Rapid detection of meticillin-resistant *Staphylococcus aureus* bacteraemia using combined three-hour short-incubation matrix-assisted laser desorption/ionization time-of-flight MS identification and Alere Culture Colony PBP2a detection test

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Meticillin-resistant *Staphylococcus aureus* (MRSA) bloodstream infection is responsible for significant morbidity, with mortality rates as high as 60% if not treated appropriately. We describe a rapid method to detect MRSA in blood cultures using a combined three-hour short-incubation BRUKER matrix-assisted laser desorption/ionization time-of-flight MS BioTyper protocol and a qualitative immunochromatographic assay, the Alere Culture Colony Test PBP2a detection test. We compared this combined method with a molecular method detecting the *nuc* and *mecA* genes currently performed in our laboratory. One hundred and seventeen *S. aureus* blood cultures were tested of which 35 were MRSA and 82 were meticillin-sensitive *S. aureus* (MSSA). The rapid combined test correctly identified 100% (82/82) of the MSSA and 85.7% (30/35) of the MRSA after 3 h. There were five false negative results where the isolates were correctly identified as *S. aureus*, but PBP2a was not detected by the Culture Colony Test. The combined method has a sensitivity of 87.5%, specificity of 100%, a positive predictive value of 100% and a negative predictive value of 94.3% with the prevalence of MRSA in our *S. aureus* blood cultures. The combined rapid method offers a significant benefit to early detection of MRSA in positive blood cultures.

INTRODUCTION

*Staphylococcus aureus* is a clinically important pathogen that causes a wide variety of diseases and accounts for more than 50% of nosocomial infections in intensive care units (NNIS System, 1999). *S. aureus* bacteraemia (SAB) is an infection of the blood associated with a mortality rate in excess of 20% (Klevens *et al.*, 2006; Lowy, 1998) while meticillin-resistant *S. aureus* (MRSA) bacteraemia is associated with mortality rates as high as 60% (Cluff *et al.*, 1968; Julander, 1985).

Until the 1980s, MRSA was almost exclusively limited to nosocomial settings and community strains were largely considered meticillin susceptible (Thompson *et al.*, 1982). However, a decade later there was a measurable increase in community-acquired meticillin-resistant *S. aureus* (CA-MRSA) infections in people with no known risk factors and an overall increase in CA-MRSA infections in general (Layton *et al.*, 1995). In Canada, there was a 17-fold increase in CA-MRSA infections from 1995 to 2007 (Simor *et al.*, 2010). In London, Ontario, the all-cause mortality...
rate among SAB patients was 27.9% and there was an increase in MRSA bacteraemia from 40% to 51% from 2008 to 2012 (Anantha et al., 2014). Populations at risk of developing MRSA bacteraemia include the elderly and patient subgroups with frequent healthcare facility contact (Laupland et al., 2008; Lodise et al., 2003). Other MRSA bacteraemia risk factors identified by Lodise et al. (2003) include prior antibiotic use, nosocomial infection, previous hospitalization and decubitus ulcers.

Currently, there are several screening methods used to determine meticillin resistance, including a cefoxitin disc induction test, an oxacillin–salt agar screening plate, molecular tests for the presence of the mecA gene, and penicillin binding protein 2a (PBP2a) culture colony test (Al-Talib et al., 2009; Cavassini et al., 1999). Some of these methods depend on well-defined colony morphology and can be very time and labour intensive. To the best of our knowledge, a combined rapid, 3 h procedure, using a short-incubation method, has not been studied. The objective of this study was to determine the ability to rapidly identify MRSA from positive S. aureus blood cultures using the CCT in combination with a 3 h SIMI protocol.

methods

Blood culture. Blood cultures submitted to the microbiology laboratory at the London Health Sciences Centre (LHSC) in London, Ontario, Canada, were incubated in the VersaTREK (TREK Diagnostic; Thermo Fisher Scientific) continuous monitoring blood culture system for a minimum of 5 days at 35°C to obtain a negative result. Extended incubation protocols for fastidious organism detection are available when requested. A set consisted of a VersaTREK REDOX 1 (aerobic) and a VersaTREK REDOX 2 (anaerobic) bottle. The VersaTREK automated microbial detection system detects positive cultures by measuring pressure changes in the bottle headspace with an external pressure sensor. Each set submitted was considered unique if received during different patient encounters or if the collection time between blood culture sets exceeded 72 h. All positive REDOX bottles were processed and tested. We defined a positive MRSA result as any positive result obtained in a unique set of blood cultures as previously defined.

