Methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) acquiring resistance to additional classes of antibiotics: potential risk to animal and human health

Undergraduate Research Thesis

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by

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Table of Contents

Abstract

Introduction: *Staphylococcus pseudintermedius,* a bacterium considered part of the normal flora in dogs, poses a serious threat to the canine health system because it carries a large assortment of pathogenicity and virulence genes, which allow it to be capable of developing multi-drug resistance. [1] However, no long-term studies have examined the evolutionary history of MRSP's resistance to antibiotics.

Objective: The research project sought to phenotypically characterize historical MRSP environmental and canine isolates collected from The Ohio State University Veterinary Medical Center from 2007 to 2013.

Results: Linear regression analysis showed that MRSP resistance increased significantly, by approximately one new class of antibiotic every 5 years, in environmental isolates pooled across all hospital locations. MRSP resistance did not increase significantly over time for canine isolates.

Conclusions: MRSP appears to have increased its resistance to different classes of antimicrobial drugs over the past 6 years, suggesting that MRSP is continuing to acquire novel genetic components and become even more pathogenic. Researchers now need to focus on preventive measures that will deter or slow this process.

Literature Review

Introduction

The following literature review summarizes the background relevant to the primary objective of the reported study, which is to determine if *Staphylococcus pseudintermedius* strains circulating in The Ohio State University Veterinary Medical Center (OSU-VMC) have increased resistance to different classes of antibiotics from 2007 to 2013. First, the *Staphylococcus* genus is described as a component of normal human and animal flora to show how it becomes an opportunistic pathogen. Then, the historical use of antibiotics is introduced to illustrate how the two primary methicillin-resistant *Staphylococcus* species of interest, methicillin resistant *S. aureus* (MRSA) and methicillin resistant *S. pseudintermedius* (MRSP), emerged. Of the two, MRSA will be considered first in light of its relationship to MRSP. Then, MRSP will be introduced as the primary species of interest due to its ability to serve as a reservoir of resistant genes for MRSA. Next, the mechanisms by which MRSP has acquired antibiotic resistance will be discussed, to illustrate the ease with which MRSP could transmit its resistant genes to MRSA. Finally, the review will conclude with an outline of the aims of the experiment.

Staphylococcus Genus

Staphylococci are gram-positive, facultative anaerobic, catalase positive, non-motile bacteria that appear as round clusters when viewed underneath a microscope. [2] *Staphylococcus* bacteria make up both human and animal flora by inhabiting carriage sites that include skin and mucosa membranes. [2] In canines, *S. pseudintermedius* is the dominant *Staphylococcus* species that asymptomatically inhabits 46 to 92% of healthy canines' skin and mucosa. [3] In humans, *S. aureus* is the dominant *Staphylococcus* species

that asymptomatically inhabits a third of the human population. [4] Importantly, while *S. pseudintermedius* infrequently colonizes human skin and mucosa, *S. aureus* colonizes about 10% of healthy canines. [4]

While *Staphylococcus* pathogens are normal inhabitants of animal and human skin and mucosa, they become opportunistic pathogens whenever the host immune response is compromised. [5] The genus is comprised of forty opportunistic pathogen species that vary in clinical importance. [2] To separate the genus into groups, staphylococci species are categorized as either coagulase positive or coagulase negative based on their ability to produce the coagulase enzyme. [2] The coagulase enzyme allows the bacterium to convert fibrinogen to fibrin, which allows blood clot formation. [2] Coagulase positive staphylococci can evade certain host immune responses because the clot surrounds the bacteria and renders it invisible to the host system. [2] Of the coagulase positive staphylococci, *S. aureus* and *S. pseudintermedius* are the most clinically relevant pathogens for humans and animals because they naturally colonize a large percentage of these populations through carriage sites.

Furthermore, the *Staphylococcus* genus is known for its ability to acquire resistance to antibiotics. [5] The emergence of methicillin resistant *S. aureus* (MRSA) and methicillin resistant *S. pseudintermedius* (MRSP) has resulted in significant problems for human and veterinary medicine. [5]

Emergence of Antibiotic Resistance

The first major widespread antibiotic, penicillin, has an intimate relationship with staphylococci because it led to the discovery of this genus' resilience. [6] Penicillin was used as a primary treatment option for staph infections and its widespread use led to the

first penicillin resistant *S. aureus* strain being isolated within one year of its use. [6] It only took an additional ten years for penicillin resistant *S. aureus* clones to spread worldwide. [6] When penicillin was no longer a viable treatment option, methicillin became the first line of treatment for staph infections. [7] However, in a fashion similar to penicillin resistance, methicillin resistant *S. aureus* (MRSA) strains quickly emerged.

MRSA. *S. aureus* developed methicillin resistance through the acquisition of an alternative penicillin binding protein (PBP2a), which lowered the bacteria's rapport for methicillin and all other beta-lactam antibiotics. [7] The resistant determining protein PBP2a is harbored on the staphylococcal cassette chromosome (SCC*mec*). [7] The significance of this mobile genetic element is that it has allowed the proliferation of eight distinct SCC*mec* types circulating among the MRSA strains. [7] These SCC*mec* types include insertion sequences, which allow for the integration of plasmids and permit the bacteria to evolve multi-drug resistance. [7] Instead of all strains being descended from a prototypic *S. aureus* strain with one introduction of SCC*mec*, modern MRSA strains are thought to represent independent procurements of SCC*mec*. [8]

MRSA infection can result in different types of clinical manifestations. [8] MRSA has the ability to attack large surgical wounds as well as small puncture wounds made by IVS or catheters, making it the most common cause of infections in hospital patients. [8] Additionally, MRSA can cause both superficial and deep skin infections that result in boils, styes, or more serious types of invasive infections like bacteremia. [8]

By the mid-1970s, MRSA had spread to many, if not all, hospitals across the United States.[9] MRSA isolates increased from 2.1% in 1975 to 35% in 1991. [10] By 2005, the CDC estimated 94,360 invasive MRSA infections occurred in the United States. [9]

MRSA cases were not only spreading through hospitals via hospital-acquired MRSA but also through the community via community associated MRSA (CA-MRSA).[9] In 1999, four deaths caused by CA-MRSA in otherwise healthy children were reported.[11] While researchers are interested in CA-MRSA because of its ability to cause severe, aggressive diseases, the distinction between HA-MRSA and CA-MRSA is somewhat blurred as CA-MRSA strains have been returning to hospital settings.[12]

Coinciding with the increase in MRSA infections in humans, the number of MRSA infections in canines has also increased over the past 10 years.[4] While *S. pseudintermedius* is the primary staphylococci species found colonizing canines, *S. aureus* is isolated in about 10% of canines.[4] Although MRSA colonization occurs in less than 1% of canines, several reports have established a rising number of canine MRSA infections resulting from open wounds and post-operative infections.[4] MRSA infections in canines share similar clinical manifestations as those in humans, including dermatitis and pyoderma.[4]

Because of the rising prevalence of MRSA infections in veterinary settings, concerns have arisen that MRSA's similarity and relatedness to MRSP may permit the species to horizontally transfer resistance factors.[1] Staphylococcal species have been generally assumed to form a gene pool from which all of the species can exchange mobile gene elements.[13] Because MRSP and MRSA share a common environment in the veterinary field, the two species could potentially transfer genetic elements to give rise to either a MRSP or MRSA clone that poses an even higher threat to human and veterinary health.

