StaphPlex System for Rapid and Simultaneous Identification of Antibiotic Resistance Determinants and Panton-Valentine Leukocidin Detection of Staphylococci from Positive Blood Cultures

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Phenotypic methods take several days for identification and antimicrobial susceptibility testing of staphylococcal isolates after gram-positive cocci in clusters (GPCC) are observed in positive blood cultures. We developed and validated a StaphPlex system that amplifies and detects 18 gene targets simultaneously in 1 reaction for species-level identification of staphylococci, detection of genes encoding Panton-Valentine leukocidin (PVL), and antimicrobial resistance determinants of staphylococci. The StaphPlex system was compared to phenotypic methods for organism identification and antimicrobial resistance detection for positive blood culture specimens in which GPCC were observed. Among a total of 360 GPCC specimens, 273 (75.8%), 37 (10.3%), 37 (10.3%), 1 (0.3%), 3 (0.8%), and 9 (2.5%) were identified by StaphPlex as coagulase-negative Staphylococcus (CoNS), methicillin-resistant Staphylococcus aureus (MRSA), methicillin-susceptible S. aureus (MSSA), or mixed infections of CoNS and MRSA, CoNS and MSSA, or nonstaphylococci, respectively, with an overall accuracy of 91.7%. The 277 CoNS-containing specimens were further identified to the species level as containing 203 (73.3%) Staphylococcus epidermidis isolates, 10 (3.6%) Staphylococcus haemolyticus isolates, 27 (9.7%) Staphylococcus hominis isolates, 1 (0.4%) Staphylococcus lugdunensis isolate, and 36 (13.0%) other CoNS isolates, with an overall accuracy of 80.1% compared to an API STAPH test and CDC reference identification. Numerous very major errors were noticed when detection of aacA, ermA, ermC, tetM, and tetK was used to predict in vitro antimicrobial resistance, but relatively few major errors were observed when the absence of these genes was used to predict susceptibility. The StaphPlex system demonstrated 100% sensitivity and specificity, ranging from 95.5% to 100.0% when used for staphylococcal cassette chromosome mec typing and PVL detection. StaphPlex provides simultaneous staphylococcal identification and detection of PVL and antimicrobial resistance determinants within 5 h, significantly shortening the time needed for phenotypic identification and antimicrobial susceptibility testing.

Staphylococci, which are initially seen as gram-positive cocci in clusters (GPCC) by Gram staining of positive blood culture bottles, comprise a group of microorganisms commonly isolated from blood cultures. Among them, Staphylococcus aureus, including methicillin-resistant S. aureus (MRSA), is the most virulent species and is considered an important pathogen in hospitals (9), and increasingly in communities, around the world (15). Moreover, the incidence of infections caused by coagulase-negative staphylococci (CoNS) appears to be increasing. For example, Staphylococcus lugdunensis has recently been recognized as an important pathogen in bloodstream infections (5, 31). Staphylococcus epidermidis and other CoNS species are normal flora of skin and mucous membranes and are therefore common contaminants in blood cultures (2, 16, 38). Therefore, it is important to identify and differentiate between potential pathogenic and contaminant Staphylococcus species in a timely manner for appropriate clinical intervention.

Blood culture using automated blood culture instruments with continuous monitoring remains the routine method for detecting bacterial bloodstream infections. Currently, definitive identification and antimicrobial susceptibility testing of GPCC is time consuming, requiring 24 to 72 h from the initial positive result for subculture, biochemical analysis, and antimicrobial susceptibility testing. This delay may lead to the unnecessary use of antimicrobial agents. Advanced laboratory techniques have been sought to rapidly identify staphylococcal isolates and determine antimicrobial susceptibility patterns. In particular, several molecular methods have been described to identify and differentiate staphylococcal isolates (12, 24, 25, 33). To take advantage of the rapid enrichment of automated blood culture instruments, several studies have reported that use of peptide and/or nucleic acid probes and conventional and real-time PCR can rapidly identify organisms from flagged blood cultures when GPCC are detected (4, 6, 8, 12, 18, 23, 30, 32, 36, 40, 43).

