Methicillin-resistant Staphylococcus aureus (MRSA) is a frequent cause of nosocomial and community-acquired infections, with rates of infection matching as high as 28% in intensive care units. The ability to quickly identify the source of an infection would have a dramatic cost savings for both the patient and the healthcare facility; however, discriminating between bacterial isolates at the clonal level in a timely manner is challenging. Several molecular typing systems are currently used for monitoring outbreaks of infection caused by S. aureus, including pulsed-field gel electrophoresis and arbitrarily-primed PCR. However, these methods may take up to 72 hours for results. This study reports the performance of a semi-automated method of repetitive element sequence-based PCR (rep-PCR) as a rapid molecular screening tool for MRSA.

**METHOD**

Over 375 well-characterized S. aureus isolates from the SENTRY Antimicrobial Surveillance Program collection (JMI Laboratories, IA) were analyzed. These isolates were collected from 31 hospitals throughout the U.S., Europe, and South America. Isolates were cultured, and the DNA was extracted using the UltraClean Microbial DNA Isolation Kit (Mo Bio Laboratories, CA). The extracted DNA was amplified using the repPRO DNA Fingerprinting Kit® and Urine-E® primer (Bacterial BarCodes, TX). The amplicons were separated by agarose gel electrophoresis. Digital images of the gels were captured with the AlphaImager (Alpha Innotech, CA) and analyzed with BioNumerics software (Applied Maths, Belgium). For all analyses, the Pearson product-moment correlation coefficient was used to describe the similarity between pairs of isolates. Cluster analysis was performed using UPGMA and reported in a dendrogram.

**RESULTS**

Cluster analysis of rep-PCR fingerprints from the same city source indicated a diverse population. The dendrogram of S. aureus isolates typed using agarose gels (Figure 1) indicates that one major clonal group of MRSA isolates and up to four independent MSSA clones are present in this geographical region. In addition, a greater degree of discrimination is seen when isolates collected from Berkeley, CA, in the SENTRY collection showing discrimination of methicillin-resistant and methicillin-sensitive isolates.

**SUMMARY**

- These data indicate that variation in the genetic background of bacterial isolates can be used to track antimicrobial resistance.
- The results suggest that this technique may be most useful at the local or city level, where clonal outbreak isolates can be tracked or predictions made about the potential resistance or susceptibility of an unknown S. aureus isolate.
- The ability of rep-PCR to distinguish S. aureus isolates at the clonal level suggests that rep-PCR can be a powerful tool for tracking nosocomial infections and may also be useful in screening isolates for resistance.
- The rapidity with which rep-PCR fingerprints can be generated and analyzed with standard bioinformatics software suggests that this typing method may be useful for intervention in nosocomial outbreaks and for reducing healthcare costs, length of hospital stay, and the spread of antibiotic-resistant organisms.
- The DiversiLab System exploits the discriminatory power of rep-PCR. The automated detection and standard algorithms for data analysis adds consistency to this molecular method. The automation also reduces interpretation errors and time to result.