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A Probe-Based Method for Confirmation of Methicillin-Resistant *Staphylococcus Aureus* and Detection of Panton-Valentine Leukocidin and *TST* Virulence Genes

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Abstract

Probe-based detection of *mecA*, *lukS/F-PV* (Panton-Valentine leukocidin) and *tst* virulence genes in 435 isolates of *Staphylococcus aureus* had comparable sensitivity and specificity to end point polymerase chain reaction as a reference standard.

Staphylococcus aureus is an important pathogen which causes a wide spectrum of clinical illnesses, including skin and soft tissue infections, bacteremia, sepsis, and toxic shock syndrome (TSS). In recent years, new strains of methicillin-resistant *S. aureus* (MRSA; Herold BC et al., 1998) and methicillin-susceptible *S. aureus* (MSSA; Rasigade JP et al., 2010) have become more prevalent in pandemic proportions, and are causing increasing morbidity in otherwise healthy individuals. The majority of these strains carry the *lukS/F-PV* (Panton-Valentine leukocidin) genes, which may contribute to its pathogenicity (Lina et al., 1999; Vandenesch et al., 2003), in association with other genes. The toxic shock syndrome toxin-I is another potent *S. aureus* exotoxin that appears to mediate the clinical manifestations of TSS (Bergdoll et al., 1981; Schlievert et al., 1981).

Rapid identification of *S. aureus* from blood and other clinical sites has implications for clinical care. The use of end point and real-time polymerase chain reaction (PCR) has been described for the characterization of *S. aureus* (Reischl et al., 2000). However, such molecular methods need specialized equipment and typically require a high level of technical expertise and significant laboratory infrastructure. Simple, rapid, and self-

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contained molecular detection systems are available, but these solutions are expensive and typically limited to organism identification and detection of the *mecA* gene (Goldmeyer et al., 2008). Probe-based detection methodologies can be performed rapidly and have limited requirements in terms of physical space, equipment, or training. We evaluated a DNA probe-based hybridization assay (EVIGENE™; Skov et al., 1999) for rapid (3.5 hour) detection of the *mecA*, *lukS/F-PV* and *tst* genes with endpoint PCR as a reference standard.

This study included isolates of *S.aureus* cultured from two hospitals: St. Jude Children's Research Hospital (SJCRH), Memphis, TN and Queen's Medical Center (QMC), Honolulu, HI. SJCRH is a National Comprehensive Children's Cancer Center treating approximately 3000 patients with cancer each year, referred from across the United States. QMC is a private, non-profit, acute medical care facility serving as the leading medical referral center for the pacific basin. Specimens from which *S.aureus* isolates were obtained included skin, respiratory tract, and blood. All patient identifiers were delinked from the isolate before testing. The study was approved by the Institutional Review Boards at both sites.

Isolates were initially identified as *S. aureus* by Staphaurex Plus® (Remel Europe Ltd., Dartford, Kent, U.K.) at SJCRH or Slidex® reagents (bioMérieux, Marcy l'Etoile, France) at QMC. Bacterial isolates were collected from clinical sites of infection from January 2000 to December 2007 (SJCRH) and in June and July 2007 (QMC). These were preserved at -80°C, until they were retrieved for the study. Fresh second passage isolates grown on blood agar plates were used for further analysis. Tested isolates included both MSSA and MRSA strains. EVIGENE™ testing was performed as described previously (Skov RL et al., 1999). Fresh colonies were buffered with lysis solution and incubated with appropriate detection probes. Hybridization was accomplished by adding conjugate and developing substrates. Results were read by absorbance measurement at 490 nm using a fixed wavelength photometer (Molecular Devices Inc, Sunnyvale, CA). Absorbance >0.5 was considered positive. Positive and negative controls for MSSA and MRSA, for *mecA*, *lukS/F-PV* and *tst* were included in each run.

Isolates were screened for methicillin-resistance using 30 µg cefoxitin disks (BD BB1 Sensi-Disc, Becton Dickinson and Company, Sparks, MD). A zone <21 mm was interpreted as confirming methicillin-resistance. The Clinical and Laboratory Standards Institute (CLSI) methodology was followed in all testing methods (CLSI., 2008).

