

# Screening the University of Wisconsin-Eau Claire Campus for Methicillin-Resistant *Staphylococcus* spp. Isolates Capable of Transferring Methicillin-Resistance



UNIVERSITY OF WISCONSIN-EAU CLAIRE

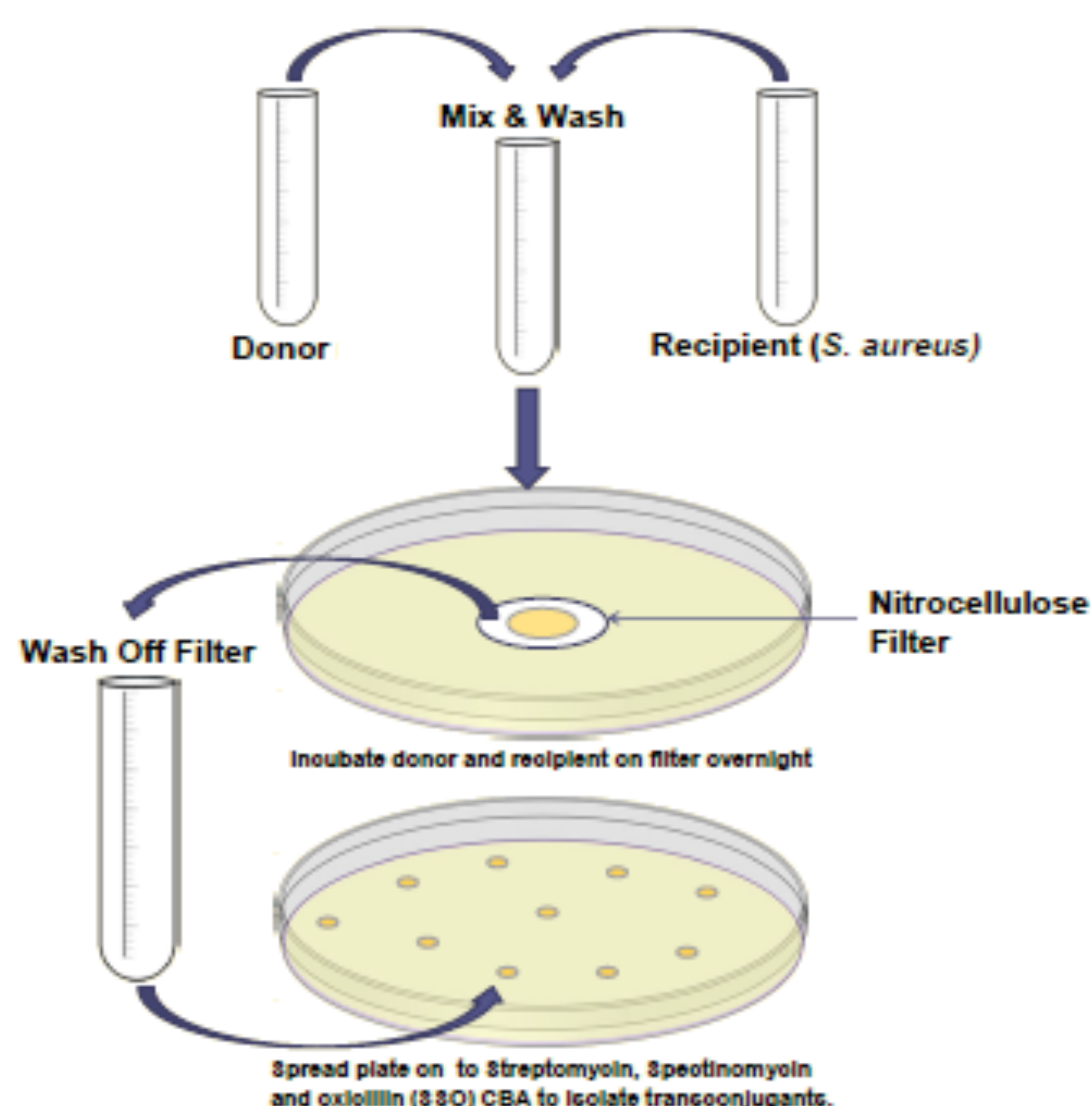
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## ABSTRACT

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 world-wide. A survey of the UW-Eau Claire campus was conducted to indicate the relative presence of Methicillin-Resistant *Staphylococcus* spp. (donor strains). We collected 125 oxacillin resistant samples. Of these, 35 samples displayed characteristics of MRSA and were designated as potential donors. Further testing determined none of these donors to be MRSA. The donors were used to determine their ability to transfer the resistance gene (*mecA*) to *Staphylococcus aureus* recipients (SAS 850 and SAS 810). To determine the ability of isolates to transfer the *mecA* gene, a series of conjugation experiments were conducted with potential donors and recipients. The resulting transconjugants (products of the donor and recipient matings) were plated on CBA plates containing streptomycin, spectinomycin (donor sensitive), and oxacillin (recipient sensitive). Colonies capable of growth on all three antibiotics were screened against the donors, recipients, and a positive MRSA control using polymerase chain reaction (PCR) and the coagulase test to genotypically distinguish the presence of *mecA*. To date, all 35 donor strains have been tested and none successfully transferred methicillin resistance to the recipient.