MRSP. Over the past decade, *S. pseudintermedius,* a bacteria considered part of the normal flora in dogs, has emerged as an opportunistic pathogen in the canine world. [1] This pathogen poses a serious threat to canine health because it carries a large assortment of pathogenicity and virulence genes, which allow it to persist in adverse environments and to be capable of developing multi-drug resistance. [1] By asymptomatically inhabiting the nostrils, mouths, and anuses of healthy companion animals, *S. pseudintermedius* has evolved biological weapons to use against the animals whenever their immune systems' ability to fight foreign bacteria is weakened. [1] For these reasons, *S. pseudintermedius* has become a significant pathogen responsible for causing severe veterinary hospital acquired infections.

At some point in the recent past, *S. pseudintermedius* transitioned from being a susceptible bacterium, which was a part of canines' normal flora, to being a microorganism resistant to beta-lactam antibiotics and additional classes of antibiotics.[1] MRSP isolates have increased from 5% to 30% of isolates collected in the United States from 2001 to 2007. [14]

Similar to MRSA, methicillin resistance in *S. pseudintermedius* is attributed to the *mec*A gene. [15] Two new staphylococcal cassette chromosome *mec* (SCC*mec*) elements, SCC*mec* II-III and VII, were detected in MRSP isolates, and represent two more reservoirs that can harbor the *mec*A gene and aid in the transfer of methicillin resistance to other staphylococcal species. [15] SCC*mec* II-III, SCC*mec* V and SCC*mec* VII-241 are all present in MRSP isolates. [13] SCC*mec* VII-241 is a novel element in MRSP isolates and is not related to any SCC*mec* elements found in *S. aureus*. [13] SCC*mec* II-III is a

hybrid of SCC*mec* II from *S. epidermidis* and of SCC*mec* III from *S. aureus*. [13] SCC*mec* V is homologous to the element in *S. aureus*. [13]

In addition to resistance to methicillin, high percentages of MRSP isolates collected from Europe and North America were found to be resistant to different classes of antibiotics. [13] In addition to *mec*-A mediated beta-lactam resistance, isolates were also found to be resistant to trimethoprim (90.3%), gentamicin/kanamycin (88.3%), streptomycin (90.3%), macrolides/lincosamides (89.3%), fluoroquinolone (87.4%), tetracyclin (69.9%), chloramphenicol (57.3%), and rifampicin (1.9%). [13]

Multi-drug resistant MRSP poses a serious threat to veterinary hospitals because all patients and personnel are at risk of carrying and transmitting the pathogen.[1] Close contact between patients, patient owners, employees, and the environment increases the likelihood of cross-contamination and transmission.[1] This contamination and continuous dissemination of MRSP increases the likelihood it will infect those it comes into contact with.[1] With the increased prevalence of MRSP in veterinary hospitals also comes increased contact with antibiotics. Bacteria must be able to adapt to changing environments to survive; in the case of *S. pseudintermedius*, this adaptation includes its ability to adapt to antibiotics.[1]

S. pseudintermedius contains numerous mobile genetic elements, including insertion elements, transposons, and an integrated plasmid and is, therefore, a bacterium that is capable of gaining and transferring DNA. [16] One study compared the changing numbers of *S. pseudintermedius* isolates resistant to antimicrobial drugs to the use of those specific antimicrobial drugs over the same time period in a veterinary clinic and found a pattern of resistance that reflected drug usage. [17] This study suggests that human

use of antimicrobial drugs poses a selective pressure on the bacteria that causes an increase in resistance that is specific to the drugs used. [17]

Data reflecting the rapid proliferation of a multi-drug resistant MRSP strain throughout Europe within a short time period highlights the need to better understand *S. pseudintermedius'* evolution and acquirement of resistant genes, if we hope to limit its spread. [18] Prudent use of antimicrobial drugs in the veterinary setting is crucial in controlling the dissemination of more successful and more resistant clones.

Transmission of Antibiotic Resistance

Antibiotic drug use causes evolution of bacteria via the mechanism of natural selection; therefore, antibiotic resistance is an outcome of the selection for resistant bacteria. [19] Antibiotic resistance can be either natural or acquired, the latter being a consequence of the selective pressure exerted by antimicrobial drugs. [19] A bacterium can acquire resistance by mutations or the acquisition of extrachromosomal DNA. [19] Thus, mobile genetic elements, which can be transferred horizontally between bacteria, play a major role in acquired antibiotic resistance. [19] For example, bacteria have evolved accessory pieces of DNA that are separate from the chromosome itself. [20] These plasmids are independently duplicating genetic elements, which allow the bacteria to alter its genetic layout in the presence of new conditions. [20] Plasmids are the essential genetic components that allow bacteria to acquire and carry antibiotic resistance. [20] Bacteria can transfer plasmids via a process known as conjugation. [20] Acquiring antibiotic resistance via the transmission of extrachromosomal DNA including plasmids is known as horizontal transmission. Therefore, horizontal transmission is a common pathway through which *S. pseudintermedius* and *S. aureus* share resistance factors.

As mentioned earlier, *S. pseudintermedius* is increasingly suspected of serving as a reservoir of resistant genes for other Staphylococcus species. Additionally, due to our inability to determine directionality of transfer, *S. pseudintermedius* could also serve as a recipient of resistant genes from other Staphylococcus species. Whether serving as a reservoir or recipient, the possibility that *S. pseudintermedius* could acquire more resistance or that other Staphylococcal species could acquire more resistance should be a major concern for animal and human health. The reason that *S. pseudintermedius* poses the ability to serve as both a reservoir and recipient is because humans share close contact with their pet animals, which allows *S. pseudintermedius* to transfer to humans and *S. aureus* to transfer to animals. [21] Several studies have examined the ability of humans to carry *S. pseudintermedius*. One study found that the *S. pseudintermedius* strains occurring in dog-owners were found to be identical to the strains found in their dogs, which suggests a possible transfer of those bacterial strains between dog and human. [21] Therefore, *S. pseudintermedius* and *S. aureus* can interact both on dogs who are carriers and humans who are carriers. While the studies do not suggest a direction of transfer or mode of transmission, bacteria carrying resistance genes can transfer them irrespective of directionality. In conclusion, because *S. pseudintermedius* harbors genes and plasmids related to those of *S. aureus*, there is a risk of genetic exchange between these staphylococcal species.