A Gram stain was performed on the positive VersaTREK REDOX blood culture bottles and the result reported as a critical result to the attending physician. All positive blood cultures were processed according to a SIMI protocol developed at London Health Sciences Centre, London, Ontario.

SIMI protocol. Positive blood cultures were sub-cultured onto a Columbia blood agar plate (BAP) (Oxoid) and incubated at 35°C in 5% CO2 for a minimum of 3 h. The SIMI was performed three times daily, at 11:00, 14:30 and 21:00. The BAP was inspected for growth after 3 h. Growth consisted of small colonies or a fine sheen film on the BAP. Colonies were transferred using the direct colony transfer method on the Bruker MALDI-TOF BioTyper instrument.

Bruker MALDI-TOF BioTyper identification. The Bruker MALDI-TOF BioTyper 96 target plate was inoculated with a bacterial test standard (no. 8255343 Bruker) and calibration was performed according to the manufacturer’s recommendations. The inoculum layer on the BAP was applied to a target spot as a thin monolayer using the direct colony transfer method. The target spot was allowed to dry and 1 µl 70% formic acid was added, followed by 1 µl of matrix (HCCA no. 8255344 Bruker) and allowed to dry at room temperature.

The identification of the isolates was performed on a Microflex LT instrument (Bruker Daltonics) with FlexControl (version 3.4) software (Bruker Daltonics) for the automatic acquisition of mass spectra in the linear positive mode within a range of 2 to 20 kDa. Automated analysis of the raw spectral data was performed by the MALDI BioTyper automation (version 3.1) software (Bruker Daltonics).

Identification criteria were followed as stipulated by the manufacturer. The identification criteria included a score, a colour grading and a consistency category. A score in excess of 2.0 with a consistency category A and a green colour grading were needed to accept the identification of the bacterial pathogen at the species level.

Blood cultures identified as S. aureus-positive, all of which complied with the above-defined identification criteria, were included in the rapid PBP2a detection study using the Alere PBP2a Culture Colony Test. All S. aureus isolates identified using the Bruker MALDI-TOF BioTyper passed the identification criteria defined previously.

CCT detection. The CCT detection test was performed immediately after the isolate was identified as S. aureus according to the manufacturer’s recommendations (Alere). The assay tube was placed on the assay card and two drops were dispensed from the dropper bottle marked Reagent 1. Fine growth from the short-incubation Columbia agar plate with 5% sheep blood (BAP) was sampled with an applicator stick and added to the reagent tube. Two drops of Reagent 2 were dispensed from the appropriate dropper bottle and the reagent tube vortexed until the solution turned from a blue colour to a clear, colourless solution. The test strip was then added with arrows pointing down (Fig. 1). After 5 min of incubation at room temperature, the assay results were read. Results were interpreted according to the manufacturer’s recommendations.

Molecular confirmation. After overnight incubation (minimum of 18 h) in 5% CO2 at 35°C, colonies from a BAP with a cefoxitin disc were sent for MRSA PCR. Susceptibility testing was performed using Vitek AST-GP67 panels (bioMérieux). Multiplex PCR for 16S rRNA, mecA and nuc genes was performed as part of our routine work-up. DNA extraction, PCR master mixture concentrations and amplification conditions were previously described by Hassain et al. (2000). The primers used for the detection of the nuc and mecA genes were published previously by Brakstad et al. (1992) and Vannuffel et al. (1995). The amplified products were detected by electrophoresis using a Caliper LabChip GX assay (Caliper Life Sciences). Each run included MSSA American Type Culture Collection (ATCC) 29213 (nuc positive, mecA negative), methicillin-resistant S. aureus (MRSA),rifampicin-resistant S. aureus (RIF-MRSA) and rifampicin-sensitive S. aureus (RIF-MRSA).
negative), MRSA ATCC 33591 (nuc positive, mecA positive) and a local meticillin-resistant Staphylococcus epidermidis strain known as QC#150 (nuc negative, mecA positive). Sterile saline was included as negative control.

**Discordant results.** Discordant results were assessed, as to potential clinical impact, by taking the colonization history and status of the patient into account.

**RESULTS**

One hundred and seventeen isolates of S. aureus were identified from the VersaTREK REDOX 1 and 2 bottles using the SIMI method. The isolates all had acceptable identifying criteria as defined in Methods. These isolates were immediately submitted for the CCT for PBP2a detection. The results of the rapid PBP2a detection were compared with our standard molecular detection method. Eighty-two (70 %) isolates were confirmed to be MSSA isolates being nuc positive and mecA negative and 35 (30 %) isolates were MRSA isolates being mecA and nuc positive. The CCT test detected 26 (77.7 %) of the 35 MRSA initial isolates correctly; two of the isolates were tested after less than 2 h of incubation. These two isolates were re-incubated and re-tested after the 3 h incubation period was completed and found to be positive. Two false negative isolates had positive results obtained from the same patient encounter and therefore had no clinical impact as the patient blood culture was correctly identified in another bottle of the set as MRSA. These four isolates were not considered clinically significant false negative results and this is reflected in our adjusted results.

