Staphylococcus haemolyticus as important hospital pathogen and carrier of methicillin resistance genes

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Running title: Antimicrobial resistance in S. haemolyticus

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Abstract
Phenotypic and molecular methods were used to characterize the antibiotic resistance of 64 clinical isolates of *Staphylococcus haemolyticus*. By PCR of the *mecA* gene, 87% were methicillin resistant. Approximately 55% harbored the SCCmecV and only one the SCCmecIV. Many isolates (75%) displayed multiresistance and pulsotype analysis showed a high diversity.

Text
Among coagulase-negative staphylococci (CoNS), *Staphylococcus haemolyticus* is the second pathogen most frequently isolated from human blood cultures (17) and with the highest level of antimicrobial resistance (4, 7). Methicillin resistance is conferred by the *mecA* gene, carried on the staphylococcal cassette chromosome mec (SCCmec) (10). Eight types (I to VIII) of SCCmec have been assigned for *Staphylococcus aureus* (9) and the SCCmec type V has already been found in CoNS, particularly in *S. haemolyticus* (11). The increase in the frequency of methicillin-resistant *S. haemolyticus* as the causal agent of hospital infections and the possibility of emergence of resistance to other antibiotics demand trustworthy characterization of the isolates and an investigation of clonal spreading within hospitals.

Here, 64 clinical strains were isolated from patients from Hospital Naval Marcílio Dias, Rio de Janeiro – Brazil, between 2006 and 2008. The strains were isolated from 31 males and 33 females of the following clinical infections or sources: bacteremia (n = 45), skin (n = 2), urine (n = 13) and unknown source (n = 4). The isolates were identified at the hospital laboratory as *S. haemolyticus* using MicroScan WalkAway PC21 panel and their identification was confirmed by the specific PCR (16).
The resistance profile of the strains was determined by disc-diffusion tests according to CLSI guidelines (6) for the main antibiotics used in Brazil, and for the antibiotic mupirocin as previously described (8, 14). The methicillin resistance was also evaluated by other phenotypic methods such as MIC for oxacillin (6) and MicroScan and by PCR of the **mecA** gene (19). The **SCCmec** type was determined in a multiplex PCR (12), except for the pair of primers mecI P2 and mecI P3 used as internal control that was replaced by MRS1 e MRS2 (19), which amplify a 154-bp fragment of the **mecA** gene.

Genetic relatedness and characterization of isolates using PFGE of genomic DNA digested with **SmaI** was carried out as previously described (20). Banding patterns were determined by visual inspection and by the Bionumerics software, version 6.0 (Applied Maths) using the Dice index and the unweighted pair group method with arithmetic average. Similar PFGE genotypes were defined using a coefficient of similarity up to 80% and the subtypes were those with less than five-band variants as recommended by van Belkun *et al.* (18).

As shown in table 1, there was much variation in the antibiotic resistance profiles. In this collection, the strains were resistant to at least one of the antibiotics tested and susceptibility to vancomycin was observed in all isolates. Moreover, 75% of the isolates were multiresistant exhibiting resistance to more than three classes of antibiotics. It is important to emphasize that 88% of the strains were classified as methicillin-resistant defined by the cefoxitin disc. When other tests to evaluate methicillin resistance were performed and considering the detection of the **mecA** gene as definitive, seven discordant results were observed. One result was false-negative for all phenotypic methods and only one for MicroScan. In fact the detection of the **mecA** gene by PCR does not allow a functional characterization of this gene. For instance, some genetic
mutations may prevent the production of active proteins. False-positive results were observed too. Five isolates with the negative results for mecA gene displayed controversial results with phenotypic methods. Three of them were classified as resistant only to MicroScan, one for disc diffusion and one for all phenotypic tests.

Among the 56 mecA-positive strains only 32 (57%) could be assigned to known SCCmec types. Thirty-one isolates (55%) had the SCCmec type V and only one had the SCCmec type IV. The SCCmec elements are common among S. haemolyticus and these microorganisms are considered to be potential SCCmec donors. Evidence has suggested horizontal transfer of the SCCmec type V from methicillin-resistant S. haemolyticus to methicillin-susceptible S. aureus, resulting in the creation of a new MRSA clone that could result in a potential outbreak (2). The remaining 24 strains (43%) were non-typeable by the method employed which can be explained by the presence of novel structures or rearrangements and recombination of the SCCmec (5, 22). The emergence of new variants of the SCCmec element found in this study and the possibility of gene transfer will be further evaluated and characterized.

