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The role of antibiotic usage in the acquisition of community-acquired methicillin-resistant Staphylococcus aureus (CA-MRSA) among Division III football players at Moravian College

Introduction

Bacteria are prokaryotic organisms that exist in all habitats. In humans, many species are transported in the anterior nasal passages (or nares) and on the epidermis. The genus Staphylococcus is characteristic of Gram-positive cocci that exist as single cells or in "grape-like" clusters. Like many bacteria, staphylococci are common flora of the skin but become infectious when granted entry through portals such as wounds, urogenital and respiratory tracts, hair follicles and glands. Bacteria in this genus are pyogenic (pus-forming) and cause local cutaneous infections (LCIs) such as folliculitis and impetigo as well as systemic infections like osteomyelitis.

Staphylococcus aureus is the most pathogenic species of staphylococci. With a generation time of 20-30 minutes and the ability to grow in conditions of high salt concentration and between 10 and 46oC, S. aureus has great potential for virulence. An example of a serious systemic epidermal infection is that of staphylococcal scalded skin syndrome, or SSSS. As is true for most bacterial infections, treatment includes antimicrobials to help the immune system combat the bacteria causing the infection. However, evolution has resulted in mutations that enable potentially harmful bacteria such as S. aureus to resist the chemical activity of antibiotics.

Resistance to therapeutic antibiotics began only a few years after Alexander Fleming's penicillin was first used in 1941. Methicillin-resistant S. aureus, or MRSA [Figure 1], is one of numerous bacteria that has mutated into a form only treatable with the highest doses of very few antibiotics. While the femA gene differentiates S. aureus from other staphylococci, mecA is one gene that differentiates MRSA from methicillin-susceptible (non-mutant) S. aureus (MSSA). Since the 1980s, MRSA has increased in prevalence more than any other antibiotic-resistant bacteria [Figure 2]. If diagnosed late, intravenous administration of medicine and surgery to remove infected tissue are two of only a few viable options for treatment. At-risk populations include hospital patients of long-term stay (i.e. ICU) and athletes in contact sports such as football and wrestling.

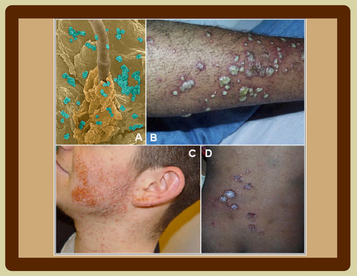

Figure 1. Images of MRSA epidermal infections collaged. a) Image of MRSA bacteria (green) localized around hair follicle; MRSA LCIs on the b) shin, c) lateral face and d) back. (Source: MRSA Resources and Metrowest Clean Gear websites.)

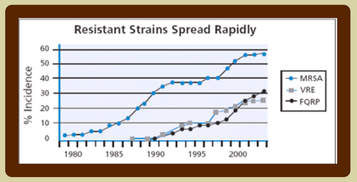

Figure 2. Chart of increasing rates of resistance for three bacteria that are of concern to public health officials: methicillin-resistant Staphylococcus aureus (MRSA), vancomycin-resistant enterococci (VRE), and fluoroquinolone-resistant Pseudomonas aeruginosa (FQRP). These data were collected from hospital intensive care units that participate in the National Nosocomial Infections Surveillance System, a component of the CDC. (Source: CDC Website.)

Numerous outbreaks have occurred within the athletic community to date. The US Centers for Disease Control and Prevention (CDC) have publicized outbreaks involving the St. Louis Rams, the Washington Redskins and the University of Southern California (USC) football team. Six Kutztown University wrestlers underwent hospital stays for MRSA infections in 2006. At the Division III level, Lycoming College lost a star wide receiver to a systemic MRSA infection in 2003. In the middle of the 2005 football season, one of Moravian College's own football players underwent surgery to remove MRSA infected tissue. During the 2006 football season alone, conference rivals Susquehanna University, Juniata College and FDU-Florham treated MRSA outbreaks and sterilized all of their facilities.

