Dear Editor,

Staphylococcus aureus is the most important cause of infection worldwide and the majority of such infections are related to methicillin-resistant S. aureus (MRSA) [1]. MRSA isolates present a staphylococcal cassette chromosome mec (SCCmec), and types II, III and IV are the most frequent [2,3,4]. Epidemiological studies conducted in recent years have shown that SCCmec IV isolates, mainly related to USA300 and European clones, are found in about 80% of MRSA isolates collected from community and hospital settings in Europe [2]. In the USA, MRSA lineages carrying SCCmec II are also very frequent [3], while the SCCmec III isolates are widespread in many countries and are found in hospitals on all continents [4].

MRSA colonization is a worldwide health problem. Moreover, in 2015 the World Health Organization prepared a global action plan against antimicrobial resistance which involves a series of strategies, including a significant innovation strategy for the diagnosis and control of pathogens [5]. A rapid identification of MRSA isolates and their SCCmec types could help track MRSA clones, allowing better control of infections as well as improvements in patient management. Here, we propose a method to detect MRSA and distinguish the types of SCCmec from nasal swabs after culture in pre-enriched selective broth.

The study

As part of a larger study, 362 nasal swabs were collected from February 2012 to November 2013, in an outpatient referral centre for atopic dermatitis in Rio de Janeiro. Swabs were collected from 159 pediatric outpatients and 203 relatives and then placed on mannitol-salt agar (MSA) (Oxoid Ltd, Basingstoke, England). After which, the swabs were inoculated into 5ml of selective broth (SB) (Mueller-Hinton broth Difco, Detroit, USA) containing 7% [wt/vol] NaCl and 2μg/ml of oxacillin (Sigma-Aldrich, St. Louis, USA) and incubated for 24h at 35° C [6]. MSA plates were incubated for 48h at 35° C. Both bacterial growths were transferred to blood agar and identified by standard tests. Methicillin-resistance was accessed by the cefoxitin disk test (Oxoid Ltd, Basingstoke, England) [7].

DNA was extracted by boiling and SCCmec typing was performed [8]. Nontypeable isolates were submitted to DNA extraction with guanidium thiocyanate and SCCmec typing [9]. Based on a previous study conducted by our group which showed that both SB and MSA presented specificity above 99% for MRSA detection [6], all isolates identified as MRSA by either method were considered true positive (gold standard detection). Multiplex PCR was carried out using DNA from SB liberated by boiling [6] plus 100μM of dNTPs (Life Technologies), Taq DNA polymerase (1.5U), Tris-HCl (20mM), KCl (50mM), MgCl2 (2mM) (Biotools, Madrid, Spain) and the
primers: Sa1/Sa2 (0.4μM) (S. aureus species) [10]; MRSA/MRS2 (1μM) [11], (mecA gene); MECIP2/MECIP3 (0.8μM) (SCCmec II and III) and CcrB1/CcrB2 (0.8μM) (SCCmecII and IV) [8]. Amplification conditions included 4min/94 ºC, 30 cycles of 94 ºC/30s, 53 ºC/30s and 72 ºC/1min and a final extension of 72º/4min.

The SB-MPCR method was validated using previously published control strains. These strains had been grown in SB until reaching turbidity 0.5 McFarland. DNA extraction and PCR procedures were as described above. Additionally, 35 MRSA clinical isolates previously characterized in relation to minimum inhibitory concentrations (MIC) to oxacillin and SCCmec types were submitted to SB-MPCR for simulation. The oxacillin MICs for the MRSA isolates recovered in the present study were also assessed [7].

The SB-MPCR method detected 42 MRSA isolates. Among the swabs that were MRSA negative according to this method, 180 coagulase-negative Staphylococcus (CoNS), 54 Gram negative bacteria (GNB) and nine methicillin-susceptible S. aureus (MSSA) isolates were detected; however 77 did not grow in the SB. On the other hand, routine tests detected 44 MRSA, 173 CoNS, 132 MSSA and six GNB. Considering that the isolates recovered from both SB and routine tests were true positives, 51 MRSA isolates were detected in this study (Table 1).

Nine MRSA isolates that were not-recovered in SB presented oxacillin MICs<4μg/ml (ranging from 0.5 to 1 μg/ml) (Table 2). Similar results were obtained for the clinical MRSA isolates (Table 2). The SB-MPCR method identified the SCCmec type of 35 MRSA isolates among a total of 42 isolates detected (Table 1). Three isolates carried one of the type II, III or nontypeable cassette, while the other 32 carried the SCCmec IV (Figure 1). Seven isolates carrying the

Table 1. Results obtained from culture in selective broth followed by the multiplex PCR (SB-MPCR) method in comparison to the routine tests for the detection of methicillin-resistant Staphylococcus aureus and their SCCmec types from 362 nasal swabs.

<table>
<thead>
<tr>
<th>Detection method</th>
<th>MRSA isolates (total=51)</th>
<th>MRSA isolates carrying SCCmec IV (total=48)</th>
<th>MRSA isolates carrying other SCCmec types (total=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TP^a</td>
<td>FN^b</td>
<td>SE^e</td>
</tr>
<tr>
<td>Routine tests</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>44</td>
<td>7</td>
<td>86.2%</td>
</tr>
<tr>
<td>SB-MPCR</td>
<td>42</td>
<td>9</td>
<td>82.3%</td>
</tr>
</tbody>
</table>

^a True positive; ^b False negative; ^c Sensibility; ^d Specificity; ^e Positive predictive value; ^f Negative predictive value; ^g All these nine MRSA isolates presented MIC to oxacillin <4μg/ml; ^h Isolates presented SCCmec types II and III and a nontypeable cassette; ^i Excluding nine isolates that did not grow in selective broth and 7 isolates that were misidentified.

