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MORPHOMETRIC ANALYSIS OF METHICILLIN-RESISTANT *Staphylococcus aureus* ISOLATED FROM THE SALIVA OF HEALTHCARE WORKERS

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ABSTRACT

The objective of this study was to evaluate the morphological changes in methicillin-resistant *Staphylococcus aureus* (MRSA) cultured in different concentrations of sodium chloride and oxacillin. In a previous study on the prevalence of MRSA and methicillin-susceptible *Staphylococcus aureus* (MSSA) in the saliva of health workers, 12 samples of MRSA were isolated and identified using conventional techniques and polymerase chain reaction (PCR). Morphological and morphometric analyses showed that the culture of methicillin-resistant *S. aureus* in different concentrations of sodium chloride (2%, 4%, 6% and 7.5%) and oxacillin (2 µg, 4 µg and 6 µg/mL) had no effect on the morphology of the bacteria.

KEY WORDS: methicillin-resistant *Staphylococcus aureus*; oxacillin; sodium chloride; morphology.

RESUMO

Análise morfométrica de *Staphylococcus aureus* resistente à meticilina isolado da saliva de profissionais de saúde

Este estudo teve como objetivo avaliar alterações morfológicas em *Staphylococcus aureus* resistente à meticilina (MRSA) após cultivo em meios contendo diferentes concentrações de cloreto de sódio e oxacilina. Em um estudo prévio sobre a prevalência de MRSA e de *Staphylococcus aureus* susceptível à meticilina (MSSA) na saliva de profissionais de saúde, 12 amostras de MRSA foram isoladas e identificadas por técnicas convencionais e pela reação em cadeia da polimerase (PCR). Análises morfológicas e morfométricas mostraram que a cultura de *S. aureus* em diferentes concentrações de

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cloreto de sódio (2%, 4%, 6% e 7,5%) e oxacilina (2 µg, 4µg e 6µg/mL) não promoveu qualquer efeito sobre a morfologia das bactérias.

DESCRITORES: *Staphylococcus aureus* resistente à meticilina; oxacilina; cloreto de sódio; morfologia.

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important etiologic agent associated with difficult-to-treat infections in hospitals and in the community (7, 16). MRSA is involved in various infections that affect humans, ranging from simple skin infections to severe and disseminated diseases such as pneumonia, meningitis and endocarditis (8, 9).

The dissemination of MRSA is attributed to the contaminated hands of healthcare workers, who help spread the organism from one patient to another within the hospital environment (1, 11). MRSA may be isolated simultaneously from samples of nasopharyngeal secretions and from the hands of the same healthcare workers. Moreover, MRSA can be identified in the saliva of healthcare professionals, indicating that the mouth is an important reservoir and potential source of its dissemination (2, 18, 19).

The mechanisms of methicillin resistance in MRSA are diverse, and include the production of altered penicillin-binding proteins (PBPs), which have less affinity to penicillin. The *mec*A gene, integrated on the chromosomes of MRSA strains, encodes the altered protein (15, 23).

One of the principal challenges facing health services is the need to characterize the methicillin susceptibility of *S. aureus* isolates as quickly and precisely as possible, since this information will guide treatment and control measures. Several methods have been used to detect MRSA; however, it still remains a difficult process (11). The objective of this study was to evaluate morphological changes in methicillin-resistant *S. aureus* cultured in the presence of different concentrations of sodium chloride and oxacillin.

MATERIALS AND METHODS

Bacterial isolates and determination of methicillin resistance

An analysis was conducted of 12 samples of MRSA, which had been isolated from the saliva of healthcare workers and stored in the Laboratory of Bacteriology, Medical Institute of Tropical Pathology and Public Health, Federal University of Goiás, Brazil. These isolates of *S. aureus* are part of a previous study on colonization in healthcare professionals (5, 6, 7, 14). *S. aureus* was identified by mannitol fermentation test and by the production of catalase, coagulase and deoxyribonuclease (DNase) (2, 12).

The methicillin-susceptible *S. aureus* (MSSA) standard strain ATCC 25923 was used as the control in all the tests. Screening tests for detecting the susceptibility of *S. aureus* to oxacillin included: a disk diffusion test using a 30 µg-cefoxitin disk and 1 µg-oxacillin disk (Oxoid, Basingstoke, England), and the agar dilution test (6 µg of oxacillin per mL supplemented with 4% sodium chloride), performed according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI) (3, 4). In addition, the Etest® was used to determine the minimum inhibitory concentration (MIC) in accordance with the manufacturer's instructions (bioMérieux).

The twelve oxacillin-resistant isolates confirmed by the Etest® were grown on tryptic soy broth (TSB) for 24 hours at 37 ºC before being submitted to PCR to detect the presence of the *mec*A gene (17).

Assessment of morphological changes in MRSA

The possible effect of sodium chloride and oxacillin on the morphology of MRSA isolates was evaluated by growing the staphylococci in different concentrations of these substances. The 12 samples of MRSA were cultured in tryptic soy broth containing salt and oxacillin, with twelve different combined concentrations of sodium chloride and oxacillin being assessed (Table 1). After incubation at 35 ºC for 72 hours, the slides were prepared from the colonies developed, with the smear being prepared in a circular motion, and then Gram stained.

Table 1. Culture media with different concentrations of sodium chloride and oxacillin

Media	Sodium chloride (%)	Oxacillin (µg/ml)
1	2.0	2
2	4.0	2
3	6.0	2
4	7.5	2
5	2.0	4
6	4.0	4
7	6.0	4
8	7.5	4
9	2.0	6
10	4.0	6
11	6.0	6
12	7.5	6

The slides of MRSA were submitted to computerized morphometric analysis, and statistical tests were performed to evaluate the effect of sodium chloride and oxacillin on the bacterial wall. Morphometric analysis was performed

using a Zeiss Axiostar microscope connected to a Sony NEX-3 digital camera. A total of 1, 260 slides were photographed using a lens with 100x magnification to review the area and assess the diameter of the bacterial cells arranged in grape-like bunches or clusters in the IMAGE J (NIH) program. A total of 75, 630 steps were performed.