Our adjusted results reflect that our method identified 85.7 % (30/35) isolates as MRSA. Five (14.3 %) of the isolates were considered to be false negatives as they were incorrectly identified as MSSA. The five isolates incorrectly identified as MSSA belonged to three different patients, two of whom were noted to have previously colonized patients; one patient was newly flagged as MRSA-positive by our infection control protocol after the result of the blood culture. All 82 isolates of the MSSA isolates were correctly identified as PBP2a-negative with the SIMI/CCT test, none of these patients was colonized with MRSA. The results are summarized in Table 1.

**Statistical analysis**

Compared with our standard molecular confirmation method, the SIMI/CCT had a sensitivity of 87.5 % [95 % confidence interval (CI): 73.2 % to 95.8 %], a specificity of 100 % (95 % CI: 95.6 % to 100 %), a positive predictive value of 100 % (95 % CI: 90 % to 100 %) and a combined negative predictive value of 94.3 % (95 % CI: 87.1 % to 98.1 %) given that the prevalence of MRSA in our S. aureus-positive blood cultures was 32.8 % (95 % CI: 24.6 % to 41.9 %).

**DISCUSSION**

Several studies have demonstrated an increase in mortality rate associated with MRSA bacteraemia, where mortality rates varied from 29 % to 63 % compared with mortality rates of 12 % to 27 % associated with MSSA bacteraemia (van Hal et al., 2012). Factors associated with this observation include pathogen-specific factors such as SCCmec-associated virulence factors, with SCCmec type II being an independent predictor of mortality (Ippolito et al., 2010; van Hal et al., 2012). Other bacterial virulence factors potentially resulting in increased mortality in MRSA bacteraemia include staphylococcal toxins such as phenol-soluble modulins (Li et al., 2009). Inefficient empirical therapy, as well as the questionable efficacy of vancomycin therapy influenced by pharmacokinetic and pharmacodynamic properties may also contribute (Ippolito et al., 2010; Kim et al., 2008).

In a meta-analysis, Cosgrove et al. (2003) showed a significant increase in mortality associated with MRSA bacteraemia compared with MSSA bacteraemia, with a pooled relative risk of 1.42. In another meta-analysis, Whitby et al. (2001) observed that bacteraemia caused by MRSA was associated with significantly higher mortality rates than bacteraemia caused by MSSA (29 % vs 12 %). The associated proportion of patients whose death was attributable to MRSA was significantly higher than that for MSSA [11.8 % vs 5.1 %; odds ratio (OR) 2.49] (Melzer et al., 2003). Bacteraemia due to MRSA is associated with both an increase in median hospital length of stay (9 days compared with 7 days) and consequently higher median hospital charges (US$ 26 424 vs US$ 19 212) (Melzer et al., 2003).

The treatment of MSSA bacteraemia with vancomycin is associated with treatment failure and higher mortality rates compared with treatment with β-lactam antibiotics.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Number tested</th>
<th>Molecular detection after 18 h of incubation</th>
<th>Rapid method: SIMI/CCT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>nuc</td>
<td>mecA</td>
</tr>
<tr>
<td>MSSA</td>
<td>82 (70%)</td>
<td>82 (100%)</td>
<td>0</td>
</tr>
<tr>
<td>MRSA</td>
<td>35 (30%)</td>
<td>35 (100%)</td>
<td>35 (100%)</td>
</tr>
<tr>
<td>Total</td>
<td>117</td>
<td>117</td>
<td>35</td>
</tr>
</tbody>
</table>
(Ippolito et al., 2010). Paul et al. (2010) found that mortality was significantly higher among patients receiving inappropriate antibiotic therapy (49.1%) compared with those receiving appropriate (33.3%) empirical antibiotic treatment.

In patients with MSSA-associated bacteraemia, Kim et al. (2008) showed in a retrospective cohort study a mortality rate of 37% in patients treated with vancomycin versus a mortality rate of 18% in patients treated with β-lactam antibiotics. In a patient population with MSSA-associated infective endocarditis, Lodise et al. (2007) showed that the mortality rate in patients who received empirical vancomycin was significantly higher (39.4%) than the mortality rate of 11.4% in patients who received empirical β-lactam therapy. Conversely, treating MRSA bacteraemia with a β-lactam antibiotic is associated with a significant increase in mortality. A meta-analysis of nine studies demonstrated that mortality rates are nearly doubled with inappropriate empirical therapy for MRSA bacteraemia compared with appropriate initial therapy (pooled OR, 1.99; 95% CI, 1.6–2.4) (Paul et al., 2010). This finding further stresses the importance of an accurate, but rapid MRSA detection method.