We developed and validated a StaphPlex system (Genaco...

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Biomedical Products, Inc., Huntsville, AL) for simultaneous species-level identification and detection of Panton-Valentine leukocidin (PVL) and several antimicrobial resistance determinants of staphylococci directly from blood culture medium in which GPCC were seen by using Gram staining. The StaphPlex system uses a unique target-enriched multiplex (Tem)-PCR method (14) to amplify and detect 18 Staphylococcus-specific genes simultaneously in 1 reaction. The tuf gene target provides identification and differentiation of CoNS, and the nuc gene target is specific for S. aureus. The mecA gene confers resistance to methicillin. The adcA gene is responsible for resistance to aminoglycosides. The ermA and ermC genes contribute to resistance to macrolides-lincosamides-streptogramins (MLS). The tetM and tetK genes are responsible for resistance to tetracycline. The amplified products are further characterized by using a Luminex 100 suspension array. The whole process, from the blood culture medium processing to results reporting, can be performed within 5 h, which greatly shortens the time usually needed for phenotypic identification and antimicrobial susceptibility testing.

**MATERIALS AND METHODS**

Reference strains and clinical specimens. A panel of staphylococcal reference strains were obtained from the American Type Culture Collection (ATCC) (Manassas, VA) or previous studies (19). They included 10 S. aureus strains (ATCC 35556, 700699, 700698, BAA38, BAA39, BAA40, BAA41, BAA42, BAA43, and MRSA52), 2 S. epidermidis strains (35984 and 12228), 1 Staphylococcus haemolyticus strain (29770), 2 Staphylococcus hominis strains (27845 and 700235), 2 Staphylococcus simulans strains (27848 and 11631), 1 S. lugdunensis strain (43809), 1 Staphylococcus capitis strain (27840), and 1 Staphylococcus warneri strain (25614), 1 Staphylococcus xylosus strain (700044), and 1 Staphylococcus saprophyticus strain (15305). A total of 360 positive blood culture specimens from a BACTEC 9240 blood culture system (Becton Dickinson Diagnostic Instrument Systems, Sparks, MD) which demonstrated GPCC by Gram staining were collected consecutively from 13 August through 13 October 2005 at Vanderbilt University Hospital and included in the study. If a patient had multiple blood cultures demonstrating GPCC within 1 week, only the first specimen from the aerobic bottle was selected. Bacterial isolates recovered from the same bottle upon subculture were also collected and saved in brain heart infusion containing 4.5% sodium chloride (37).

**RESULTS**

Specimen processing and DNA extraction. Total nucleic acids were extracted from the contents of the positive blood culture bottles using a NucliSens easyMAG system (bioMerieux, Inc., Durham, NC). Briefly, 100 μl of contents were spun down, the supernatant was discarded, and the pellets were mixed with 100 μl of lysis buffer (bioMerieux, Inc.). After a thorough vortex mix, the mixture was placed in the instrument using the default extraction protocol (41). Total nucleic acids were eluted in 55 μl of elution buffer (bioMerieux, Inc.) and 5 μl of the extracts were used for nucleic acid amplification.

**Templex multiplex amplification.** The Templex StaphPlex I assay system (catalog no. 021-01-S; Genaco Biomedical Products, Inc., Huntsville, AL) was used according to the manufacturer’s instructions. In brief, 6 μl Templex StaphPlex I Superprimers was added to 25 μl of QIAGEN HotStarTaq master mix (QIAGEN, Inc., Valencia, CA). Next, 5 μl of extracted DNA and 14 μl of water were added for a final volume of 50 μl. Amplification was carried out with the five-stage Templex cycling program: (i) Hot-start; 95°C, 15 min; (ii) enrichment stage consisting of 15 cycles of 94°C, 30 s; 55°C, 1 min 30 seconds; and 72°C, 1 min; (iii) tagging stage consisting of six cycles of 94°C, 30 s; 70°C, 1 min, 30 s; (iv) amplification stage consisting of 35 cycles of 94°C, 20 s; 55°C, 30 s; and 72°C for 20 s; and (v) extension stage, 72°C for 3 min (14). The molecular targets included in this StaphPlex are listed in Table 1.