Frozen crude lysates of pelleted, liquid cultures were transported to the laboratory of another investigator (Dr. Seifried), for assay by PCR-based genotyping. Testing was performed using a multiplex PCR protocol previously described (Perez-Roth et al., 2001) for the detection of the *mecA* gene (encoding the gene for methicillin-resistance). The presence of the *lukS/F-PV* was determined by PCR as previously described (Jarraud et al., 2002). The *tst* gene was amplified by PCR as previously described (Lovseth et al., 2005). Amplified products were detected following electrophoresis on a Bio-Rad Gel (Bio-Rad Laboratories, Hercules, CA) after ethidium bromide staining and visualized using a Bio-Rad GelDoc system.

Probe hybridization testing was performed separately by two independent investigators at each site, in duplicate. PCR testing was performed by another investigator at a third site who was blinded to the results of the hybridization testing at both sites. Samples with discrepant results were re-assayed in duplicate for the specific target, using both methods. Observation of a minimum of one PCR amplification product that exhibited the expected electrophoretic mobility was sufficient to consider that isolate positive for the target gene in the PCR arm of the discrepant analysis.

A total of 435 *S.aureus* isolates were tested. These included 212 MSSA isolates and 223 MRSA isolates. There were 237 *S.aureus* isolates (108 MSSA; 129 MRSA) tested at SJCRH and 198 *S.aureus* isolates (104 MSSA; 94 MRSA) tested at QMC.

The assay including data from both sites had an aggregate sensitivity of 99%, 96%, and 95% and a specificity of 96%, 97%, and 99% for *mecA*, *lukS/F-PV* and *tst* respectively compared with PCR, which was used as the reference standard method (Table 1). All three targets had a comparable sensitivity (94%-100%) and specificity (94%-99%), between the two sites. However, there was a lower prevalence of *lukS/F-PV* (25%) at SJCRH than at QMC (41%).

These findings show a high sensitivity and specificity for probe-based testing in comparison with PCR for the detection of *mecA*, *lukS/F-PV*, and *tst* in clinical *S.aureus* isolates.

Advantages of such commercially produced hybridization probes include ease of use, rapid throughput with results available within 4 hours similar to real-time PCR, and limited equipment and space requirements. Such characteristics enable rapid integration of these tests in a clinical microbiology laboratory setting, without extensive training or the need for prior experience in molecular diagnostic methods.

Although sensitivity and specificity were comparable between the two study sites, SJCRH, which included predominantly children with cancer, showed a lower prevalence of isolates carrying the PVL genes. An increasing incidence of PVL-positive MRSA infections in children with cancer at this institution has been reported previously (Srinivasan et al., 2009). Rapid detection of MRSA infections, particularly with PVL-positive strains, which have emerged in pandemic proportions, may enable earlier institution of appropriate therapy, potentially reducing the high morbidity associated with such infections.

Other studies have evaluated the use of hybridization probes for detecting *mupA* (Rasmussen et al., 2010) and *mecA* (Levi and Towner., 2003; Poulsen et al., 2003; Kerttula et al., 2008), with similar results. Our study has the advantage of including the largest cohort of samples studied retrospectively to date using this method, with validation by independent investigators at two separate sites in two unique patient populations.

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Table 1
**Comparison between probe-based method and PCR for detection of *mecA*, *lukS/F-PV*,
and *tst* genes**

	<i>mecA</i>	<i>lukS/F-PV</i>	<i>tst</i>
–			
–			
Tested	435	435	435
Present	223	140	21
Initial Disc ^a	20	54	24
Final Disc ^b	11	16	6
aPCR/pPR ^c	9	10	6
pPCR/aPR ^d	2	6	1
pPCR/pPR	221	134	20
aPCR/aPR	203	285	408
Prevalence	0.51	0.32	0.05
Sensitivity	0.99	0.96	0.95
Specificity	0.96	0.97	0.99
PPV ^e	0.96	0.93	0.77
NPV ^f	0.99	0.98	1.00

^aInitial discrepant between probe-based method and PCR requiring re-analysis.

^bFinal discrepant remaining after re-analysis.

^cAbsent by PCR.

^dPresent by probe-based method.

^ePositive predictive value.

^fNegative predictive value.