Aims

Thus, given time and exposure to antibiotics, MRSP has been postulated to be increasing its resistance to antibiotics.[1] However, no long-term studies have directly examined the evolutionary history of MRSP's resistance to antibiotics. Therefore, the

objective of the proposed research project is to phenotypically characterize historical MRSP isolates from the environment and from canines that were collected at OSU-VMC from 2007 to 2013. The phenotypic analysis will allow us to determine if the strains circulating in the OSU-VMC have increased their resistance over the years. The central hypothesis is that MRSP has been accumulating antibiotic resistance to an increasingly large number of classes of antimicrobial drugs. This research will also contribute to the larger objective of understanding the epidemiology of MRSP, with an end goal of controlling the dissemination and maintenance of this emerging nosocomial pathogen in veterinary hospitals.

Materials and methods

Source of Isolates

A total of 211 MRSP isolates were collected at OSU-VMC between 2007-2013 through active and passive surveillance. Isolates came from both the environment (130 isolates) and from incoming canine patients (81 isolates). From November 2007 to October 2008, samplings of canines and the environment were conducted once every month. From November 2009 to November 2013, samplings of the environment were conducted once every 3 months.

Specific services that were targeted for the environmental sampling were community practice (examination room and treatment area), dermatology (treatment room and wards), intensive care unit, surgery (pre-surgery room, anesthesia room, and surgery suites and wards), and rehabilitation. Within each hospital service, specific surfaces were chosen based on amount of contact during routine daily activities. High-contact surfaces were chosen as they had a higher likelihood of being contaminated.

Furthermore, a surface was labeled as a human contact surface if it was regularly touched by humans throughout the day and generally out of reach from direct contact with animals (e.g., computers). In comparison, a surface was labeled as an animal contact surface if it was primarily in direct contact with multiple animals (e.g., cages). Table 1 (see next page) outlines which environmental surfaces were considered human or animal contact surfaces.

During only the first year, in addition to the environmental samples, incoming dogs admitted to the hospital were sampled upon their arrival if they had not been in the hospital in the past 6 months. For each enrolled canine, sterile pre-moistened cotton swabs were used to collect samples from the nasal cavity, ear canals, external surface of the perianal area, and any skin lesions (if present).

S. pseudintermedius samples were identified using standard microbiological and biochemical tests. MRSP isolation and identification began with samples being incubated at 35°C in pre-enrichment media for 24 hours and then streaked onto mannitol salt agar (BD BBL™ Mannitol Salt Agar, Dickinson and Company) with 2 μg/mL of oxacillin. After 24 to 48 hours, 1 to 3 colonies per sample were selected and streaked onto trypticase soy agar with 5% sheep blood (Remel®, Blood Agar [trypticase soy agar, TSA, with 5% sheep blood], Lenexa, KS). Identification of *S. pseudintermedius* was performed by standard colony morphology and biochemical tests reactions that included mannitol fermentation, gram stain, catalase, tube coagulase, anillin fermentation, Polymyxin B susceptibility, acetoin production (Vogues-Proskauer test) and latex agglutination (Sure-Vue® Color Staph ID, Biokit USA, Inc., Lexington, MA). [22] Phenotypic MRSP confirmation was performed by growth on Oxacillin Screen Agar® (OSA) plates

containing 6 μg/mL of oxacillin supplemented with NaCl (BD BBLTM, Becton Dickinson and Company) following the Clinical Laboratory Standards Institute (CLSI) protocols.[23]

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility profiles of 130 environmental isolates and 81 canine isolates were determined by testing against 16 antibiotics frequently used in both the veterinary and human field of medicine, using the Kirby Bauer Disk Diffusion technique established by CLSI. [23] Antimicrobials included were: amikacin 30 μg, ampicillin 10 μg, amoxicillin with clavulanic acid 20/10 μg, cefpodoxime 10 μg, cephalothin 30 μg, chloramphenicol 30 μg, ciprofloxacin 2 μg, clindamycin 2 μg, doxycycline 30 μg, enrofloxacin 5μg, erythromycin 15 μg, gentamicin 1 μg, oxacillin 1 μg, sulfamethoxazole with trimethoprim 1.25/23.75 μg, and tetracycline 30 μg. Vancomycin resistance was tested using Vancomycin Screen Agar plates (6 mg/L) (BD BBL™ Vancomycin Screen Agar, Dickinson and Company, Sparks, USA). Inducible Clindamycin resistance was tested using the D-test. [24] Antimicrobial susceptibility patterns were grouped in profiles based on their pattern similarity, and later classified as multidrug resistant (MDR) if they were resistant to 3 or more classes of antimicrobials (including beta-lactams). For quality control purposes, six strains were included in each round: *S. aureus* (ATCC 43300), *S. aureus* (ATCC 29213), *S. aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 23212), *Escherichia coli* (ATCC 25922), and *Pseudomonas aeruginosa* (ATCC 27853). All samples were processed in the Diagnostic and Research Laboratory of Infectious Diseases (DRLID), which is a Bio Safety Level 2 laboratory (rooms 367, 371 and 375) located at the Veterinary Medicine Academic Building (VMAB) of OSU.

Table 1: Human and Canine Contact Surfaces Sampled with Electrostatic Cloths (✖) or Sterile Swabs (★) by Service at the Small Animal Hospital in the Ohio State University Veterinary Medical Center during Surveillance.

Hospital service	*Human contact surface*	*Animal contact surface*
Community practice	Doors ✖ Computers ✖[a,b] Otoscope ★	Exam tables ✖[a] Floor (exam) ✖[a] Muzzles ★
Dermatology	Doors (ward) ✖ Exam lights ✖ Fax/phone ★ Computer ★[b] Microscope ★ Otoscope ★ Paper towel dispenser ★[a] Alcohol-gel dispensers ★[a]	Cages (ward) ✖ Floors (exam) ✖ Muzzles (ward) ★ Water bowels (ward) ★
Intensive care unit	Doors ✖ IV pumps ★ Computer ★[a,b] Laptop ★	Cages (2) ✖ Muzzles ★ Water bowls ★
Surgery	Clippers ★ Doors ✖ Drawer handles ✖ Exam lights ✖[a] Light switches ✖[a] Table knobs ✖[a]	Cages ✖ Exam tables ✖ Muzzles (ward) ★ Oxygen monitors ★ Warming pads ✖[c] Water bowls ★
General	NA	Carts/gurney ✖ (3)
Rehabilitation	Doors ✖	Walls of the pool ✖ Floaties ✖ Mats ✖ Balls ✖ Slings ✖ Muzzles ★ Laser ★

[a]Samples collected as a pool within the same service.
[b]Included keyboard and mouse.
[c]Multiple warming pads located in the same room were sampled as a pool.
IV, intravenous; NA, not applicable.