## CONJUGATION EXPERIMENT

- 125 samples were identified as possible *Staphylococcus* species. 35 possible methicillin resistant donors were selected for subsequent conjugation experiments.
- Conjugation procedure:



## METHODS

- Swabs were collected from general student and athlete-only access areas on campus.
- The samples were screened using cultural isolation techniques to determine presumptive *Staphylococcus* spp. isolates.
  - Growth on mannitol salt agar (Figure 1).
  - Gram staining (Figure 2).
  - Testing for the presence of catalase (Figure 3).
  - Agglutination testing for protein A associated with *Staphylococcus aureus* strains (Figure 4).



Figure 1. Mannitol Salt Agar (MSA). MSA is a selective and differential growth media that selects gram positive bacterium, and differentiates mannitol fermenters (yellow) and non-fermenters (red).



Figure 3. Catalase test. The catalase test determines the presence of the enzyme catalase. Catalase catalyzes the decomposition of hydrogen peroxide to water and oxygen. Negative catalase (left) and positive catalase (right).

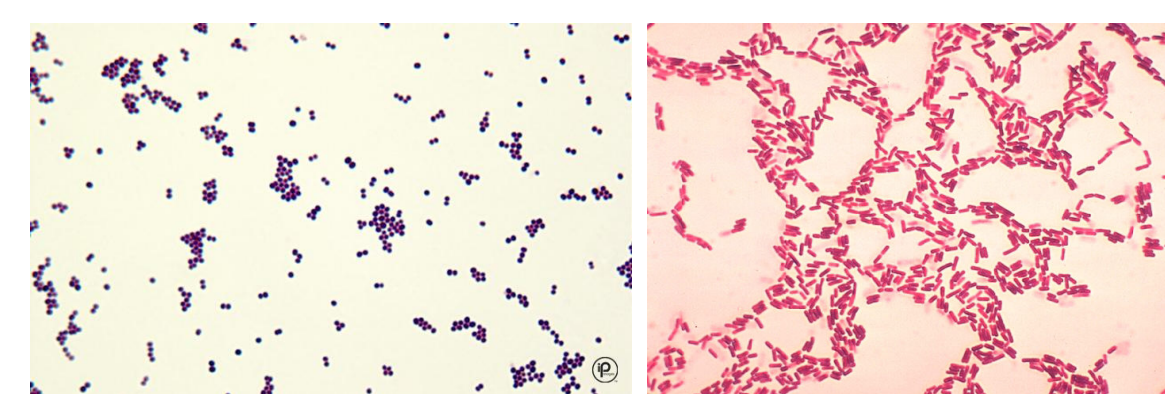


Figure 2. Gram Stain. Gram staining is a staining technique that differentiates bacteria based on physical properties. Gram positive bacterium (left), gram negative bacterium (right).

