Methicillin-Resistant *Staphylococcus aureus* Isolates, Obtained from the University of Wisconsin—Eau Claire Campus, Capable of Transferring Methicillin-Resistance

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Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an antibiotic-resistant strain of the bacterium *Staphylococcus aureus* that is responsible for many community and hospital-acquired infections worldwide. A survey of the UW—Eau Claire campus was conducted to indicate the relative presence of MRSA in academic and recreational buildings. Fifteen samples were identified as possible MRSA. Of these fifteen samples, one potential methicillin-resistant donor was selected (36-14).

To determine the ability of MRSA isolates to transfer the methicillin-resistance, a series of conjugation experiments were conducted with the potential donor (36-14) and a recipient *Staphylococcus aureus* strain (SAS 805). The resulting transconjugant samples (products of the donor and recipient matings) were then plated on MSA plates containing spectinomycin (donor sensitive) and oxacillin (recipient sensitive). Colonies capable of growth on both oxacillin and spectinomycin were comparatively screened against the donor (36-14), recipient (SAS 805) and a positive MRSA control using polymerase chain reaction (PCR) based identification to genotypically distinguish the presence of the methicillin-resistance gene (*mecA*). The frequency of transformation of the methicillin-resistance gene was determined to be 7.6x10⁻¹. The results of this study have provided evidence supporting horizontal transfer of the *mecA* gene between staphylococcal species.

Methods

- Samples were collected from general student and athlete-only areas on campus.
- The samples were screened using cultural isolation techniques to determine presumptive MRSA isolates.
- Colonies that fermented mannitol were selected from MSA plates containing 4ug/mL oxacillin and streaked for isolation (Figure 1).
- Gram Staining was used to select for Gram-positive cocci (Figure 2).
- Catalase testing was performed to determine the presence of the enzyme catalase (Figure 3).
- Agglutination testing was used to determine the presence of coagulase and protein A associated with *Staphylococcus aureus* strains (Figure 4).

Conjugation Experiments

- Fifteen samples were identified as possible MRSA. One potential methicillin-resistant donor (36-14) was selected for subsequent conjugation experiments.
- Minimum inhibitory concentration data were collected on the methicillin-resistant donor (36-14) and the recipient *Staphylococcus aureus* strain (SAS 805).
- Conjugation Procedure: (Figure 5)

Polymerase Chain Reaction

- Standard bacterial DNA isolation procedure was completed.
- A standard PCR master mix was prepared with forward and reverse primers for amplification of the *mecA*, *femB* and 16S rRNA genes; identifying oxacillin-resistance, *Staphylococcus* and aureus strains, respectively.

Gel Electrophoresis

- A 2% agarose gel was prepared with a 1X Tris Borate EDTA (TBE) running buffer.
- The samples were run at 90 volts for 45-60 minutes.

Results

Table 1. Prevalence of MRSA from UW—Eau Claire

<table>
<thead>
<tr>
<th>Sample Source</th>
<th>Total Oxacillin-Resistant Colonies</th>
<th>Presumptive MRSA Colonies</th>
</tr>
</thead>
<tbody>
<tr>
<td>General Student Population</td>
<td>59</td>
<td>11</td>
</tr>
<tr>
<td>Student Athlete Population</td>
<td>15</td>
<td>4</td>
</tr>
</tbody>
</table>

Minimum Inhibitory Concentrations (MIC)

- Methicillin-resistant donor (36-14)
- Oxacillin® (39µg/mL) and Streptomycin® (2.393µg/mL)
- *Staphylococcus aureus* strain (SAS 805)
- Streptomycin® (2.393µg/mL) and Spectinomycin® (12.500µg/mL)

Conjugation Results

- Transformation frequency: \( \frac{\text{Transconjugants}}{\text{Donor}} = 3.6 \times 10^{-3} \)  Transconjugants \( \text{Recipient} = 7.6 \times 10^{-4} \)

Discussion/Conclusion

- Of the 59 oxacillin-resistant colonies collected from the general student areas, 18.6% of the isolates were presumptively MRSA.
- Of the 15 oxacillin-resistant colonies collected from the student athlete areas, 26.7% of the isolates were presumptively MRSA.
- The prevalence of community acquired MRSA strains suggests that the epidemiology of MRSA is changing as isolation is not restricted to hospitals.
- The results of this study have provided evidence supporting horizontal transfer of the *mecA* gene between staphylococcal species, as 36-14 was not identified as MRSA.

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