veterinary Teaching Hospital 2007 – 2009. *JAVMA* Accepted for publication 2/4/2011.

Hanselman BA, Kruth SA, Rousseau J, et al. Methicillin-resistant *Staphylococcal aureus* Colonization in Veterinary Personnel. *EID* 2006;12:1933-1938.

Hanselman BA, Kruth S & Weese JS. Methicillin-resistant staphylococcl colonization in dogs entering a veterinary teaching hospital. *Vet. Microbiol* 2008;126;277-281.

Heller J, Armstrong SK, Girvan EK, Reid SW, Moodley A, Mellor DJ. Prevalence and distribution of Methicillin-resistant *Staphylococcus aureus* within the environment and staff of a university veterinary clinic. *J Small Anim Pract* 2009; 50: 168-173

Heller J, Kelly L, Reid SWJ, et al. Qualitative Risk Assessment of the Acquisition of Meticillin-Resistant *Staphylococcus aureus* in Pet Dogs. *Risk Analysis* 2010;30:458-472.

Huycke MM, Sahm DF & Gilmore MS. Multiple-Drug Resistant Enterococci: The Nature of the Problem and an Agenda for the Future. *EID* 1998;4:239-249.

Ishihara K, Shimokubo N, Sakagami A, et al. *Staphylococcus pseudintermedius* in an Academic Veterinary Hospital. *Appl Env Microbiol* 2010;76:5165-5174.

Klevens RM, Edwards JR, and Gaynes RP. The Impact of Antimicrobial-Resistant, Health Care-Associated Infections on Mortality in the United States. *Clin. Infect. Dis*. 2008;47:927-30.

Lanz R, Kuhnert P, Boerlin P. Antimicrobial resistance and resistance gene determinants in clinical Escherichia coli from different animal species in Switzerland. *Vet Microbiol* 2003, 91: 73-84.

Lin Y, Barker E, Kislow J, et al. Evidence of Multiple Virulence Subtypes in Nosocomial and Community-Associated MRSA Genotypes in Companion Animals from the Upper Midwestern and Northeastern United States. *Clin Med Res* 2010: doi:10.3121/cmr.2010.944

Loeffler A, Boag AK, Sung J, et al. Prevalence of Methicillin-Resistant *Staphylococcus aureus* among Staff and Pets in a Small Animal Referral Hospital in the UK. *J. Antimicrob. Chemother.* 2005;56:692-697.

Loeffler A & Lloyd DH. Companion animals: a reservoir for methicillin-resistant *Staphylococcus aureus* in the community? *Epidemiol. Infect*. 2010;138:595-605.

Mertz D, Frei R, Jaussi B, et al. Throat swabs are necessary to reliably detect carriers of *Staphylococcus aureus*. *Clin Infect Dis*. 2007;45:475-7.

Miller K, Bolin C, Stobierski MG, et al. Infection control knowledge, attitudes and practices of VTH faculty and staff, and CVM students [poster]. In: Proceedings of the Annual Phi Zeta Research Day; 2010 Oct 1; East Lansing, Michigan: Michigan State University, College of Veterinary Medicine; 2010. p 12. Poster nr 30.

Moodley A, Stegger M, Bagcigil AF, et al. *spa* typing of methicillin-resistant *Staphylococcus aureus* isolated from domestic animals and veterinary staff in the UK and Ireland. *J Antimicrob Chemother* 2006;58:1118-1123.

Moodley A, Nightingale EM, Stegger M, Nielsen SS, Skov RL, Guardabassi L. High risk for nasal carriage of Methicillin-resistant *Stahylococcus aureus* among Danish veterinary practitioners. *Scand J Work Enviorn Health* 2008; 34(2): 151-157

Murphy CP, Reid-Smith RJ, Boerlin P, et al. *Escherichia coli* and selected veterinary and zoonotic pathogens isolated from environmental sites in companion animal veterinary hospitals in southern Ontario. *Can Vet J* 2010a;51:963-972

Murphy CP, Reid-Smith RJ, Weese JS, et al. Evaluation of Specific Infection Control Practices Used by Companion Animal Veterinarians in Community Veterinary Practices in Southern Ontario. *Zoonoses Public Health* 2010b;57:429-438.

Murray BE. Diversity among Multidrug-Resistant Enterococci. *EID* 1998;4:37-49.

Nilsson P & Ripa T. *Staphylococcus aureus* Throat Colonization is More Frequent than Colonization in the Anterior Nares. *J Clin Microbiol*. 2006;44:3334-9

Nseir S, Blazejewski C, Lubret R, et al. Risk of acquiring multidrug-resistant Gram-negative bacilli from prior room occupants in the intensive care unit. *Clin Microbiol Infect*, no. doi: 10.1111/j.1469-0691.2010.03420.x.

Ogeer-Gyles J, Mathews KA, Sears W, et al. Development of antimicrobial drug resistance in rectal *Escherichia coli* isolates from dogs hospitalized in an intensive care unit. *JAVMA* 2006;229:694-699.

