



# Isolation and Characterization of MRSA at UW-Eau Claire

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## Abstract

The UW-Eau Claire campus was surveyed for the presence of MRSA. The results indicate that of all the bacteria isolated, the percentage of *S. aureus* isolated from the student athlete equipment was 43% of isolates. Of those *S. aureus* isolates, 3 (1.8%) were tentatively confirmed as MRSA isolates. Similarly, of all the bacterial isolates from general student athletics, we isolated up to 16% *S. aureus* from which we tentatively confirmed 3 additional MRSA isolates. In the general student population areas, up to 36% of the isolates were *S. aureus*, with all of the strains testing negative for methicillin resistance. We are currently in the process of gathering more samples of potential MRSA isolates, confirming the identities of isolates by polymerase chain reaction (PCR) and assaying for the transfer ability of the methicillin resistance gene.

## Introduction

Presence of methicillin-resistant *Staphylococcus aureus* (MRSA) was first reported in 1961 shortly after the introduction of the antibiotic methicillin. *S. aureus* is normally found in the nasal cavities of humans making it fairly transmittable through mucosal secretions. Recently there have been concerns about the spread of antibiotic resistances in bacteria, more specifically, methicillin resistant *S. aureus* (MRSA). *S. aureus* are gram positive cocci able to ferment mannitol.

There are two general strains of MRSA, a strain acquired by nosocomial infections (hospital acquired) and a community acquired strain. The CDC reported in 2005, that there were 94,000 MRSA cases in the United States, and of those cases 19,000 resulted in death. Approximately 85% of MRSA cases in 2005 were the result of nosocomial infections while the remaining 15% were as a result of community acquired infections. Because of this, there has been increased awareness of MRSA in the general population as well as in the hospital setting.

We looked at the incidence of MRSA in the community, more specifically the UW-Eau Claire campus. We surveyed the general student population (desks, vending machines, water fountains, etc), the general student athletic only population (treadmills, free weights, exercise machines, etc), and athletic team equipment only (volleyballs, gymnastic beams, etc).

## Materials & Methods

**Sampling.** Sterile cotton swabs were dipped into sterile water and a 4 in x 4 in square was swabbed. The cotton swab was then streaked onto both a Mannitol Salt Agar (MSA) (Difco MI) plate and a MSA containing 2ug/mL oxacillin. Plates were then incubated for 48 hours prior to counting colonies.

**Filter-mating.** Filter Mating procedure was followed as diagramed in Fig. 1. Donors were the isolates and the recipients were *Staphylococcus aureus* SAS 800 (Strep')

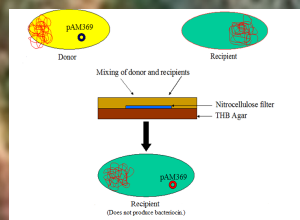


Fig 1. Conjugation and Selection of Transconjugate

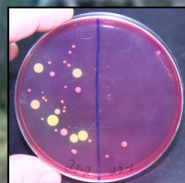


Fig. 2. Mannitol Salt Agar



Fig 4. Catalase Test



Fig. 3 Agglutination test

**Agglutination Test.** We used the BactiStaph (Remel, Lenexa,KS) to the directions supplied. The BactiStaph (Remel, Lenexa,KS) kit tested for the presence of coagulase and protein A associated with *S. aureus* strains.

**Antibiotic Resistance Test.** Serial 2-fold dilutions of *S. aureus* grown in Todd-Hewitt Broth (THB) (Difco MI) were performed to determine the minimum inhibitory concentration (MIC) of Methicillin (oxacillin) and other antibiotics.

**Polymerase Chain Reaction (PCR).** A reaction mixture containing the target DNA sequence to be analyzed and 2 primers (forward and reverse *mecA* and 16S for known *S. aureus*) were used to identify the *oxiI* gene and *S. aureus* specific *mecA* respectively. A 2% agarose gel was to separate the resulting PCR products.

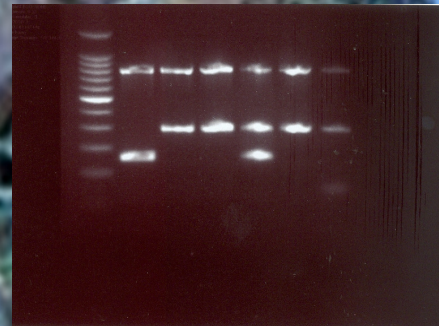


Fig 5. 63F Transconjugate Gel Electrophoresis Results

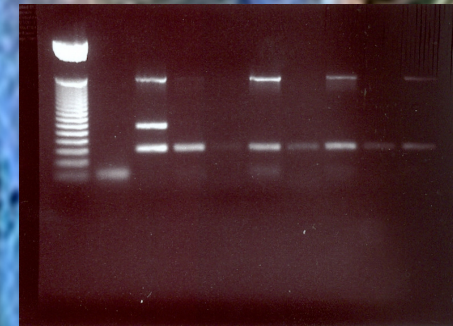


Fig 6. Donors Gel Electrophoresis Results

## Results

**Sampling.** Mannitol positive colonies were found and selected for isolation (Fig 1). 428 mannitol positive colonies were found in the general student population, of which 5.14% were presumed to be MRSA. In the general student athletic population, 142 mannitol positive colonies were found and of those, 26.76% were presumed to be MRSA. Athletic Team only equipment revealed 283 mannitol positive colonies and of those 16.25% were presumed to be MRSA. Our data are presented in Table 1.

**Presumptive *S. aureus* Tests.** Presumptive test results for isolates are shown in Table 2 and Figs 2, 3, 4, 5 and 6.

Table 1. Sample Results From Various Sources

Sample Source	Total Colonies (MSA)	Mannitol Positive (MSA) (% of Total)	Oxacillin Resistant (% of mannitol positive)
General Student Population	2640	428 (16.21%)	22 (5.14%)
General Student Athletic only Population	2376	142 (5.98%)	38 (26.76%)
Athletic Team only equipment	1862	283 (15.20%)	46 (16.25%)
Total	6878	853 (12.40%)	106 (12.43%)

Table 2. Presumptive Test Results For Isolates

Strain ID #	Donor Potential	Catalase Test	Agglutination Test	<i>S. aureus</i>	MIC (ug/mL)
3-3A	-	+	++	-	1250
3-3B	-	+	-	-	312
7	-	+	++	-	156
23	-	+	++	-	312
2-7	-	+	+	-	156
8	-	+	++	-	ND
69	-	+	++	-	-
59F	-	+	++	-	-
63F	+	+	+++	-	-

ND : Not detected + : positive (catalase)/ weak positive (agglutination)  
- : negative ++ : moderate positive (agglutination) +++ : strong positive (agglutination)

## Discussion

Of 6878 total colonies, 1.54% were presumed to be MRSA (Table 1). MIC's, agglutination, and catalase tests were done on all donors (Table 2). Strain 63F served as a potential donor of *oxiI*. However, gel electrophoresis analysis demonstrated that it was not (Fig 5). 63F transconjugate's bands were compared against those of a known *S. aureus* (FAA1000 MRSA). The *S. aureus mecA* and 16S ribosomal RNA primers when used on donor isolates did not produce *S. aureus* banding patterns indicating they were not *S. aureus*. All donor strains tested were *mecA* positive (Fig 6).

## References

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