Amplification product identification. The amplified products were further characterized by using a suspension array for multiplex detection (catalog no. 021-01-D; Genaco Biomedical Products, Inc., Huntsville, AL) performed with a Luminex 100 instrument (Luminex, Austin, TX). In brief, 35 μl hybridization buffer was mixed with 10 μl bead mix, and 5 μl PCR product was then added. The mixture was incubated at 52°C for 10 min for hybridization. Ten microliters of a freshly made 1:2 dilution of Streptavidin-PE was added to the mixture. After an additional incubation for 5 min, 120 μl of prewarmed stopping buffer was added to the mixture and the results were read immediately using the Luminex machine. A red laser identifies each bead (or target) by its color-coding, while a green laser detects the hybridization signal associated with each bead. Results for each channel are expressed as the median fluorescence intensity value (14). The cutoff value for each target was determined as the sum of the mean plus four times the standard deviations of the negative controls.

SCCmec typing and PVL detection by real-time PCR. The reference method to determine staphylococcal cassette chromosome mec (SCCmec) types I, II, III, and IV and to detect PVL was a real-time TaqMan PCR performed with the 7700 ABI Prism sequence detector (Applied Biosystems, Foster City, CA) as described previously (11) with modification. In brief, 5 μl of the extracted nucleic acid was added to 24 μl of reaction mixture containing 0.8 μM of each primer and 0.4 μM fluorophore probe (final concentration) and mixed with 25 μl of TaqMan Universal PCR master mix (Applied Biosystems). The primers and fluorophore TaqMan probes for SCCmec types I, II, III, and IV and PVL and the real-time PCR protocol were published previously (11, 19).

Statistical analysis. Statistical comparisons were performed with EpInfo software (version 6; CDC, Atlanta, GA). The accuracy of StaphPlex for identification of CoNS, MRSA, and methicillin-susceptible S. aureus (MSSA) and species identification of CoNS was determined in comparison to the reference identification determined at the CDC. Sensitivity, specificity, and positive predictive values of the StaphPlex method for SCCmec typing and PVL detection were determined in comparison to real-time PCR results. Very major, major, and minor error rates for antimicrobial resistance determinants were calculated in comparison to the phenotypic antimicrobial susceptibility results. P values of ≤0.05 were considered statistically significant.
the internal control gene IDS, indicating that they were free of amplification inhibitors.

We first compared the identification obtained with StaphPlex or phenotypic methods for the ability to detect and differentiate between CoNS, MRSA, and MSSA. Among a total of 360 GPCC specimens, 273 (75.8%), 37 (10.3%), 37 (10.3%), 1 (0.3%), 3 (0.8%), and 9 (2.5%) were identified by StaphPlex as CoNS, MRSA, MSSA, mixed infections of CoNS and MRSA, MSSA-MRSA coinfection, and 3 negatives and an additional 11 enterococci, 5 E. coli isolates, 4 group A streptococci, 3 Pseudomonas aeruginosa isolates, 2 Serratia marcescens isolates, and 1 Peptostreptococcus sp. isolate.

The overall accuracies of StaphPlex were 90.8% (perfect match) or 91.7% (one match in coinfection) in comparison to standard identification. The overall accuracy of StaphPlex for species-level identification of CoNS was 80.1% (Table 3).