Statistical Analysis

All results were organized in an Excel spreadsheet and basic frequencies and distributions were generated. Boxplots were used to compare distributions of mean number of resistant classes between services (community practice, dermatology, general, intensive care unit, rehabilitation, and surgery). A one-way ANOVA model was used to compare the mean number of resistant classes for each of the six locations. Simple linear regression models were used to fit basic time series models, using months as the independent variable and number of resistant classes as the dependent variable. Multiple regression models were considered to incorporate the possible impact of location and time on the prediction of resistance. Regression coefficients were obtained using the statistical software Minitab statistical software (Version 16.2.4). Relationships were considered statistically significant when their *P*-value (two-sided) was ≤ 0.05.

Results

Phenotypic characterization of MRSP environmental isolates

A total of 130 MRSP environmental isolates that were collected from December 2007 to November 2013 at OSU-VTH were phenotypically characterized. As expected, all of the MRSP environmental isolates were resistant to antibiotic class beta-lactams. The vast majority of the MRSP environmental isolates (94%) were multi-drug resistant or resistant to 3 or more classes of antibiotics. Lincosamide (90%), potentiated sulfonamide (81%), macrolides (78%), and quinolone (78%) were the most frequent classes of antibiotics to which MRSP environmental isolates were resistant. Around half of the MRSP environmental isolates (52% and 41%) were resistant to tetracycline and aminoglycoside, respectively. All MRSP environmental isolates except for 8 were

susceptible to antibiotic class phenicol (94%) and all of the MRSP environmental isolates were susceptible to antibiotic class glycopeptides (100%). The most frequent class profile of resistance (28%) included beta-lactams, quinolone, macrolides, lincosamide, aminoglycoside, and potentiated sulfonamide (see Table 1). All MRSP environmental isolates were susceptible to amikacin and vancomycin (see Table 2).

Antibiotic resistance by hospital surface for MRSP environmental isolates

The distributions of the number of antibiotic classes to which MRSP environmental isolates were resistant across different hospital surfaces were inspected using box and whisker plots (see Figure 1). The mean number of resistant antibiotic classes did not vary significantly by location ($F= 0.64$, $P= 0.671$), suggesting that antibiotic resistance was not a function of the specific hospital surface that was sampled.

Antibiotic resistance over time for MRSP environmental isolates

MRSP environmental isolates increased their antibiotic resistance over time, as reflected in a significant positive relationship between the number of resistant antibiotic classes and the month when samples were obtained ($F= 9.05$, $P= 0.003$) (see Figure 2). The number of classes to which the MSRP environmental isolates were resistant increased by roughly 1 class every 4 to 5 years. To determine if a non-linear model would account for more of the variance in antibiotic resistance, locally weighted scatterplot smoothing was used to obtain an alternative model that shows the best possible fit to the data (Figure 3). The resulting model showed that antibiotic resistance increased linearly after the first 10 months of data collection, suggesting that the linear regression model provides an adequate fit to the data.

Next, the possible impact of hospital service on the relationship of antibiotic resistance to time of sampling was explored. For dermatology only, the number of resistant classes of antibiotics was positively related to the month when sampling occurred (F= 6.08, *P*= 0.018) (see Figure 4). The relationship of antibiotic resistance to time of sampling was not significant in any of the other locations; this may be because of the small number of samples available for most specific locations.

Antibiotic resistance by type of contact surface (human versus animal) for MRSP environmental isolates

The number of antibiotic classes showing MRSP resistance did not differ significantly between human and animal contact surfaces. When controlling for the month when sampling occurred, the mean number of resistant antibiotic classes did not vary significantly across human and animal contact surfaces (F= 0.319, *P*= 0.573).

Phenotypic characterization of MRSP canine isolates

A total of 81 MRSP canine isolates that were collected from November 2007 to October 2008 at OSU-VTH were phenotypically characterized. The vast majority of canine isolates (91.4%) were multi-drug resistant. As expected, all of the canine isolates were resistant to antibiotic class beta-lactams. Lincosamide (99%), macrolides (81%), potentiated sulfonamide (64%), and quinolone (63%) were the most frequent classes of antibiotics to which MRSP canine isolates were resistant. A little under half of the isolates (42% and 33%) were resistant to aminoglycoside and tetracycline, respectively. All of the MRSP canine isolates except for one were susceptible to phenicol, and all of the MRSP canine isolates were susceptible to glycopeptides (see Table 3). All of the

isolates were susceptible to vancomycin, and 98% of the isolates were susceptible to amikacin and chloramphenicol (see Table 2).

Antibiotic resistance over time for MRSP canine isolates

The number of antibiotic classes to which MRSP canine isolates were resistant did not increase significantly as a function of when sampling occurred (F= 2.19, *P*= 0.143) (see Figure 5). The number of resistant antibiotic classes across different anatomical locations-ears, lesion, nose, and perianal-were inspected with box and whisker plots (see Figure 6). The mean number of resistant antibiotic classes did not vary significantly across anatomical locations (F= 0.64, *P*= 0.671).

Discussion

While MRSP infections continue to rise in prevalence and an increasing number of MRSP strains are reported to be multi-drug resistant, no long-term studies have tracked changes in MRSP's resistance to antibiotics over time to determine whether MRSP is indeed acquiring resistance to additional classes of antibiotics. Therefore, this study sought to phenotypically characterize historical MRSP canine and environmental isolates that were collected from OSU-VTH over the span of six years to examine MRSP's antibiotic resistance during that period. The study's objective was accomplished, as 211 isolates were phenotypically characterized and changes in susceptibility patterns were observed. The importance of phenotypically characterizing these MRSP isolates stems from the risk MRSP poses to public health because of its unique relationship with MRSA.

First, the results indicated that both environmental and canine MRSP isolates show high rates of antimicrobial resistance. Ninety four percent of MRSP environmental

isolates and 91% of MRSP canine isolates were multi-drug resistant. This high rate of antimicrobial resistance is reflected in a similar multi-center study conducted by Perreten et al. [13] They found MRSP isolates to be resistant to trimethoprim (90.3%), gentamicin (88.3%), macrolides and/or lincosamide (89.3%), tetracycline (69.9%), and chloramphenicol (57.3%). [13] Similarly, our study found MRSP environmental and canine isolates to be resistant to trimethoprim (81/64%), gentamicin (41/42%), macrolides (78/80%), lincosamide (78/78%), tetracycline (51/33%), and chloramphenicol (6/1%) respectively (see Table 2). Although our MRSP isolates showed slightly lower proportions of resistance than those reported by Perreten et al., both studies found MRSP isolates to have high rates of antimicrobial resistance and, more importantly, resistance to multiple antimicrobials regularly used in veterinary medicine. [13] Furthermore, no other studies exist to suggest that MRSP has low resistance to antimicrobials. Therefore, the current results highlight a serious problem that can potentially impact animal health when trying to treat MRSP infections, as clinically available treatment options are extremely limited.