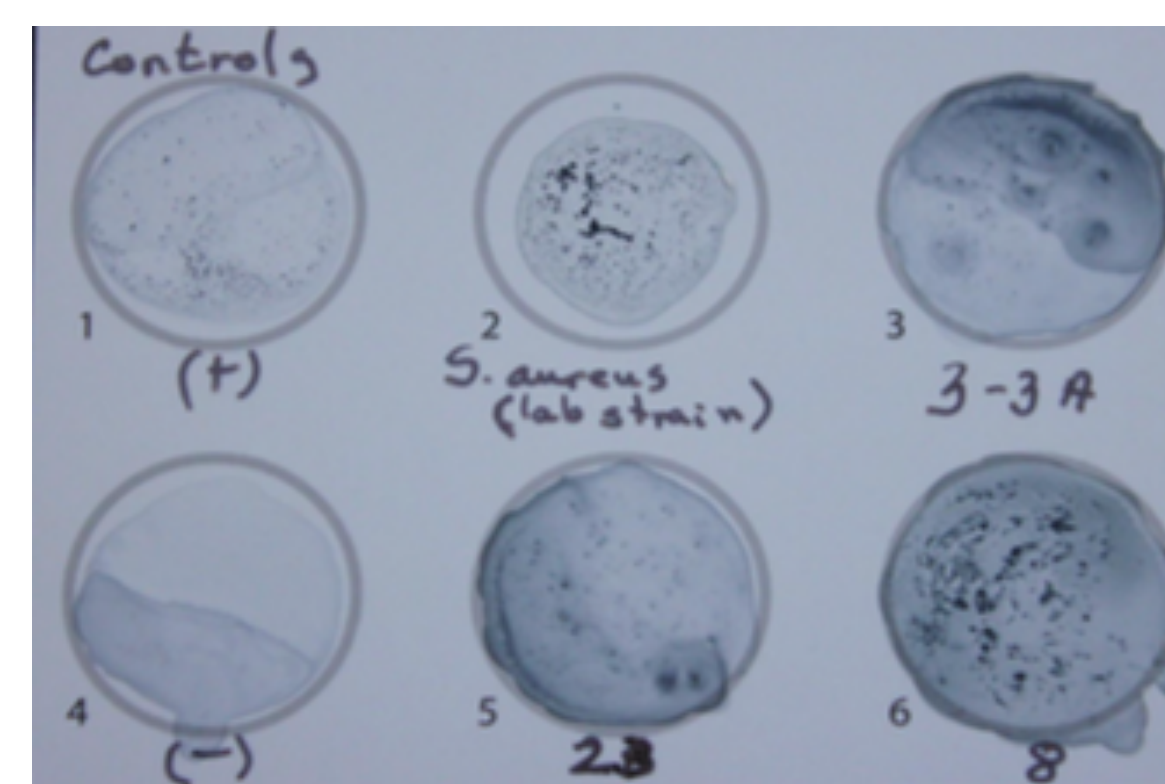


Figure 4. Agglutination test. Agglutination testing was used to determine the presence of coagulase and protein A associated with *S. aureus* strains. A BactiStaph latex reagent coated with fibrinogen and IgG was used to screen for coagulase-positive staphylococci. Clumping as shown above in screen 1, 2, 5, and 6 are positive results for *S. aureus*, whereas screens 3 and 4 are negative.

## BACKGROUND

- Recent research has shown that MRSA is no longer limited to hospitals (nosocomial infections). Community acquired MRSA (CA-MRSA) has increased in prevalence in public facilities.
- The first reported case of MRSA was in 1961, shortly after the introduction of methicillin. Since then, MRSA infections have increased in prevalence throughout the U.S.
- MRSA has continued to develop resistances to antibiotics through natural selection, in particular beta-lactam antibiotics.
- The project aimed to identify the incidence of MRSA at the University of Wisconsin-Eau Claire
- General student populations, along with student athlete populations were surveyed.

## BACTERIAL CONJUGATION

- Horizontal gene transfer of antibiotic resistance genes has played an important role in bacterial evolution. The presence of a mobile staphylococcal cassette chromosome (SCCmec) in *S. aureus* has been shown to encode for methicillin resistance (*mecA*), along with a number of other antibiotic resistance genes.
- The *mecA* gene encodes for a penicillin binding protein 2a (PBP2a), which is poorly acylated by beta-lactam antibiotics. The gene is a mobile genetic element inserted into the SCCmec.
- To determine the ability of potential methicillin-resistant *Staphylococcus* spp. isolates to transfer the methicillin resistance, a series of conjugation experiments were conducted.

## DISCUSSION

- Of the 102 oxacillin resistant colonies collected from the general student access areas, 25% of the isolates were presumptively *Staphylococcus* spp.
- Of the 23 oxacillin resistant colonies collected from the student-athlete access areas, 39% of the isolates were presumptively *Staphylococcus* spp.
- The increased prevalence of community acquired MRSA strains suggests that the epidemiology of MRSA is changing as isolation is not restricted to hospitals
- None of the oxacillin resistant donors successfully transferred the *mecA* gene.

## ACKNOWLEDGEMENTS

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## RESULTS

### PREVALENCE OF METHICILLIN-RESISTANT STAPHYLOCOCCUS SPP. AT UW-EAU CLAIRE (TABLE 1)

Sample Source	Total Oxacillin Resistant Colonies	Potential <i>S. aureus</i> Colonies	Percent Potential <i>S. aureus</i> Colonies	Number of Potential Oxacillin Resistant Donors	Number of Potential <i>S. aureus</i> Donors
General Student Access Area	102	26	25%	ND	ND
Student-Athlete Access Area	23	9	39%	ND	ND

Table 1. Prevalence of oxacillin resistant bacteria (including MRSA) and their ability to transfer oxacillin resistant gene.

### CONJUGATION RESULTS

- Thus far, potential methicillin donors have not been identified.