Paul NC, Moodley A, Ghibaudo G, et al. Carriage of Methicillin-Resistant *Staphylococcus pseudintermedius* in Small Animal Veterinarians: Indirect Evidence of Zoonotic Transmission. *Zoonoses Public Hlth*, no. doi: 10.1111/j.1863-2378.2011.01398.x

Perreten V, Kadlec K, Schwarz S, et al. Clonal Spread of Methicillin-resistant *Staphylococcus pseudintermedius* in Europe and North America: an International Multicentre Study. *J Antimicrob Chemother* 2010:doi:10.1093/jac/dkq078.

Peterson AE, Davis MF, Krain LJ, et al. Discordance Between Epidemiologic and Molecular Classifications of Health Care- Versus Community- Associated MRSA [poster]. In: Proceedings of the 48th Annual Meeting of the Infectious Disease Society of America; 2010 Oct 21-24; Vancouver, British Columbia, Canada; 2010. Poster nr LB-33.

Qi W, Lacher DW, Bumbaugh AC, et al. *Ec*MLST: an Online Database for Multi Locus Sequence Typing of Pathogenic *Escherichia coli.* Proc. of the 2004 IEEE Computational Systems Bioinformatics Conference (CSB 2004): Stanford, CA.

Sahm DF, Brown NP, Yee YC, et al. Stratified Analysis of Multidrug-Resistant *Escherichia coli* in the US Health Care Institutions. *Postgrad. Med.* 2008;120 (Suppl): S3: ISN – 0032-5481, e-ISSN – 1941-9260.

Sanchez S, McCrackin Stevenson MA, Hudson CR, et al. Characterization of Multdrug-Resistant *Escherichia coli* Isolates Associated with Nosocomial Infections in Dogs. *J Clin Microbiol* 2002; 40:3586-3595.

Sasaki T, Kikuchi K, Tanaka Y, et al. Reclassification of Phenotypically Identified *Staphylococcus intermedius* Strains. *J Clin Microbiol* 2007;45:2770-2778.

Scheftel, JM, Elchos, BL, Cherry, B, et al. Compendium of Veterinary Standard Precautions for Zoonotic Disease Prevention in Veterinary Personnel: National Association of State Public Health Veterinarians Veterinary Infection Control Committee 2010. *JAVMA* 2010; 237:1403-1422.

Sidhu MS, Oppegaard H, Devor TP, et al. Persistence of Multidrug-Resistant *Staphylococcus haemolyticus* in an Animal Veterinary Teaching Hospital Clinic. *Microb. Drug Resist.* 2007;13:271-280.

Silbergeld EK, Graham J & Price LB. Industrial food animal production, antimicrobial resistance, and human health. *Annu Rev Public Health.* 2008;29:151-69.

Simjee S, White DG, McDermott PF, et al. Characterization of Tn1546 in vancomycin-resistant *Enterococcus faecium* isolated from canine urinary tract infections: evidence of gene exchange between human and animal enterococci. *J. Clin. Microbiol.* 2002;40:4659-4665.

Tamura K, Dudley J, Nei M & Kumar S. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* 2007;24:1596-1599.

Tenover FC, Arbeit RD, Goering RV, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J. Clin. Microbiol.* 1995;33:2233–2239.

Teshale EH, Painter J, Burr GA, et al. Environmental Sampling for Spores of *Bacillus anthracis. EID* 2002;8:1083-1087.

Tolan RW, Steele RW, Baorto E, et al. (2001, September 2). Staphylococcus Aureus Infection. Retrieved September 13, 2011 from http://emedicine.medscape.com/article/971358.

Trott DJ, Filippich LJ, Bensink JC, et al. Canine model for investigating the impact of oral enrofloxacin on commensal coliforms and colonization with multidrug-resistant *Escherichia coli*. *J Med Microbiol* 2004;53:439-443.

van Duijkeren E, Wolfhagen MJHM, Heck MEOC, et al. Transmission of a Panton-Valentine Leucocidin-Positive, Methicillin-Resistant *Staphylococcus aureus* Strain between Humans and a Dog. *J. Clin. Microbiol.* 2005;43:6209-6211.

Vincze S, Paasch A, Walther B, et al. Multidrug- and Methicillin resistant *Staphylococcus pseudintermedius* as a cause of canine pyoderma: a case report. *Berl Munch Tieraztl Wochenschr* 2010;123:353-358.

Walther B, Wieler LH, Friedrich AW, Kohn B, Brunnberg L, Lubke-Becker A. *Staphylococcus aureus* and MRSA colonization rates among personnel and dogs in a small animal hospital: Association with nosocomial infection. *Berl Munch Tieraztl Wochenschr* 2009; 122: 178-185.

Warren A, Townsend K, King T, et al. Multi-drug resistant *Escherichia coli* with extended-spectrum β-lactamase activity and fluoroquinolone resistance isolated from clinical infections in dogs. *Aust Vet J* 2001;79:621-623.

Weese JS, DaCosta T, Button L, et al. Isolation of Methicillin-Resistant *Staphylococcus aureus* from the Environment in a Veterinary Teaching Hospital. *J Vet Intern Med* 2004;18:468-470.

Weese JS, Dick H, Willey BM, et al. Suspected transmission of methicillin-resistant *Staphylococcus aureus* between domestic pets and humans in veterinary clinics and in the household. *Vet Microbiol.* 2006;115:148-155.

Weese JS. Antimicrobial Resistance in Companion Animals. *Animal Health Research Reviews* 2008;9:169-172