Second, the results indicated that the distribution of antibiotic resistance across hospital services (community practice, dermatology, ICU, surgery, and rehabilitation) and between type of contact surface (animal versus human) for MRSP environmental and canine isolates did not vary significantly. No studies exist showing that the distribution of antibiotic resistance for MRSP isolates vary significantly between type of contact surface and across services. However, prior studies have illustrated the ability of MRSA to contaminate both human and animal contact surfaces, consistent with the current findings involving MRSP. [25] Our finding, paired with van Balen et al., [25] highlights the concern

that MRSA and MRSP potentially share three common environments- human carriage sites, canine carriage sites, and environmental surfaces contacted frequently by animals and/or humans. In any of these environments, MRSA and MRSP could potentially share resistance genes.

Third, the results suggest that MRSP environmental isolates are becoming resistant to one new antibiotic class approximately every 5 years. Although we found that a large proportion of MRSP isolates are resistant to many antimicrobials frequently used in veterinary medicine, the increase in resistant classes over the study period indicates that MRSP environmental isolates are continuing to acquire resistance. No long-term studies have tracked changes in MRSP's resistance to antibiotic classes over time. Therefore, it is quite difficult to compare our results due to the lack of publications. In any case, this finding adds to the pressure on personnel within the veterinary field to create a system that slows the spread of resistance in this type of pathogen. If this trend of becoming resistant to more and more classes of antibiotics continues, then MRSP infections may become incurable, as no treatment options will remain.

For these reasons, veterinarians need to be aware of the effectiveness of particular antibiotics when combating MRSP infections. The continued use of ineffective antibiotics could be potentially harmful to their patients, and the overuse of effective antibiotics could hasten the process by which MRSP isolates gain resistance. As a result, veterinarians face a dilemma: Do they introduce new antibiotics into the system that will likely result in the bacteria becoming resistant to them or continue to rely upon antibiotics that will continue to become less effective as resistance spreads? Additionally, the

process of developing a new antibiotic is a long, resource intensive, and complex process that many pharmaceutical companies are not willing to undertake. [6]

Therefore, veterinarians need to combine a rigorous antimicrobial drug use policy with a stringent cleaning procedure of the environment and hygiene protocol for veterinary hospital personnel if they want to limit the spread of antibiotic resistance among MRSP isolates. The drug use policy needs to require MRSP infections to be phenotypically characterized before a specific antibiotic is chosen, and canines need to be followed closely over the course of treatment to ensure minimal spread of the bacteria to the environment, other dogs, and humans. Prudent use of antimicrobials combined with cleaning and hygiene protocols are the primary methods for controlling the spread of resistant bacteria.

One of the primary limitations of the study is that the design is correlational/observational and not experimental, which precludes any definitive conclusions about causation. Nevertheless, the results show that MRSP antibiotic resistance is increasing, even though they cannot serve as a basis for identifying specific mechanisms. Second, the number of MRSP canine isolates was relatively small because they were collected over a shorter amount of time (1 year) as compared to the MRSP environmental isolates (6 years). This limitation makes the power of statistical analysis lower for the MRSP canine isolates than that for the analysis of the MRSP environmental isolates. However, high antimicrobial resistance was clearly demonstrated in both types of isolates. Lastly, the generalizability of our results to other hospitals within the US is uncertain, as all of our data were collected from a single teaching hospital. However, the

findings of antimicrobial resistance are consistent with those reported from other hospitals.

In conclusion, this study has demonstrated the importance of tracking the evolution of a bacterium that poses serious issues to veterinary medicine, and indirectly, to public health. For animal health, if MRSP continues to acquire resistance, MRSP infections will become ever more challenging to prevent and treat. For public health, if MRSP and MRSA potentially transfer antimicrobial resistant genes between one another because they are both present in multiple common environments, then MRSA might also become more resistant and difficult to treat in human and canine cases. Our findings highlight the importance of antimicrobial stewardship, cleaning, and hygiene protocols and continuing to perform surveillance of MRSP isolates.

This thesis represents the retrospective component of a much larger study. The results of my study will be compared to current isolates being collected at OSU-VMC. The larger research project will be a comprehensive study, with both prospective and retrospective components, that analyzes MRSP in a veterinary teaching hospital. A second team member will be performing the analysis of the current MRSP isolates, and the studies will be combined prior to publication. Molecular characterization will be performed as well. The long-term goal is to increase our knowledge of the changing epidemiology of MRSP and thereby foster the development of an infectious disease control plan that will decrease the dissemination of MRSP throughout the OSU-VMC, and that can be shared with other veterinary hospitals worldwide.

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Appendix

Table 1: MRSP environmental isolates categorized into groups by antibiotic class profile. The number of isolates (frequency) and percentage of each profile is listed.

Antibiotic Class Profile	Number of Antibiotics Classes	Frequency	Percent
B, Q, M, A, L, S	6	37	28.5
B, Q, M, L, T, S	6	36	27.7
B, Q, M, L, S	5	14	10.8
B, Q, L, T	4	7	5.4
B, Q, M, A, L, T, S, P	8	4	3.1
B, L, T	3	4	3.1
B	1	4	3.1
B, Q, M, L, T, S, P	7	3	2.3
B, A	2	3	2.3
B, M, L, T, S	5	2	1.5
B, M, L, T	4	2	1.5
B, M, A, L	4	2	1.5
B, L, S	3	2	1.5
B, A, T, S	4	2	1.5
B, A, T	3	2	1.5
B, A, L, T, S	5	2	1.5
B, T, S	3	1	0.8
B, T	2	1	0.8
B, M, L, T, S, P	6	1	0.8
B, M, A, L, T, S	6	1	0.8

B=Beta-lactams, Q=Quinolone, M=Macrolides, A=Aminoglycoside, L=Lincosamide, S=Potentiated Sulfonamide, T=Tetracycline, P=Phenicol

Table 2: Description of the relative effectiveness of each antibiotic against both MRSP environmental (VTH) and canine (CAN) isolates.