It is believed that transmission of antibiotic-resistant bacteria is through poor hygiene both in hospitals and in the community. The CDC has published protocols which include frequent hand washing, avoidance of any infected individuals and wearing gloves at all times when treating any patients in healthcare settings to control and prevent the spread of MRSA.

The goals of my research are:

1. To examine the prevalence of MRSA through nasal carriage among members of the 2006 Moravian College football team through various laboratory methods.
2. To determine the efficiency of Hibiclens® in preventing LCIs.
3. To investigate possible correlations of susceptibility to MRSA based on past antibiotic usage of each player.

Materials and Methods

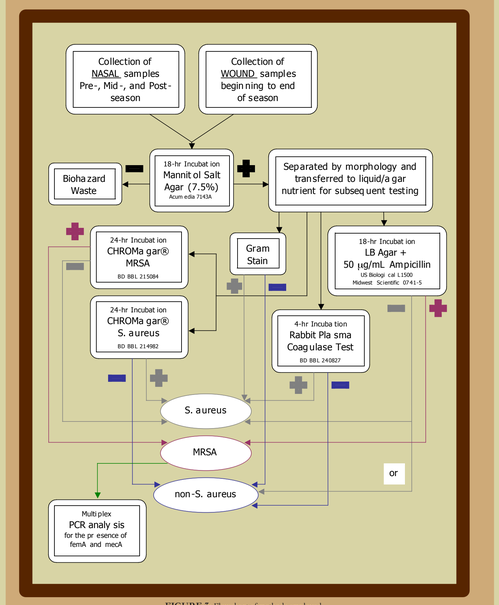

FIGURE 3: Flowchart of methods employed.

Test	Positive (+)	Negative (–)
Mannitol Salt Agar (7.5% NaCl)	Fermentation of MSA to acid; change in pH as shown by phenol red-colored agar made yellow.	No fermentation of MSA; no color change or change to pink (indicating non-*S. aureus*).
LB Agar + 50 µg/mL Ampicillin	Growth; white/cream-colored colonies.	No growth.
Rabbit Plasma Coagulase Test	Coagulation; any degree of gel at room temperature.	Liquid at room temperature.
CHROMagar® *S. aureus*	Growth; mauve-colored colonies.	Growth; blue-green colonies or inhibition (indicates non-*S. aureus*)
CHROMagar® MRSA [see Figure 5]	Growth; mauve-colored colonies.	No growth (indicates *S. aureus*) or blue-colored colonies (indicates non-*S. aureus*).

Table I. Descriptions of test results: positive (+) and negative (–).

Growth; mauve-colored colonies.

No growth (indicates S. aureus) or blue-colored colonies (indicates non-S. aureus).

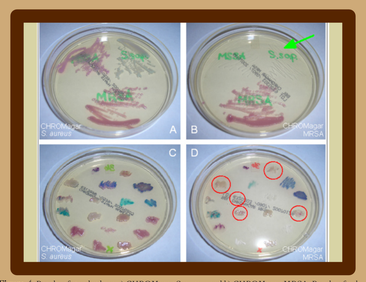

Figure 4. Results of standards on a) CHROMagar S. aureus and b) CHROMagar MRSA. Results of cultured specimen on c) CHROMagar S. aureus and d) CHROMagar MRSA. Green arrow indicates few blue/green colonies and red circles indicate possible MRSA samples on CHROMagar MRSA plates.

Results

Of the total 658 samples acquired over the course of the season, there were 402 wounds (abrasions), 247 nasals, four locker room cultures and two washer samples. Due to a number of athletes opting to leave the study, periodic nasal samples did not remain consistent at 88.

Locker room and washer samples. Of the four locker room samples, three tested MSA positive (indicating S. aureus) and were recorded as positive results. Two of the three (+) MSA samples were coagulase negative, but all three tested positive with CHROMagar S. aureus. Figure 5 shows that of the three MSA positive samples, none showed a positive CHROMagar MRSA test. Therefore, none of the six randomly chosen locations tested positive for MRSA.

