

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is becoming more commonly encountered in clinical settings. The prevalence of methicillin resistance in *Staphylococcus* species other than *Staphylococcus aureus*, and coagulase-negative staphylococci (CNS) in general, has not been studied in great detail.

The purpose of this investigation was to estimate the frequency of methicillin resistance in CNS isolated from students at Minnesota State University, Mankato. This information will be used as part of a long-term project at MSUM to determine the likelihood of CNS serving as a reservoir of methicillin resistance.

Methods

The strains used in this experiment were *Staphylococcus* species isolated from students as part of a class exercise in Medical Microbiology (BIOL 475/575) in Fall 2012. One isolate was obtained from each student registered for the course.

- Isolates were screened for resistance to oxacillin using oxacillin screening agar. Oxacillin is similar to methicillin and is prescribed much more frequently.

- Isolates that tested oxacillin resistant in screening were identified using biochemical tests

- The minimum concentration of oxacillin required to inhibit bacterial growth (MIC) was determined by inoculating suspected oxacillin resistant isolates into twofold serial dilutions of oxacillin in Mueller-Hinton broth.

- DNA was extracted from suspected oxacillin resistant isolates and used for Polymerase Chain Reaction (PCR) testing to detect *mecA*, the penicillin binding protein-2 (PBP2) allele responsible for oxacillin and methicillin resistance. Carriage of *mecA* was established by the presence of a 533 bp band in electropherograms of PCR products.