Antibiotic Effectiveness Against Canine and VTH MRSP Isolates								
			Resistant (%)		**Intermediate (%)**		**Susceptible (%)**	
Antibiotic Class	**Antibiotic**	**Abr.**	**Can**	**VTH**	**Can**	**VTH**	**Can**	**VTH**
Aminoglycoside	Gentamicin	GEN	34 (42%)	53 (41%)	7 (9%)	19 (15%)	40 (49%)	58 (45%)
	Amikacin	AMK	1 (1%)	0 (0%)	1 (1%)	0 (0%)	79 (98%)	130 (100%)
Beta-Lactams	Ampicillin	AMP	81 (100%)	130 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	Amoxicillin with Clavunate	AMC	39 (48%)	62 (48%)	0 (0%)	0 (0%)	42 (52%)	68 (52%)
	Oxacillin	OXA	57 (70%)	101 (78%)	6 (7%)	1 (1%)	18 (22%)	28 (22%)
	Cephalotin	CEP	19 (23%)	43 (33%)	9 (11%)	12 (9%)	53 (65%)	75 (58%)
	Cefpodoxime	CPD	66 (81%)	111 (85%)	12 (15%)	9 (7%)	3 (4%)	10 (8%)
Glycopeptides	Vancomycin	VAN	0 (0%)	0 (0%)	0 (0%)	0 (0%)	81 (100%)	130 (100%)
Lincosamide	Clindamycin	CLI	63 (78%)	102 (78%)	2 (2%)	0 (0%)	16 (20%)	28 (22%)
Macrolides	Erythromycin	ERY	65 (80%)	102 (78%)	0 (0%)	0 (0%)	16 (20%)	28 (22%)
Phenicol	Chloramphenicol	CHL	1 (1%)	8 (6%)	1 (1%)	0 (0%)	79 (98%)	122 (94%)
Quinolone	Enrofloxacin	ENO	49 (60%)	101 (78%)	2 (2%)	6 (5%)	30 (37%)	23 (18%)
	Ciprofloxacin	CIP	51 (63%)	101 (78%)	2 (2%)	6 (5%)	28 (35%)	23 (18%)
Tetracycline	Tetracycline	TET	27 (33%)	66 (51%)	1 (1%)	0 (0%)	53 (65%)	64 (49%)
	Doxycycline	DOX	27 (33%)	66 (51%)	0 (0%)	0 (0%)	54 (67%)	64 (49%)
Potentiated Sulfonamide	Sulfamethoxazole with Trimethoprim	SXT	52 (64%)	105 (81%)	1 (1%)	7 (5%)	28 (35%)	18 (14%)

Table 3: MRSP canine isolates categorized into groups by antibiotic class profile. The number of isolates (frequency) and percentage of each profile is listed.

Antibiotic Class Profile	Number of Antibiotic Classes	Frequency	Percent
B, A, L, M, Q, S	6	25	30.9
B, L, M, Q, S	5	10	12.3
B, L, M	3	8	9.9
B, L, M, T	4	7	8.6
B, L	2	7	8.6
B, L, M, Q, T, S	6	6	7.4
B, L, T	3	5	6.2
B, A, L, M, Q, T, S	7	4	4.9
B, A, L, Q, S	5	3	3.7
B, L, M, T, S	5	2	2.5
B, L, Q	3	1	1.2
B, L, M, P, Q, T, S	7	1	1.2
B, A, M, Q, T, S	6	1	1.2
B, A, L, M, T	5	1	1.2

B=Beta-lactams, Q=Quinolone, M=Macrolides, A=Aminoglycoside, L=Lincosamide, S=Potentiated Sulfonamide, T=Tetracycline, P=Phenicol

Figure 1: Box and whisker plot used to compare distribution of the number of classes of antibiotics that MRSP environmental isolates were resistant to by location.

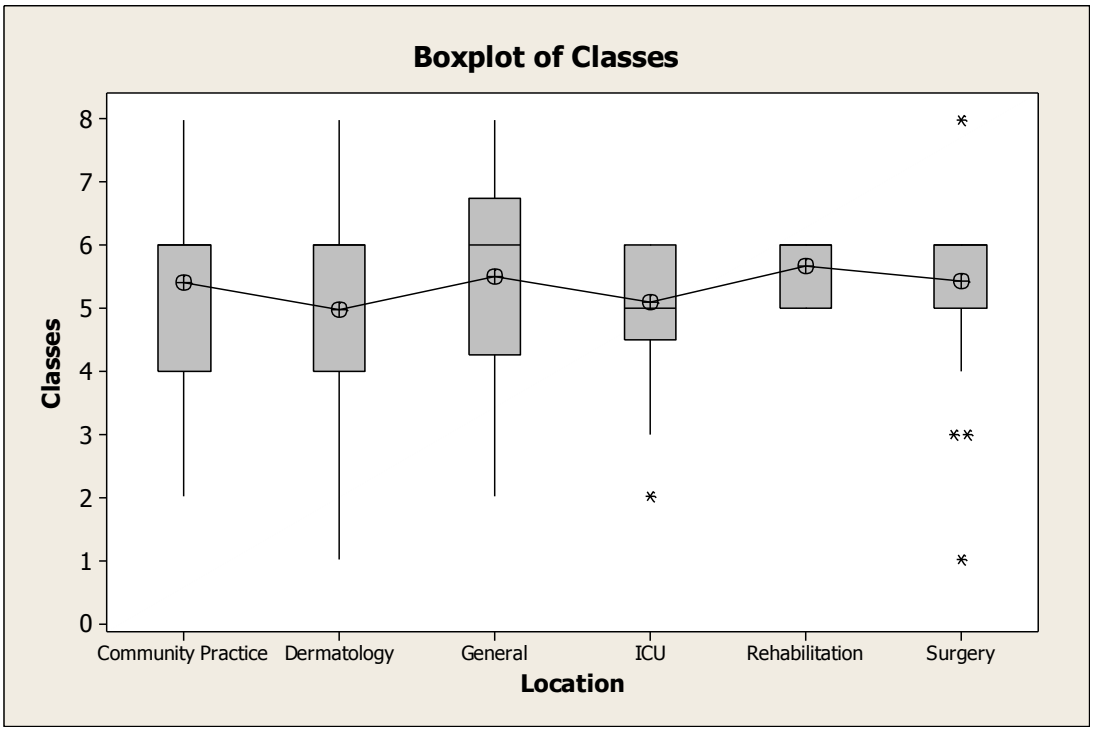


Figure 2: Regression analysis of antibiotic classes versus months for MRSP environmental isolates.