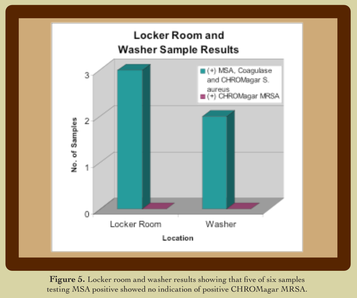

Figure 5. Locker room and washer results showing that five of six samples testing MSA positive showed no indication of positive CHROMagar MRSA.

Nasal cultures.

Although 247 nasal swabs were acquired, only 218 can be analyzed due to coding error with 29 samples. 59 samples were taken at pre-season, 80 at mid-season and 79 at post-season.

> Pre-season – 25 of 59 (42.4%) tested MSA positive. Blood plasma coagulated in 21 of the 59 samples (35.6%) and 22 tested positive on CHROMagar S. aureus media. None of these samples tested positive on CHROMagar MRSA plates.
>
> Mid-season – MSA, coagulase and CHROMagar S. aureus resulted in the same number of positive tests throughout (31 of 80; 38.8%). Five of 80 samples (6.3%) showed positive results on CHROMagar MRSA indicating that MRSA may be present in those samples.
>
> Post-season – 34 of 79 (43.0%) of post-season nasal samples were MSA positive. 31 (39.2%) coagulated rabbit blood plasma and 33 of 79 (41.8%) tested positive on CHROMagar S. aureus. CHROMagar MRSA media tested positive three times, indicating possible MRSA presence.

Figure 6 shows the trend in percent positive nasal culture results over the course of the season. By the end of the season, each test was yielding more positive results than at the start of the season, with the exception of CHROMagar MRSA media. Although there appears to be no rising trend of possible MRSA carriage through the course of the season, the low percentage of samples testing positive is not necessarily indicative of a decreasing trend in MRSA. Rather, MRSA may have been present in the anterior nares and not successfully cultured.

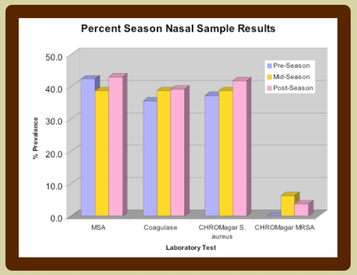

Figure 6. Trends of positive laboratory test results on nasal samples acquired over the course of the 2006 football season.

Wound cultures.

Throughout the football season, 402 wound cultures were acquired and analyzed. 169 or 42.0% of all samples tested positive on MSA. Of the total 402 wound samples, 15 (3.7%) harbored bacteria that present morphology similar to standard MRSA on CHROMagar MRSA media, suggesting MRSA presence in these wounds. Figure 7 shows the results of wound culture analysis, each as subsets of the total.

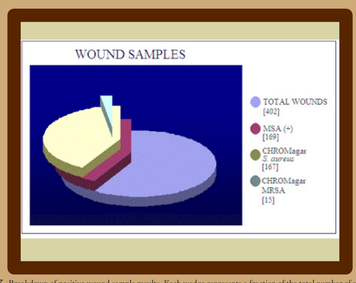

Figure 7. Breakdown of positive wound sample results. Each wedge represents a fraction of the total number of samples.

Although Hibiclens® was used throughout the season on all wounds, only 131 wounds were swabbed both before and after treatment. Figure 8 illustrates the results of wound sample analysis relative to total cultures before and after Hibiclens® treatment. Table II shows positive MSSA and MRSA results in numerical form.

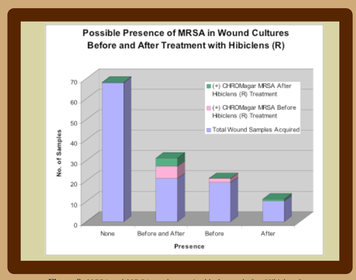

Figure 8. MSSA and MRSA results acquired before and after Hibiclens® treatment to investigate the efficiency with which wounds are cleaned.