Phenotypic antimicrobial susceptibility results were available for five clinically important CoNS species, S. epidermidis, S. haemolyticus, S. hominis, S. simulans, and S. lugdunensis. The 277 CoNS-containing specimens were further identified as 203 (73.5%) S. epidermidis isolates, 10 (3.6%) S. haemolyticus isolates, 27 (9.7%) S. hominis isolates, 1 (0.4%) S. lugdunensis isolate, and 36 (13.0%) other CoNS isolates by StaphPlex. S. simulans was not detected by StaphPlex in 360 specimens studied. CoNS was subcultured and identified from 277 specimens by phenotypic methods and included 271 pure cultures and 6 mixed cultures with either MRSA or MSSA (Table 2). CoNS isolates were identified using the API STAPH test. Isolates with discrepant results between API STAPH and StaphPlex were further identified at the CDC by reference methods. The overall accuracy of StaphPlex for species-level identification of CoNS was 80.1% (Table 3).

| TABLE 2. Accuracy of StaphPlex for identification of CoNS, MRSA, and MSSA directly from positive blood culture |
| --- |
| Standard identification (n) (a) | CoNS (265) | MRSA (40) | MSSA (32) | Nonstaphylococci (11) | CoNS-MRSA coinfection (12) | CoNS-MSSA coinfection (0) |
| CoNS (273) | 258 | 4 | 2 | 2 | 7 | 0 |
| MRSA (37) | 3 | 33 | 0 | 0 | 1 | 0 |
| MSSA (37) | 1 | 3 | 29 | 2 | 2 | 0 |
| Negatives or nonstaphylococci (9) | 2 | 0 | 1 | 6 | 0 | 0 |
| CoNS-MRSA coinfection (1) | 0 | 0 | 0 | 0 | 1 | 0 |
| CoNS-MSSA coinfection (3) | 1 | 0 | 0 | 1 | 1 | 0 |

(a) Overall correct identification rate of StaphPlex is 90.8% (perfect match) or 91.7% (one match in coinfection) in comparison to standard identification. Subculture identified 3 negatives and an additional 11 enterococci, 5 E. coli isolates, 4 group A streptococci, 3 Pseudomonas aeruginosa isolates, 2 Serratia marcescens isolates, and 1 Peptostreptococcus sp. isolate. 
(b) Specimens with discrepant identifications between phenotypic and StaphPlex tests were further identified by CDC-recommended methods (20, 21). 
(c) n, no. of specimens. 
(d) Total no. of specimens for each StaphPlex group is given in parentheses.
for 345 of 360 specimens studied. We next assessed the correlation of phenotypic antimicrobial susceptibility profiles with detection of antibiotic-resistance genes, including \textit{aacA}, \textit{ermA}, \textit{ermC}, \textit{tetM}, and \textit{tetK}. Since phenotypic results for minocycline resistance were not available, we assessed the concordance between detection of \textit{aacA}, \textit{ermA}, and \textit{ermC} with antimicrobial susceptibility profiles for gentamicin, erythromycin, and clindamycin. Resistance gene detection by StaphPlex demonstrated poor sensitivity in comparison to phenotypic antimicrobial susceptibility testing for 345 specimens analyzed, resulting in high very major error rates (range, 6.9% to 93.1%). In contrast, the specificity of this assay correlated well with phenotypic antimicrobial resistance, resulting in low major error rates for gentamicin, erythromycin, and clindamycin (Table 4).

Of 37 specimens yielding only MRSA, neither \textit{ermA} nor \textit{ermC} was detected in erythromycin- or clindamycin-susceptible specimens, resulting in 0 major errors and a positive predictive value of 100% (Table 4) for MRSA.

A total of 56 specimens containing MRSA were found by either culture or StaphPlex. We next assessed the accuracy of StaphPlex for SCC\textit{mec} typing and PVL detection compared to results obtained with a real-time TaqMan assay. As shown in Table 5, the sensitivity and specificity of StaphPlex were 100.0% and 95.8% for determining SCC\textit{mec} II (traditionally health care-associated MRSA) and 100.0% and 95.5% for determining SCC\textit{mec} IV (traditionally community-associated MRSA). All specimens except two were interpreted as negative for PVL by both the TaqMan and StaphPlex methods, giving a specificity of 100.0%. The number of PVL-positive specimens was so low that no conclusion can be made regarding its sensitivity (Table 5).