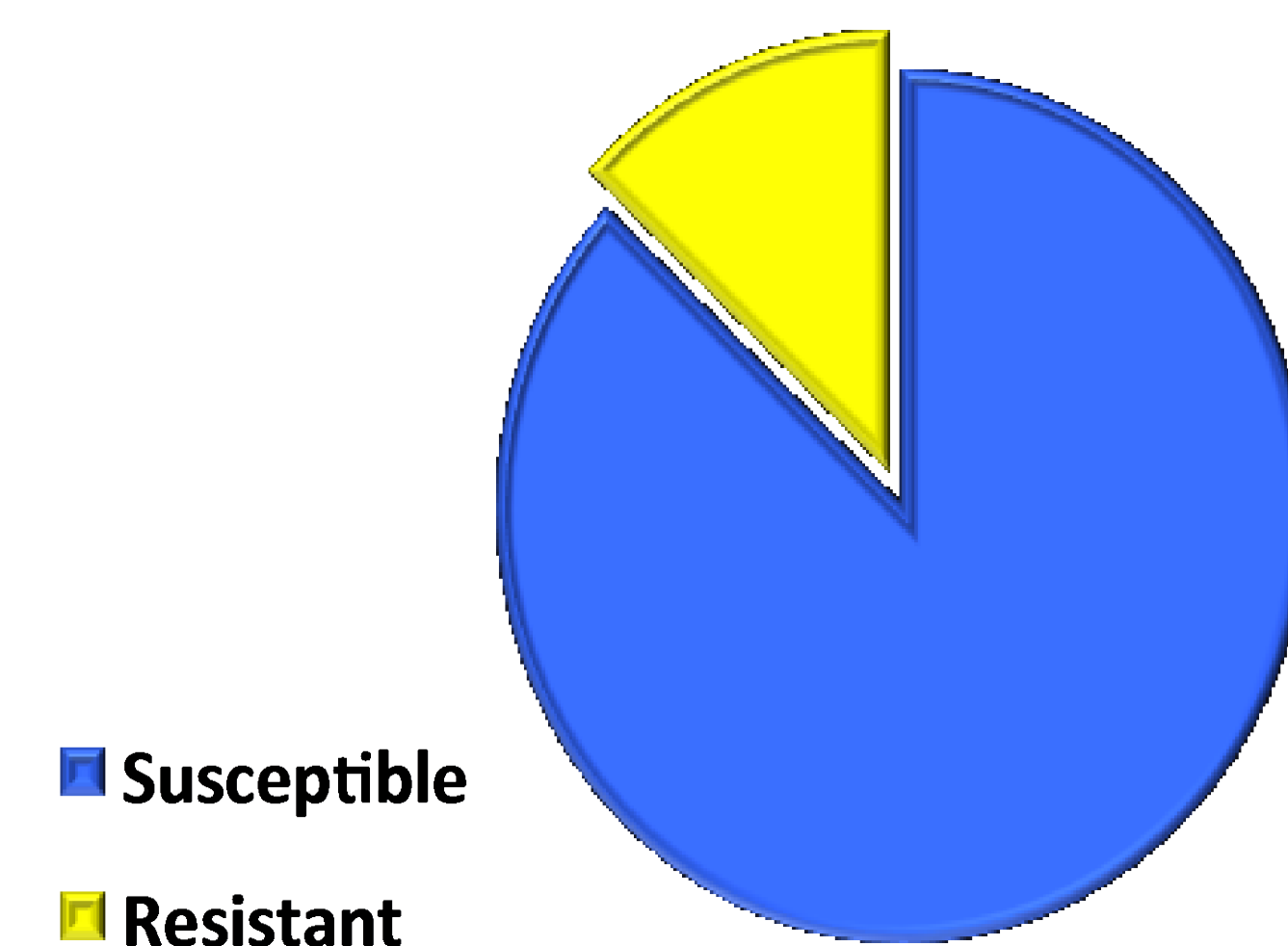


Figure 1. Frequency of oxacillin resistance in staphylococci recovered from students in Medical Microbiology, Fall 2012. Of 46 isolates obtained, six (13%) screened positive for oxacillin resistance.



Figure 2. Isolates growing on oxacillin screening agar. This medium contains 6 µg/ml oxacillin; isolates are classified as oxacillin resistant at MIC ≥ 4 µg/ml.

Table 1 – Identification of oxacillin-resistant staphylococci

Isolate #	Species
5	<i>S. cohnii</i> subsp. <i>cohnii</i>
8	<i>S. caprae</i>
10	<i>S. capitis</i> subsp. <i>urealyticus</i>
11	<i>S. cohnii</i> subsp. <i>urealyticum</i>
30	<i>S. caprae</i>
45	<i>S. caprae</i>



Figure 3. MIC testing. Two-fold serial dilutions ranging from 32 to 0.016 µg/ml (left to right) were prepared. The isolates in the top 4 rows had an MIC of < 0.016 µg/ml, and the MIC of the isolate in the bottom 4 rows was > 32 µg/ml.