	Before Hibiclens®	No. samples	After Hibiclens®	No. samples
S. aureus results	-	---	-	---
	+	6	+	4
	+	2	-	0
	-	0	+	1

Table II. Possible MRSA presence in wounds that test positive for S. aureus before and/or after Hibiclens® treatment.

A second evaluation of Hibiclens® was performed by swabbing wounds both at Day 0 (the initial day of abrasion) and approximately Day 5. After analyzing 10 randomly chosen instances in which follow-up swabs were acquired, one sample was MRSA positive on Day 0 before showering, but no samples were potentially MRSA positive after treatment or on Day 5.

Results summary.

The 658 total samples were broken down into 247 from the anterior nares and 402 were cultured from wounds. 60 of 60 (100%) randomly selected samples (nasal and wound) showed the appearance of Gram positive cocci after staining, performed as a confirmatory test of Staphylococcus, spp. as per Figure 9. MSA, coagulase and CHROMagar S. aureus tests jointly showed that approximately 40% of all players sampled carry MSSA in the nose but only a few appear as carriers of MRSA.

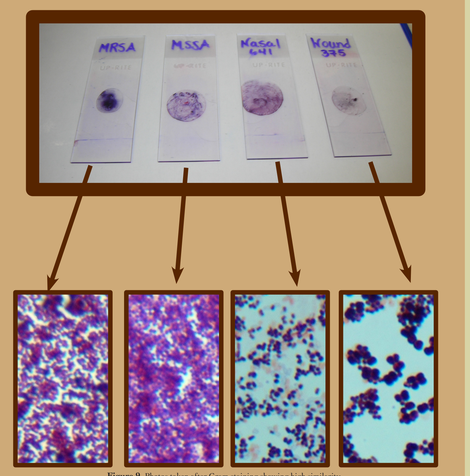

Figure 9. Photos taken after Gram staining showing high similarity between standard MRSA, MSSA, nasal and wound specimen.

Discussion

Virtually the same number of wound samples, 169 and 167, tested positive on both MSA and CHROMagar S. aureus media, respectively. Combining nasal results, MSA, CHROMagar S. aureus and coagulase testing identified 39.5% ± 2.44 of samples as S. aureus. These consistent percentages of positive results show that all three tests perform equally well in determining the identity of S. aureus bacteria from clinically isolated samples.

Unlike several other teams within the conference, Moravian's football team did not experience an outbreak of MRSA during the 2006 season. With such a small sample size of possible MRSA, discrepancies with use of Hibiclens® and the high likelihood that all wounds were not reported, conclusions cannot be drawn as to whether carriage of MRSA or presence of MRSA in wounds was spread or controlled within the team over the course of the season. Even though results of this study suggest that Hibiclens® is not 100% effective, it is important to keep in mind the potential for error associated with this data due to methods of using Hibiclens®. Although a number of samples tested positive for MRSA in wounds, no LCIs occurred over the course of the season. This suggests that the use of Hibiclens® is effective in the control of MRSA.

In an effort to investigate increased susceptibility to MRSA based on past antibiotic usage, players were contacted numerous times to retrieve their family physician contact information, but few responded. Of responders, physicians were contacted and only one sent back a report. This player had not received any antibiotic prescriptions from that office. The Moravian College Health Center was also contacted to retrieve antibiotic prescription information. Data collection from this location is ongoing.

At this time, CHROMagar MRSA results have shown that eight of the 88 players who enrolled in the study may be carriers of MRSA and 21 may have been exposed to it at some point during the season. PCR analysis of these 29 samples is now being conducted to determine the presence of fem A (indicating MSSA) and mecA (indicating MRSA) in each sample. Regardless of these test results, no athletes required treatment for LCIs caused by MRSA during the 2006 season, therefore indicating that Hibiclens® may serve as a sufficient means with which to prevent MRSA from infecting players in the locker room.

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Acknowledgments

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