Table 2. Comparative results obtained from culture in selective broth followed by the multiplex PCR (SB-MPCR) method in clinical MRSA isolates carrying different SCCmec types and presenting different oxacillin MIC values in the MRSA isolates detected in the present study.

<table>
<thead>
<tr>
<th>SCCmec type/ oxacillin MIC (n° of MRSA isolates)</th>
<th>MRSA isolates</th>
<th>SCCmec types detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical isolates (total=35)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II/ ≥4μg/ml (5)</td>
<td>5</td>
<td>100%</td>
</tr>
<tr>
<td>III/ ≥4μg/ml (10)</td>
<td>10</td>
<td>100%</td>
</tr>
<tr>
<td>IV/ &lt;4μg/ml (10)</td>
<td>7</td>
<td>100%</td>
</tr>
<tr>
<td>IV/ ≥4μg/ml (10)</td>
<td>10</td>
<td>100%</td>
</tr>
<tr>
<td>Total</td>
<td>32</td>
<td>100%</td>
</tr>
</tbody>
</table>

| Isolates detected in the present study (total=51) |               |                        |
| IV/ ≥4μg/ml (39)                                 | 39            | 100%                   |
| IV/ <4μg/ml (9)                                 | 0             | 91.4%                  |
| Other/ ≥4μg/ml (3)                              | 3             | 100%                   |

^a True positive; ^b False negative; ^c Sensibility; ^d Specificity; ^e These isolates did not grow in selective broth and presented oxacillin MICs ranging from 0.5 to 1μg/ml; ^f These isolates did not grow in selective broth and presented oxacillin MICs ranging from 0.5 to 2μg/ml; na - not applicable
SCCmec IV showed only the mecA gene band in the test.

The detection of MRSA from clinical specimens has been proposed elsewhere [6,12,13]. However, these proposed methods were not able to distinguish the different SCCmec types. Here, we successfully described a rapid PCR-based method to detect the mecA gene and segments of the SCCmec types II, III and IV in MRSA isolates grown in a selective broth.

In the present study conventional and SB-MPCR methods presented excellent specificity although failed to detect some MRSA isolates. The SB-MPCR presented a sensitivity of 82.3% to detect MRSA. This value was similar to the one obtained by Yam et al. [13] in the evaluation of a commercial PCR-based method for MRSA detection (83.3%). Our method failed to detect nine isolates, whose oxacillin MICs were lower than 4µg/ml (range of 0.5 to 1µg/ml), values commonly found among community isolates [14]. However, MRSA clinical isolates with very low oxacillin MICs are not common. Moreover, to investigate the role of MIC in the accuracy of the SB-MPCR method, 35 MRSA clinical isolates previously characterized were analyzed by this method and similar results were found, confirming that type IV isolates presenting very low oxacillin MICs cannot be detect by the SB-MPCR method. According to Andriesse et al. [12], false negative in conventional methods are related to low counts in community patients. They found 86% of sensitivity for bacterial culture agreeing with the present study.

The SB-MPCR method was also designed to distinguish the types of SCCmec prevalent worldwide, characterizing the methicillin resistance quickly. SB-MPCR identified the SCCmec types in 68.8% of the 51 MRSA isolates. Seven SCCmec IV isolates showed only the mecA gene band. The great genetic diversity of SCCmec IV, probably due to recombination and enhanced mobility, is a possible explanation for the difficulty to detect these isolates [15].

To avoid false-positive results from the coexistence between methicillin-resistant CoNS and MSSA isolates, Schuenck et al. [6] recommended 4µg/ml of oxacillin to inhibit MSSA. However, in the present study, this concentration inhibited type IV isolates (data not shown). Although dubious results can occur, Schuenck and coworkers did not find any questionable results even at concentrations of 2µg/ml of oxacillin.

A number of studies using various multiplex PCRs for SCCmec typing have been described [8,9]. These techniques present good sensitivity and specificity and can identify a large number of SCCmec types. However, these methods were designed to detect only SCCmec types after the identification of S. aureus followed by DNA extraction, which requires a minimum of 72 hours.

Here we present a rapid detection of MRSA and SCCmec types from nasal swabs which is faster than the conventional methodologies. The SB-MPCR showed high specificity, low cost and a sensitivity similar to the commercial PCR-based methods. Detection of S. aureus species, methicillin resistance and SCCmec type was performed within 24h after culture in selective broth. False negative results were associated to isolates with very low oxacillin MICs, situations that are not clinically common. Moreover, the method characterized the SCCmec types of the majority of isolates, showing that it can provide fast and reliable results to help control MRSA infections.

Acknowledgements
This study was supported by Brazilian grants from Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ), Conselho Nacional de Desenvolvimento Científico e Tecnológico ( CNPq), Coordenação de Aperfeiçoamento Pessoal de Nível Superior (CAPES), Fundação Universitária José Bonifácio (FUJB) and Programa de Núcleos de Excelência (PRONEX).

References


**Corresponding author**

Kátia Regina Netto dos Santos
Laboratório de Infeção Hospitalar, Departamento de Microbiologia Médica
Instituto de Microbiologia Paulo de Góes, Universidade Federal do Rio de Janeiro, CCS, Bloco I, Sala 010. Rio de Janeiro, RJ, Brazil.
CEP: 21941-590
Phone: 55-21-2560-8344
Email: santoskrn@micro.ufrj.br

**Conflict of interests:** No conflict of interests is declared.