Statistical Analysis

Differences between groups were compared using the nonparametric Kruskal-Wallis test and p-values <0.05 were considered statistically significant.

RESULTS

Of the twelve *S. aureus* isolates evaluated, all proved resistant to cefoxitin and oxacillin in the disk diffusion tests, and were shown to grow in oxacillin screen agar containing 4% sodium chloride and 6μg/mL oxacillin. The resistance profile of the isolates was confirmed by the results of the Etest®, showing MICs that ranged from 24 to >256 μg/mL. Genotypic analysis showed the presence of the *mec*A gene in all 12 samples analyzed (Figure 1).

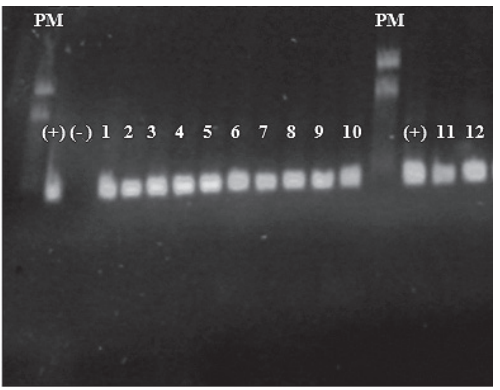

Figure 1. Electrophoresis of the *mec*A gene of MRSA in 1% agarose gel. PM: molecular weight marker; (+): positive control; (-): negative control (1-10) MRSA. PM: molecular weight marker; (+): positive control for the *mec*A gene (11 - 12) MRSA.

Morphometric Analysis

All the isolates were observed as gram-positive cocci arranged in pairs, short chains or grouped in irregular shaped clusters measuring 0.5 to 1.5 mm in diameter.

The area and diameter of the 12 MRSA isolates grown in different concentrations of oxacillin (2 μg, 4 μg or 6 μg) and sodium chloride (2%, 4%, 6% and 7.5%) were analyzed, and no statistically significant morphological changes were observed (Tables 2 and 3).

Table 2. Median values found in the area of *S. aureus* cells cultured in different concentrations of sodium chloride and oxacillin

Oxacillin	Sodium chloride			
	2%	4%	6%	7.5%
2 μg	0.67 (1.02-0.33)	0.63 (1.01-0.39)	0.66 (1.14-0.43)	0.69 (1.03-0.38)
4 μg	0.65 (1.51-0.36)	0.64 (2.01-0.33)	0.65 (1.57-0.35)	0.63 (1.51-0.34)
6 μg	0.66 (1.17-0.31)	0.67 (1.13-0.32)	0.75 (1.07-0.30)	0.66 (1.21-0.32)

Results expressed as median and range.

Table 3. Median values found in the diameter of *S. aureus* cells cultured in different concentrations of sodium chloride and oxacillin

Oxacillin	Sodium chloride			
	2%	4%	6%	7.5%
2 μg	0. 61 (1.08-0.47)	0.58 (1.07-0.50)	0.61 (1.12-0.52)	0.74 (1.03 0.54)
4 μg	0.60 (1.11-0.54)	0.59 (1.25-0.50)	0.62 (1.12-050)	0.58 (1.10-0.52)
6 μg	0.60 (1.18-0.47)	0.60 (0.99-0.49)	0.63 (1.13-0.50)	0.63 (1.03-0.44)

Results expressed as median and range.

DISCUSSION

Possible variations in cell morphology were analyzed by computerized morphometry, with no significant morphological changes being observed when the cells were cultured in different concentrations of sodium chloride and oxacillin. Morphologically, the cells analyzed ranged in diameter from 0.58 mm to 0.75 mm, within the range described in the literature of 0.5 mm to 1.5 mm in diameter (16, 22).

The cell development of MRSA in the different concentrations of sodium chloride (2%, 4%, 6% and 7.5%) and oxacillin (2 μg, 4 μg and 6 μg) was satisfactory, showing that concentrations higher than 7.5% sodium chloride and oxacillin do not affect morphology. Using similar methodology, Raju et al. (2007) conducted a study with MRSA and noted significant growth at concentrations of 20 to 50 μg of oxacillin.

Jones et al. (1997) and Raju et al. (2007) reported a delay in MRSA cell development when the samples were cultured in the presence of sodium chloride and oxacillin. Partial inhibition was detected in MRSA strains cultured in the presence of sodium chloride, showing that microbial growth was slower due to low teichoic acid synthesis, thus increasing susceptibility to autolysis.

The present study showed that different concentrations of sodium chloride and oxacillin had no effect on the cell morphology of the MRSA isolates evaluated. However, other studies have detected the presence of morphological changes in MRSA cells cultured in the presence of sodium chloride and oxacillin. According to those authors, the cells stressed by sodium chloride have a shorter and looser interpeptide bridge compared to non-stressed cells, thus disrupting the peptidoglycan and leading to morphological changes in bacterial cells (13, 20, 24, 25).

No changes were observed in relation to the morphological appearance of the cells when MRSA isolates were cultured in different concentrations of sodium chloride (2%, 4%, 6% and 7.5%) and oxacillin (2 µg, 4 µg and 6 µg/mL). None of the concentrations of sodium chloride and oxacillin tested induced morphological changes in the cells that could be detected by the method used.

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