Based on PFGE cluster (Figure 1), the 64 isolates were typed into 51 PFGE profiles (PFPs). Only two pairs of isolates (strains 54 and 57; strains 60 and 63) exhibited identical PFPs. There was no single clone with a fixed pulsotype disseminated among these patients, although the strains with different PFPs often harbored the same SCCmec element, type V.

S. haemolyticus is highly prevalent in the hospital environment, with a tendency to develop resistance to multiple antibiotics (13, 21). This was observed in this study and the highest resistance rates were found for the β-lactams. There are several methods to detect methicillin resistance and some of them were used here. As in other studies some false positives and false negatives results were found. In fact, a novel mecA homologue
in *S. aureus* was described recently which was phenotypically resistant to methicillin but tested negative for the *mecA* gene. This gene was located in a novel *SSCmec* designated type XI (1). Resistance to oxacillin, without the *mecA* gene, may be due to either the overproduction or overexpression of penicillinase or alteration of other penicillin-binding proteins (3). With respect to the *SCCmec* typing, the results are comparable to the distribution of the *SCCmec* types among *S. haemolyticus* strains that appeared to be major reservoirs of type V (11, 15). Besides, the large genetic diversity among the samples, including those resistant to methicillin, highlights the possibility of horizontal spread of the *SCCmec* among the *S. haemolyticus* strains. The extreme plasticity of the *S. haemolyticus* genome was inferred through the complete genome sequencing of strain JCSC1435 which identified as many as 82 insertion sequences in its chromosome. This characteristic may result in frequent genomic rearrangements, phenotypic diversification and acquisition of antibiotic resistance. This revealed how the medically important staphylococcal species diversified themselves to successfully colonize or infect the human host (17).

In conclusion, our tests showed a high prevalence of multiresistance among *S. haemolyticus*. The phenotypic tests had good correlation with the genotypic characterization of methicillin resistance, but some discrepant results were observed. The *SCCmec* type V was the most prevalent although many strains were non-typeable. Despite the great genetic diversity, *S. haemolyticus* plays an important role as an efficient recipient and/or carrier of the *SCCmec* elements.

This study was supported by Brazilian grants (FAPERJ, CNPq, CAPES and PRONEX).

**References**


Figure 1: Dendrogram from computer-assisted analysis of PFGE profiles obtained for 64 *S. haemolyticus* isolates.
Table 1: Antibiogram patterns of the 64 *S. haemolyticus* strains by the disc-diffusion test

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Number of <em>S. haemolyticus</em> isolates (%)</th>
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<th></th>
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<tbody>
<tr>
<td></td>
<td>Susceptible</td>
<td>Intermediate</td>
<td>Resistant</td>
</tr>
<tr>
<td>Penicillin</td>
<td>3 (5)</td>
<td>0</td>
<td>61 (95)</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>3 (5)</td>
<td>0</td>
<td>61 (95)</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>8 (12)</td>
<td>0</td>
<td>56 (88)</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>8 (12)</td>
<td>0</td>
<td>56 (88)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>16 (25)</td>
<td>1 (2)</td>
<td>47 (73)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>18 (28)</td>
<td>0</td>
<td>46 (72)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>21 (33)</td>
<td>2 (3)</td>
<td>41 (64)</td>
</tr>
<tr>
<td>Trimethoprim-Sulfamethoxazole</td>
<td>27 (42)</td>
<td>3 (5)</td>
<td>34 (53)</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>34 (53)</td>
<td>0</td>
<td>30 (47)</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>48 (75)</td>
<td>0</td>
<td>16 (25)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>50 (78)</td>
<td>2 (3)</td>
<td>12 (19)</td>
</tr>
<tr>
<td>Rifampin</td>
<td>54 (84)</td>
<td>3 (5)</td>
<td>7 (11)</td>
</tr>
<tr>
<td>Mupirocin</td>
<td>58 (90)</td>
<td>1 (2)</td>
<td>5 (8)</td>
</tr>
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