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Staphylococcus haemolyticus as an Important Hospital Pathogen and Carrier of Methicillin Resistance Genes

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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 found to be methicillin resistant. Approximately 55% harbored staphylococcal cassette chromosome *mec* element (SCC*mec*) type V, and only one SCC*mec* type IV. Many isolates (75%) displayed multiresistance, and pulsotype analysis showed a high diversity.

Among coagulase-negative staphylococci (CoNS), *Staphylococcus haemolyticus* is the second most frequently isolated from human blood cultures (18) and has the highest level of antimicrobial resistance (3, 8). Methicillin resistance is conferred by the *mecA* gene, carried on the staphylococcal cassette chromosome *mec* element (SCC*mec*) (12). Eight types (I to VIII) of SCC*mec* have been assigned for *Staphylococcus aureus* (11), and SCC*mec* type V has already been found in CoNS, particularly in *S. haemolyticus* (13). 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.

In the studies reported here, 64 clinical strains were isolated from patients at Hospital Naval Marcílio Dias, Rio de Janeiro, Brazil, between 2006 and 2008. The strains were isolated from the following clinical infections or sources in 31 males and 33 females: 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* by using the MicroScan WalkAway PC21 panel, and their identification was confirmed by specific PCR (17).

The resistance profiles of the strains for the main antibiotics used in Brazil were determined by disc diffusion tests according to CLSI guidelines (5). However, the mupirocin susceptibility testing was not preconized by CLSI, so the results for this antibiotic were interpreted as previously described (7, 9). The methicillin resistance was also evaluated by other phenotypic methods, such as the MIC for oxacillin (5), the MicroScan, and PCR of the *mecA* gene (6). The SCC*mec* type was determined in a multiplex PCR as previously described (14), except that the pair of primers mecI P2 and mecI P3 used as the internal control were replaced by MRS1 and MRS2 (6), which amplify a 154-bp fragment of the *mecA* gene.

Analysis of genetic relatedness and characterization of isolates using pulsed-field gel electrophoresis (PFGE) of genomic DNA digested with SmaI were carried out as previously described (20). Banding patterns were determined by visual inspection and by using 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 of up to 80%, and the subtypes were those with less than five-band variants, as recommended by van Belkun et al. (19).

As shown in Table 1, there was great 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 as defined by the cefoxitin disc diffusion assay. 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 the 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 negative results for the *mecA* gene displayed controver-

TABLE 1 Antibiogram patterns of the 64 *S. haemolyticus* strains determined by the disc diffusion test

Antimicrobial agent	No. (%) of *S. haemolyticus* isolates that were:		
	Susceptible	Intermediate	Resistant
Penicillin	3 (5)	0	61 (95)
Ampicillin	3 (5)	0	61 (95)
Oxacillin	8 (12)	0	56 (88)
Cefoxitin	8 (12)	0	56 (88)
Gentamicin	16 (25)	1 (2)	47 (73)
Ciprofloxacin	18 (28)	0	46 (72)
Erythromycin	21 (33)	2 (3)	41 (64)
Trimethoprim-sulfamethoxazole	27 (42)	3 (5)	34 (53)
Clindamycin	34 (53)	0	30 (47)
Chloramphenicol	48 (75)	0	16 (25)
Tetracycline	50 (78)	2 (3)	12 (19)
Rifampin	54 (84)	3 (5)	7 (11)
Mupirocin	58 (90)	1(2)	5 (8)