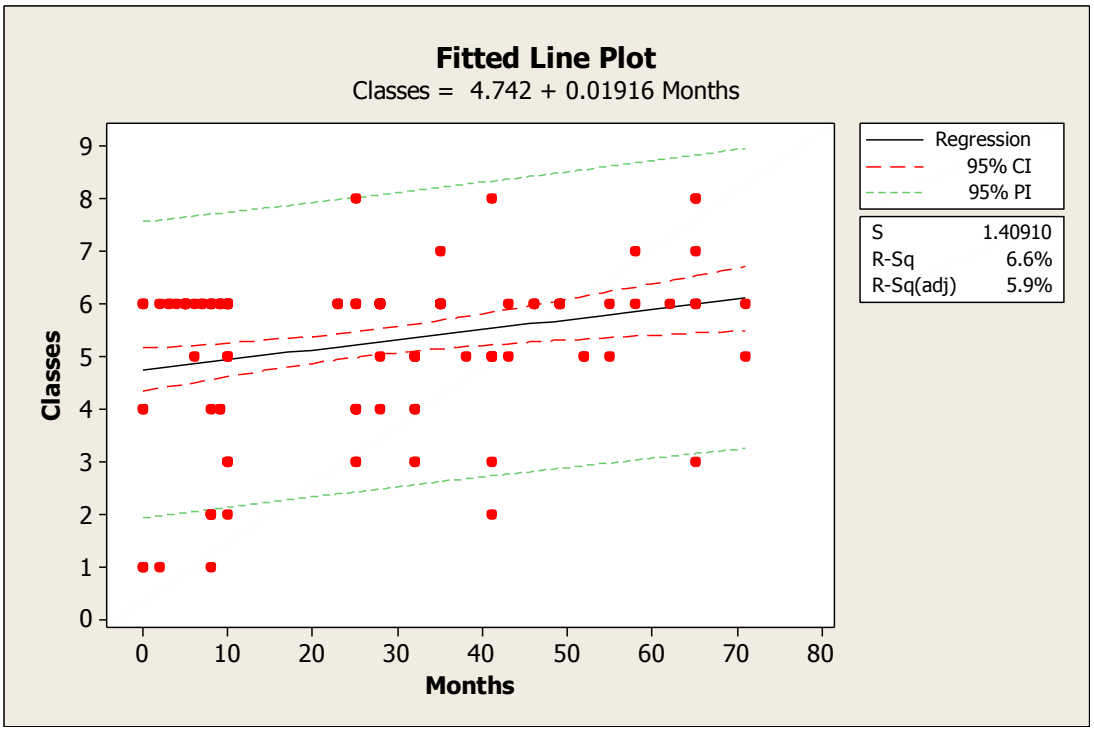


Figure 3: Nonparametric regression for antibiotic classes versus months for MRSP environmental samples.

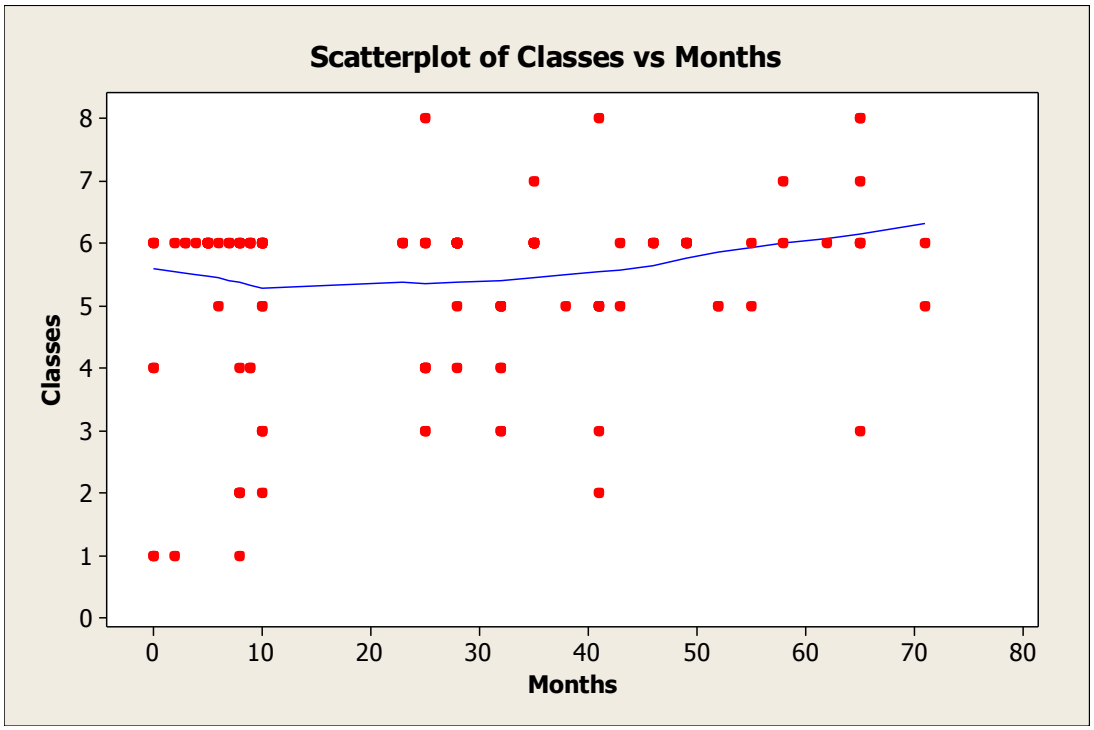


Figure 4: Regression analysis for MRSP environmental isolates collected from dermatology.