**DISCUSSION**

We describe here a novel strategy for rapid detection and characterization of staphylococci directly from flagged blood culture bottle contents when GPCC are recognized by Gram staining. The StaphPlex system provides simultaneous staphylococcal identification, antibiotic resistance determinant detection, detection of PVL, and determination of SCC\textit{mec} types I to IV within 5 h. This may potentially better direct antibiotic usage when GPCC are detected and thus lead to an overall

**TABLE 3. Accuracy of StaphPlex for CoNS species determination directly from positive blood culture**

| Standard identification\(^{b}\) (\(n\)) | S. epidermidis (203) | S. haemolyticus (10) | S. hominis (27) | S. lugdunensis (1) | Other (36) |
|------------------------------------------|---------------------|---------------------|----------------|-------------------|-----------|
| S. epidermidis (203)                     | 186                 | 0                   | 0              | 1                 | 0         |
| S. haemolyticus (12)                     | 0                   | 10                  | 0              | 0                 | 2         |
| S. hominis (34)                          | 7                   | 0                   | 25             | 0                 | 2         |
| S. lugdunensis (1)                       | 0                   | 0                   | 0              | 1                 | 0         |
| S. auricularis (1)                       | 0                   | 0                   | 0              | 0                 | 1         |
| S. capitis (10)                          | 7                   | 0                   | 0              | 0                 | 1         |
| S. pasteurii (2)                         | 0                   | 0                   | 0              | 0                 | 1         |
| S. warneri (2)                           | 1                   | 0                   | 0              | 0                 | 1         |
| "S. pettenkoferi" (9)                    | 0                   | 0                   | 0              | 0                 | 0         |
| S. aureus (1)                            | 1                   | 0                   | 0              | 0                 | 0         |
| Other, nonstaphylococcal species (2)     | 1                   | 0                   | 0              | 0                 | 0         |

\(^{a}\) Overall correct identification rate of StaphPlex is 80.1% in comparison to phenotypic identification.  
\(^{b}\) API STAPH was used first as the phenotypic identification standard. Specimens with discrepant identifications between API STAPH and StaphPlex were further identified by CDC reference methods (20, 21).  
\(^{c}\) n, no. of isolates.  
\(^{d}\) Total no. of isolates in each StaphPlex group is given in parentheses.  
\(^{e}\) Proposed name.

**TABLE 4. Performance of StaphPlex for SCC\textit{mec} typing and PVL detection compared to results obtained with a real-time TaqMan assay**

| Antibiotic and gene | Specimen category (\(n\)) | AST, R; SP, + | AST, R; SP, − | AST, S; SP, + | AST, S; SP, − | Very major error (%) | Major error (%) | PPV (%) | NPV (%) |
|---------------------|-----------------------------|---------------|---------------|---------------|---------------|---------------------|----------------|---------|---------|
| Gentamicin; \textit{aacA} | All (345)                  | 32            | 32            | 20            | 261           | 50.0               | 7.1            | 61.5    | 89.1    |
|                     | MRSA (37)                   | 1             | 2             | 1             | 33            | 66.7               | 2.9            | 50.0    | 94.3    |
| Erythromycin; \textit{ermA} | All (345)                  | 70            | 185           | 3             | 87            | 72.5               | 3.3            | 95.9    | 32.0    |
|                     | MRSA (37)                   | 27            | 6             | 0             | 4             | 18.2               | 0.0            | 100.0   | 40.0    |
| Erythromycin; \textit{ermC} | All (345)                  | 110           | 145           | 7             | 83            | 56.9               | 7.8            | 94.0    | 36.4    |
|                     | MRSA (37)                   | 3             | 30            | 0             | 4             | 90.9               | 0.0            | 100.0   | 11.5    |
| Clindamycin; \textit{ermA} | All (345)                  | 66            | 123           | 7             | 149           | 65.1               | 4.5            | 90.4    | 54.8    |
|                     | MRSA (37)                   | 27            | 2             | 0             | 8             | 6.9                | 0.0            | 100.0   | 80.0    |
| Clindamycin; \textit{ermC} | All (345)                  | 104           | 85            | 13            | 143           | 45.0               | 8.3            | 88.9    | 62.7    |
|                     | MRSA (37)                   | 2             | 27            | 0             | 8             | 93.1               | 0.0            | 100.0   | 22.9    |