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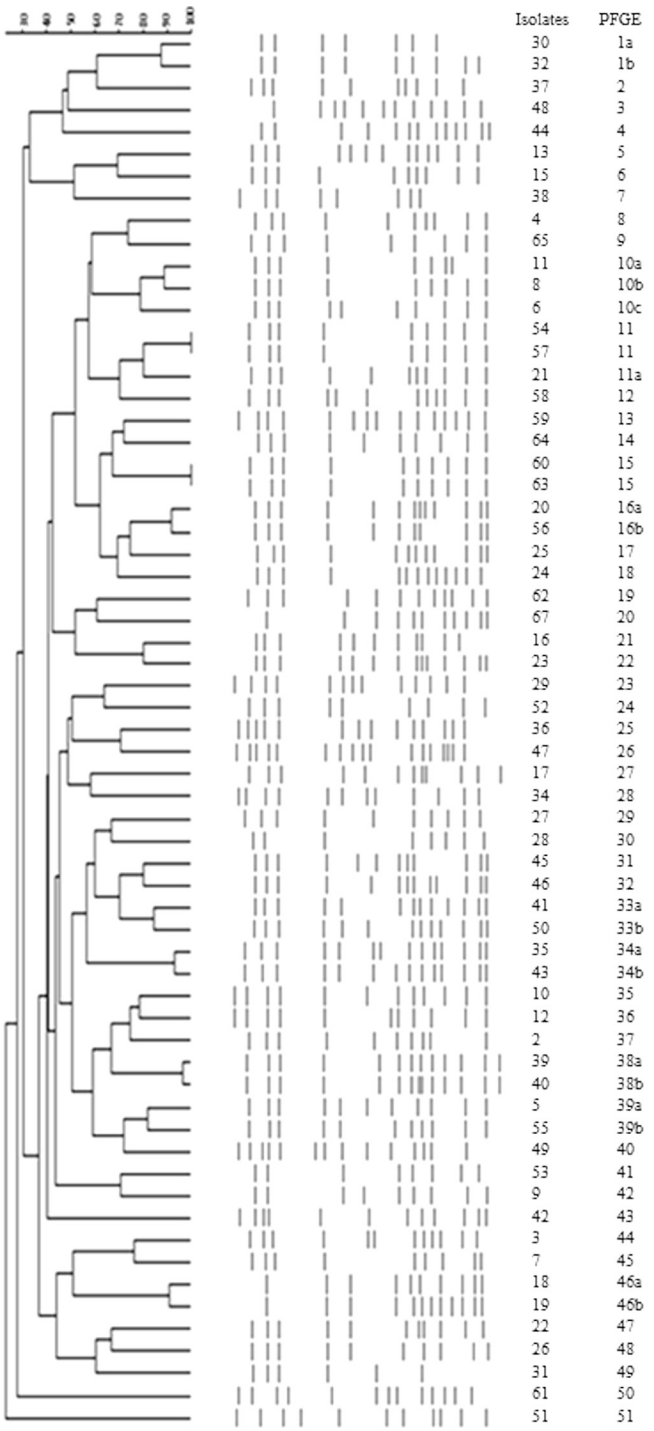

FIG 1 Dendrogram from computer-assisted analysis of PFGE profiles obtained for 64 *S. haemolyticus* isolates.

sial results with phenotypic methods. Three of them were classified as resistant only by the MicroScan, one by disc diffusion, and one by all phenotypic tests.

Among the 56 *mecA*-positive strains, only 32 (57%) could be assigned to known SCC*mec* types. Thirty-one isolates (55%) had SCC*mec* type V, and only one had SCC*mec* type IV. The SCC*mec* elements are common among *S. haemolyticus* isolates, and these microorganisms are considered to be potential SCC*mec* donors. Evidence has suggested horizontal transfer of SCC*mec* type V from methicillin-resistant *S. haemolyticus* to methicillin-susceptible *S. aureus*, resulting in the creation of a new methicillin-resistant *S. aureus* (MRSA) clone that could result in a potential outbreak (1). The remaining 24 strains (43%) were nontypeable by the method employed, which can be explained by the presence of novel structures or rearrangements and recombination of the SCC*mec* (4, 22). The emergence of new variants of the SCC*mec* element found in this study and the possibility of gene transfer will be further evaluated and characterized.

Based on PFGE clustering (Fig. 1), the 64 isolates were typed into 51 PFGE profiles (PFPs). Only two pairs of isolates (strains 54 and 57 and 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 SCC*mec* element, type V.

S. haemolyticus is highly prevalent in the hospital environment, with a tendency to develop resistance to multiple antibiotics (15, 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-positive and false-negative 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 SSC*mec* designated type XI (10). 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 (2). With respect to the SCC*mec* typing, the results are comparable to the distribution of the SCC*mec* types among *S. haemolyticus* strains that appeared to be major reservoirs of type V (13, 16). Besides, the large genetic diversity among the samples, including those resistant to methicillin, highlights the possibility of horizontal spread of the SCC*mec* 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 diversify themselves to successfully colonize or infect the human host (18).

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

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