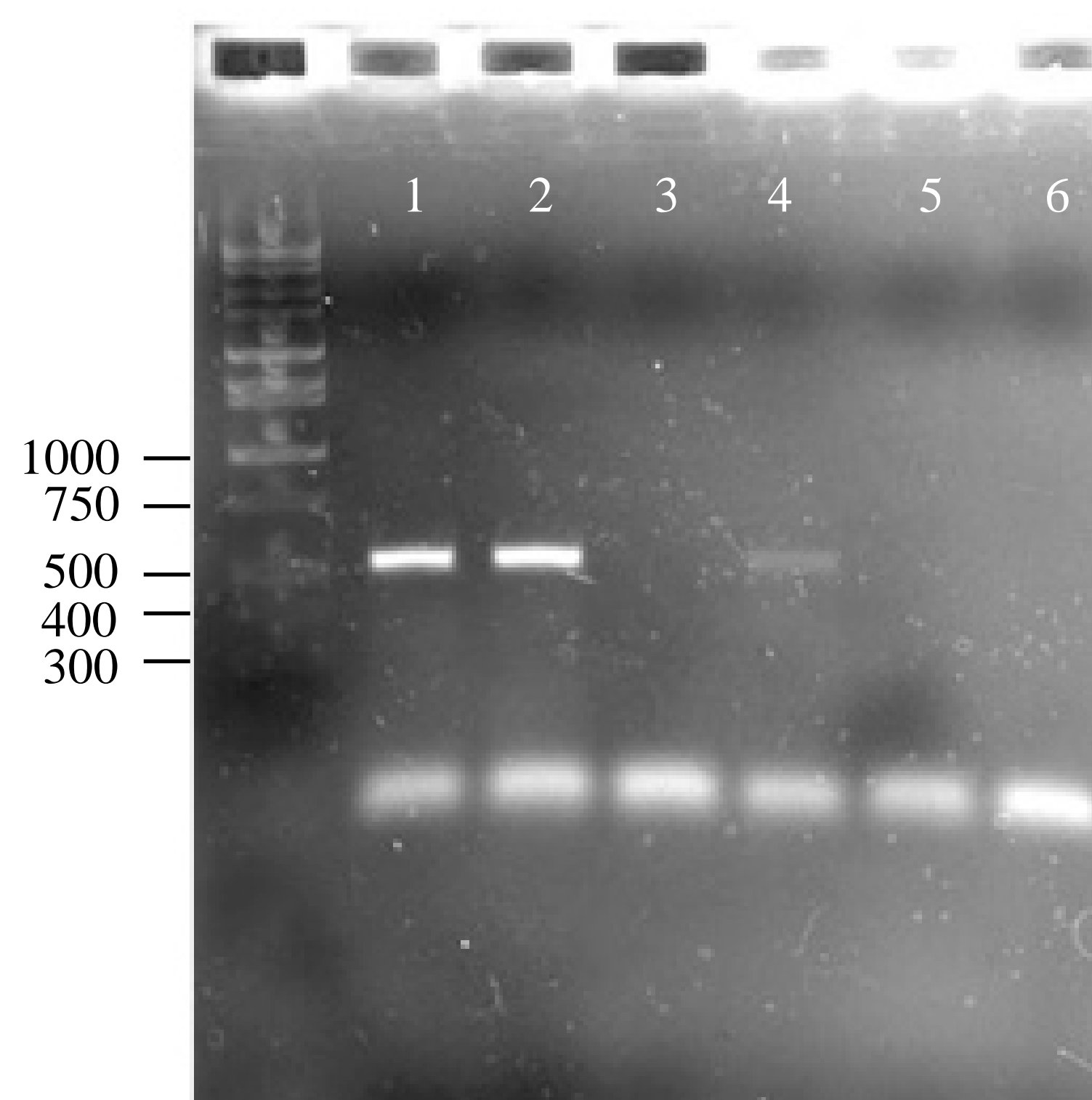


Figure 4. PCR testing for *mecA*. DNA was amplified using *mecA*-specific primers. Organisms yielding a product that migrates as a 533 bp band (such as those in lanes 1, 2, and 4) were considered positive. Lane 1, isolate #8; lane 2, isolate #30; lane 3, isolate #45; lane 4, isolate #10; lane 5, isolate #11; lane 6, negative control.

Results

- Six of 46 *Staphylococcus* isolates (13%) were oxacillin resistant when tested on oxacillin screening agar (Figs. 1 and 2). None of the resistant isolates was identified as *S. aureus* (Table 1).
- Four of 5 isolates had oxacillin MIC > 32 mg/ml (Fig. 3). The sixth isolate did not survive for MIC testing.
- 3 of 5 isolates tested contained the *mecA* gene, supporting the characterization of these isolates as oxacillin resistant (Fig. 4).

Discussion

- The prevalence of oxacillin resistance in CNS was much higher than expected. Whether these results are representative of the population at large or a characteristic of the cohort studied will require longer term studies.
- Poor quality DNA extracts may explain the failure to detect *mecA* in 2 strains with high oxacillin MICs.
- Three of the isolates that screened as oxacillin resistant were identified as *S. caprae*. While these were recovered from different individuals, it is possible that these people were colonized from a common source. Additional testing will be necessary to determine if these are a single strain or multiple strains.
- While we cannot determine if the presence of *mecA* in these isolates originated from MRSA or other CNS, these data demonstrate the potential for coagulase negative Staphylococci to serve as a reservoir for oxacillin resistance.

Acknowledgements

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References

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