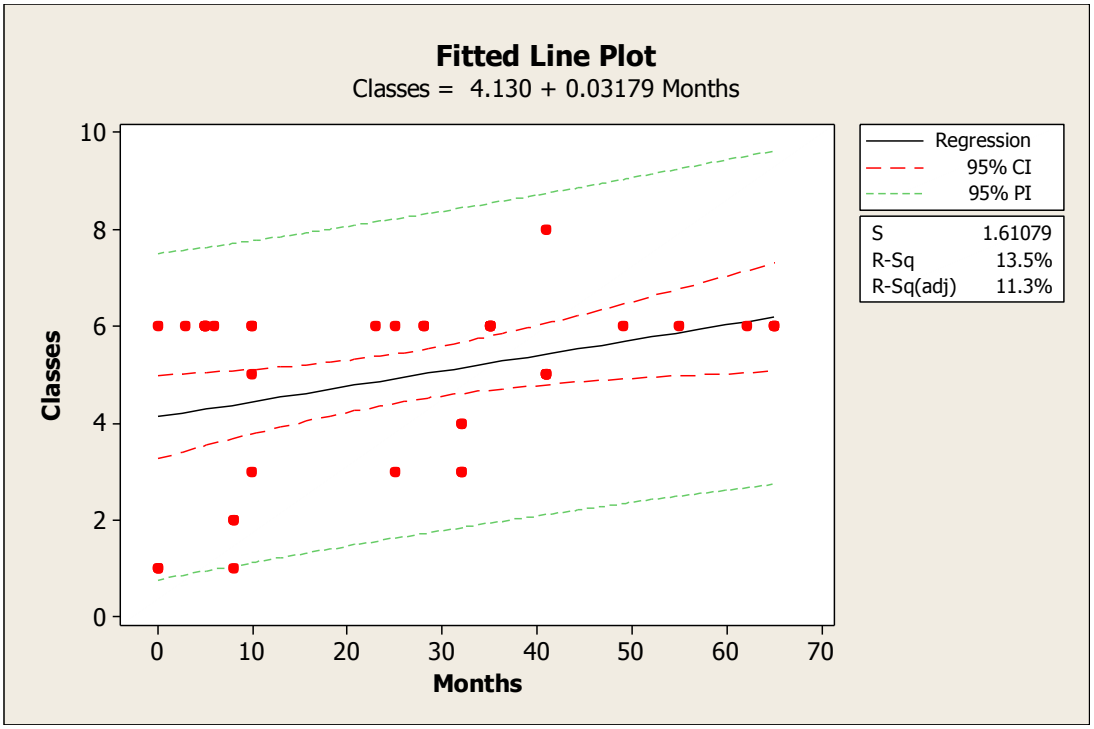


Figure 5: Regression analysis of antibiotic classes versus months for MRSP canine isolates.

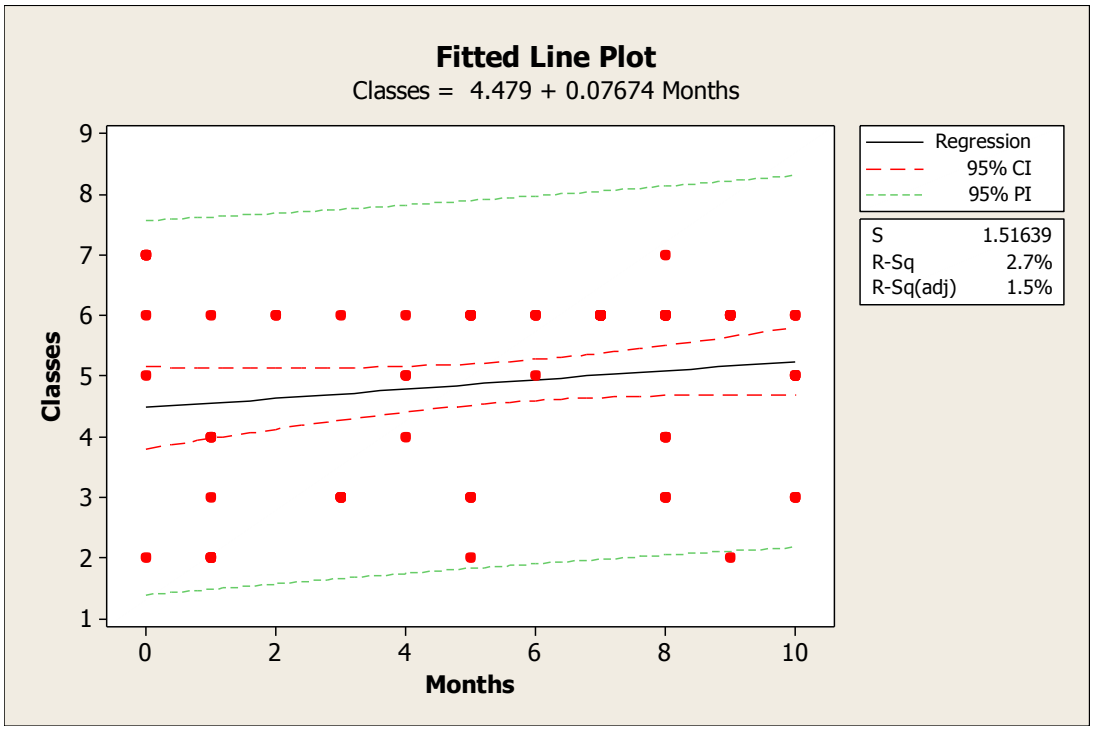
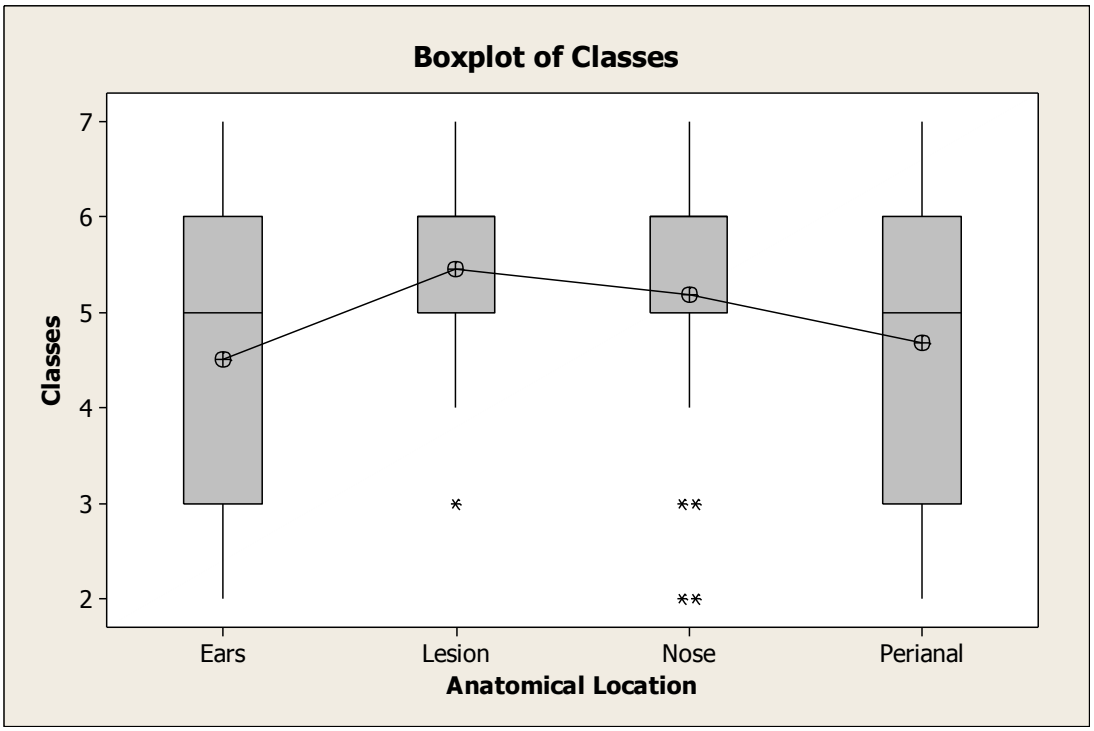


Figure 6: Boxplot of antibiotic classes versus anatomical location for MRSP canine isolates.



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