\(^{a}\) AST, phenotypic antimicrobial susceptibility testing; SP, StaphPlex resistance-related gene detection; R, resistant; S, susceptible; +, gene detected; −, gene not detected; PPV, positive predictive values; NPV, negative predictive values.  
\(^{b}\) n, no. of specimens.
reduction of use of vancomycin, which is often used empirically to treat patients until susceptibility results are available.

Templex products were developed using proprietary Tem-PCR (target-enriched multiplex PCR) technology. Tem-PCR was developed to overcome the three most difficult problems associated with conventional multiplex PCR: incompatible primer sets, high background amplification/detection, and poor reproducibility. First, nested primers increase compatibility among loci. Instead of forcing all targets to accept only one amplification condition, Templex uses a nested primer design that allows each target four opportunities to be amplified. Second, low concentrations of unlabeled, gene-specific primers reduce background. Third, use of low-concentration, unlabeled primers improves reproducibility. A major advantage of the Templex technology is its ease of use. Since it is unnecessary to perform post-PCR cleanup or posthybridization washes, hands-on time is limited to less than 2 h. The entire procedure can be automated easily because only reagent addition is required. Eliminated are complicated PCR manipulations, such as gel electrophoresis, centrifugation to remove unused primers, and vacuum filtration for posthybridization washes.

Six resistance gene targets were included in the StaphPlex panel. The mecA gene confers resistance to methicillin in both CoNS and S. aureus and is used to differentiate methillin-resistant staphylococci from susceptible isolates. When mecA is detected in specimens containing both CoNS and S. aureus, it is important to correctly differentiate between MRSA and MSSA. We made this determination based on the reading of median fluorescence intensities and reached satisfactory results in comparison to phenotypic methods, which resulted in a relatively low concordance between phenotypic and StaphPlex methods for MRSA and MSSA detection. In addition, for these incorrectly determined specimens, SCCmec typing was also affected, since SCCmec genes can be detected in some CoNS. An S. aureus-specific gene located adjacent to the SCCmec element should be included to provide accurate differentiation between MRSA and methicillin-resistant CoNS when a specimen contains a mixture of staphylococci and is positive for mecA (17).

Five additional gene targets were included in the StaphPlex panel to predict resistance to MLS and tetracycline in MRSA (13, 26, 28, 34, 35). The bifunctional enzyme AacA-AphD, encoded by aacA, inactivates a broad range of clinically useful aminoglycosides, such as gentamicin, tobramycin, and amikacin, and is the most frequently encountered aminoglycoside resistance mechanism among staphylococcal isolates (35). Two MLS resistance determinants, ermA and ermC, included in the StaphPlex panel encode methylases which dimethylate adenine residues in the bacterial 23S rRNA. This dimethylation leads to a conformational change in the ribosome, rendering resistance to most antibiotics in the MLS group (26, 34). Two mechanisms of tetracycline resistance in staphylococci are ribosomal protection mediated by transposon-located or chromosomal determinants, e.g., tetM (28), and active efflux resulting from acquisition of plasmid-located genes, e.g., tetK (13). S. aureus strains carrying tetK are resistant only to tetracycline, while tetM confers resistance to both tetracycline and minocycline (3). When resistance gene detection was used to predict phenotypic susceptibility results, a relatively small major error rate was noticed, but the very major error rates were quite high compared to phenotypic antimicrobial susceptibility profiles. This may not be surprising, since multiple mechanisms can confer antimicrobial resistance. Unless all resistance mechanisms are known and are covered by a genetic assay, early determination of antibiotic resistance by detecting associated genes is not clinically useful. In our limited specimens yielding only MRSA, 0 major errors and a 100.0% positive predictive value were noticed. The poor sensitivity of this component of the StaphPlex assay suggests that it would not be a reliable tool for the clinical microbiology laboratory, although the positive predictive value implies it may be useful when resistance determinants are detected.