The current gold standard to detect and identify S. aureus-associated bacteraemia remains blood cultures and routine identification methods; this, however, usually takes longer than 2 days to complete. Peters et al. (2007) evaluated the performance of real-time PCR assays for the quantitative detection of S. aureus bacteraemia directly in blood samples. The sensitivity and specificity for bacteraemia of the S. aureus PCR were 75% and 93%, respectively. PCR and other molecular methods have variable reported success rates from positive blood culture bottles with percentage sensitivity and specificity rates in the lower to high nineties for different commercial and in-house-developed assays (Davis & Fuller, 1991; Ellem et al., 2015).

In our study, we compared our standard culture and MRSA PCR with an Alere PBP2a Culture Colony Test (CCT) in combination with a 3 h short-incubation MALDI-ToF identification (SIMI) protocol. This method had a significant advantage in turnaround time with results being reported within 3–9.5 h after the blood culture was flagged positive compared with 18–24 h with conventional testing. Although there were no false positive results, five out of 35 MRSA isolates were not identified as such. These five isolates were obtained from three different patients, two being previously colonized with MRSA.

In our institution, treatment of S. aureus takes into account MRSA colonization status and previous cultures; therefore, we reviewed patient charts to assess the impact that the false negative results would have had. Four of the false negative isolates were not considered to be false negatives; two isolates tested too early (at 1.5 h) were positive after additional incubation and two other isolates actually had positive results in one of the other blood culture bottles in the set.

It is clear that consideration of previous MRSA infections and colonization status is important for interpreting a negative result because only one patient had a negative colonization status. As this test has acceptable diagnostic criteria (sensitivity 87.5%, specificity 100% and negative predictive value of 94.3%) consideration should be given to de-escalating to a β-lactam antibiotic after MRSA is ruled out. Clinicians, however, ideally require a negative predictive value closer to 100% and this may be obtainable if colonization status is taken into account. In our study, only one result would have been considered a false negative result if colonization status was part of the diagnostic consideration, resulting in a sensitivity of 97.2%, positive predictive value of 100% and a negative predictive value of 98.8%.

Although a negative PBP2a result is not currently reported by our laboratory, consideration is given to continuation of a combination of vancomycin and a relevant anti-staphylococcal penicillin (oxacillin and nafcillin) or cephalosporin, as vancomycin single therapy still appears inferior to initial β-lactam therapy for MSSA (McConahy et al., 2013; Mongkolrattanothai et al., 2009). If a positive result is obtained, de-escalation to vancomycin as a single agent is warranted.

We established that a rapid method for detection of MRSA with the SIMI/CCT combination is of value in diagnosing the presence of MRSA in positive blood cultures. In order to avoid false negatives, further studies need to be performed to improve on the SIMI/CCT method. A negative predictive value in excess of 95% is desirable and this was achieved in our study when patient colonization status was taken into account. In addition, although not reported, improved sensitivity may be obtained by increasing the incubation time to 5 or even 7 h, thereby improving the negative predictive value of the test.

In this study, the negative predictive value is currently 94.3%, which highlights the importance that consideration needs to be given to MRSA colonization status, co-morbidities and disease burden in the local community as these will influence the negative predictive value and may guide physicians if a negative result is obtained in a population with a low burden of disease.

Clinical trials advocating combination therapy between vancomycin and a β-lactam (such as nafcillin, oxacillin or cefazolin) for staphylococcal bacteraemia are currently lacking. There may be clinical benefit demonstrated in such randomized control studies as the treatment of a MSSA with vancomycin alone is clearly associated with a more complicated disease prognosis (McConahy et al., 2013; Mongkolrattanothai et al., 2009). If such an approach is adopted, the SIMI/CCT may be of benefit for either discontinuing the β-lactam with a positive result or discontinuing vancomycin in the case of a negative result. As demonstrated in our results, this method is superior to studies reporting PCR identification directly from blood culture specimens without prior incubation and comparable to molecular detection from incubated blood cultures if
colonization status is taken into account. Considering that this method is simple to implement in the routine microbiology laboratory with MALDI-ToF technology, this protocol represents a cost-effective and time-efficient alternative to molecular methods and conventional culture identification of MRSA- and MSSA-associated bacteremia.

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REFERENCES