Although many CoNS isolates recovered from blood cultures are considered contaminants, some of them have been identified as important pathogens causing bloodstream infections (2, 5, 16, 31, 38). A rapid identification of several potentially pathogenic CoNS isolates to the species level is clinically desirable. We chose API STAPH as the primary CoNS identification method (6, 22); CDC reference methods were used for further identification of isolates with discrepant results between the API STAPH and StaphPlex tests. An overall accuracy of 80.1% was achieved when StaphPlex was used for CoNS species determination directly from positive blood culture bottles. Currently StaphPlex provides species-level identification of only five commonly encountered CoNS species. Rapid and accurate CoNS identification based on the genetic information may have implications for interpretation of antimicrobial susceptibility assays and may provide a relevant tool in the routine clinical microbiology service.

SCCmec typing provides evidence for the derivation of MRSA clones traditionally associated with health care or the community (9, 29). SCCmec types I, II, and III have been found in strains associated with health care, whereas SCCmec types IV and V have been associated with community disease throughout the world (1, 10). Strains isolated from community-associated MRSA disease are believed to be hypervirulent, predominantly causing skin and soft tissue infection but also causing serious necrotizing pneumonia. The increased viru-

| Gene        | No. of specimens with result | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) |
|-------------|------------------------------|----------------|-----------------|---------|---------|
| SCCmec II   | TaqMan, +; StaphPlex, +     | 32             | 100.0           | 95.8    | 97.0    | 100.0   |
|             | TaqMan, +; StaphPlex, −     | 0              | 100.0           | 95.5    | 85.7    | 100.0   |
|             | TaqMan, −; StaphPlex, +     | 1              | 23              | 95.5    | 85.7    | 100.0   |
|             | TaqMan, −; StaphPlex, −     | 23             | 100.0           | 95.5    | 85.7    | 100.0   |
| SCCmec IV   | TaqMan, +; StaphPlex, +     | 12             | 100.0           | 95.5    | 85.7    | 100.0   |
|             | TaqMan, +; StaphPlex, −     | 0              | 100.0           | 95.5    | 85.7    | 100.0   |
|             | TaqMan, −; StaphPlex, +     | 2              | 42              | 95.5    | 85.7    | 100.0   |
|             | TaqMan, −; StaphPlex, −     | 2              | 54              | 100.0   | 100.0   | 100.0   |
| PVL         | TaqMan, +; StaphPlex, +     | 2              | 100.0           | 100.0   | 100.0   | 100.0   |
|             | TaqMan, +; StaphPlex, −     | 0              | 100.0           | 100.0   | 100.0   | 100.0   |
|             | TaqMan, −; StaphPlex, +     | 0              | 100.0           | 100.0   | 100.0   | 100.0   |
|             | TaqMan, −; StaphPlex, −     | 0              | 100.0           | 100.0   | 100.0   | 100.0   |
lence is believed to be due, in part, to the PVL, which, along with superantigens, can cause severe tissue necrosis (10, 27). We incorporated five genetic targets, SCCmec I to IV and PVL, to determine the SCCmec type and PVL presence for MRSA strains. In comparison to the real-time TaqMan PCR assays, StaphPlex provides satisfactory specificity and sensitivity for rapid SCCmec typing, which may provide prompt information for directing further clinical and epidemiologic intervention. More PVL-positive specimens need to be included in larger-scale studies to determine StaphPlex sensitivity for PVL detection.

The StaphPlex system implements a one-test strategy for rapid and simultaneous identification, detection of antibiotic resistance determinants and PVL, and SCCmec typing of staphylococci from positive blood cultures. The antimicrobial susceptibility results were poor, with extremely high very major errors identified. Further improvements are needed to reach satisfactory performance in the future.

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