

# Methicillin- and vancomycin-resistant *Staphylococcus aureus* in health care workers and medical devices

## *Staphylococcus aureus* resistentes a metilicina (MRSA) e vancomicina (VRSA) em profissionais da saúde e artigos médicos

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### ABSTRACT

**Introduction:** Cross-contamination by *Staphylococcus aureus* among patients, professionals and medical supplies in health facilities is a constant concern, leading many researchers to study the prevalence of this pathogen in asymptomatic carriers. **Objectives:** We investigated the colonization and the antimicrobial susceptibility profile of *Staphylococcus* spp. on surfaces of medical articles and in professionals from two basic health units in the city of Rio de Janeiro. **Materials and methods:** Seventy-nine samples resulted in 49 isolates which underwent phenotypic and molecular characterization by polymerase chain reaction (PCR) of *coa*, *mecA* and *femA* genes. **Results:** According to the phenotypes, the isolates were identified as *S. aureus* ( $n = 35$ , 71.42%) and coagulase-negative *Staphylococcus* (CoNS) ( $n = 14$ , 28.57%). Among these 14 isolates, 42.85% were methicillin-resistant coagulase negative *Staphylococcus* (MRCoNS). Among the 35 *S. aureus*, 31.42% were methicillin resistant (MRSA), and 2.8% were vancomycin resistant, characterized as VRSA. Sixty-eight percent were susceptible to methicillin (MSSA). Genes *coa*, *femA* and *mecA* were amplified from 75.51%, 71.42% and 30.61% of the isolates, respectively. After amplification of the *mecA* gene, 20.41% were characterized as MRSA, and 10.20% as MRCoNS. The vancomycin-resistant strain was characterized as VRSA after detection of the *vanB* gene. **Conclusion:** Our results show a higher frequency of MSSA and MRCoNS among *S. aureus* and CoNS respectively, colonizing devices and health professionals. However, the already described transfer of the staphylococcal cassette chromosome *mec* (SSC*mec*) from MRCoNS to MSSA may alter these results, increasing the frequency of MRSA strains.

**Key words:** *Staphylococcus aureus*; MRSA; VRSA.

### INTRODUCTION

*Staphylococcus aureus* is considered an opportunistic pathogen responsible for great morbidity and mortality; man is its main reservoir. It can be present in several sites of the human body, including oropharynx, intestines, hands, skin, and nasal cavity, which is pointed as one of the areas where colonization occurs more frequently<sup>(1,2)</sup>.

In health care settings, this pathogen may contaminate furniture, clothes and equipment around colonized or infected patients, which function as sources or reservoirs<sup>(3)</sup>. In this context, Murray *et al.* suggested that the health staff should use adequate techniques for hand washing, aimed at preventing *S. aureus* cross-infection among devices, professionals, and patients<sup>(4)</sup>. Gialluly *et al.*

stressed the urgent need to alert and inform health care workers about the potential risk of hospital infection due to, mainly, lack of hand hygiene and handling of contaminated medical devices<sup>(5)</sup>.

Since the study by Dr. Semmelweis, in the XIX century, health professionals' hands have been implicated as a source of microorganism transmission in health care settings<sup>(6)</sup>. In 1847, Semmelweis instituted hand-washing with chlorinated water as mandatory for all physicians, medicine students, and nurses, reducing mother mortality by puerperal fever from 12.2% to 2.4%, in the first month of intervention<sup>(7)</sup>. Since then, this procedure has been recommended as a primary measure to control dissemination of infectious agents.

Hand microbiota of mothers and health care workers at a maternity hospital revealed, among other organisms, the presence

of *S. aureus* and coagulase-negative *Staphylococcus*, which have been pointed in the literature as associated with hospital infection outbreaks in nurseries<sup>(8)</sup>.

Methicillin-resistant *Staphylococcus aureus* (MRSA) emerged as a nosocomial pathogen in the beginning of the 1960<sup>(9)</sup> decade, being first isolated in 1961<sup>(10)</sup>. In Brazil, MRSA isolates were detected in Barretos (São Paulo), in the hands of 73% of dentists and 52% of other dental professionals at basic health units in that city<sup>(11)</sup>.

The main MRSA propagation mechanism in hospital settings operates by the hands of health care workers<sup>(12, 13)</sup>. There are reports in the literature about prevalence of MRSA colonization (4.6%) among physicians and nurses<sup>(14)</sup>. Braga *et al.*, in 2004, highlighted the importance of hands as reservoir of microorganisms associated with cross-transmission of *S. aureus* by health care personnel<sup>(15)</sup>.

MRSA strains are endemic in many American and European hospitals, representing around 30%-35% of all clinical isolates, in which infected or colonized patients are the main reservoir<sup>(16)</sup>. In Brazil there are scarce microbiological criteria for diagnosing hospital-acquired infections (HAIs)<sup>(17)</sup>, but there is evidence that *S. aureus* is the most frequent agent and the most commonly transmitted by the hands of health professionals. However, few studies have been conducted to quantify this transmission<sup>(18)</sup>. Hand hygiene remains as the simplest and most important measure to prevent and reduce the risk of microorganism transmission between patients, and thus, the development of HAIs<sup>(19)</sup>.

Dissemination of MRSA and oxacillin-resistant *Staphylococcus aureus* (ORSA) has been an object of studies in Brazil, aimed at verifying the frequency of resistance and implications in the hospital system due to the high percentage of HAIs caused by these microorganisms, what corresponds to 40%-80%, principally in intensive care units (ICUs)<sup>(20, 21)</sup>.

Methicillin/oxacillin resistance in *S. aureus* and coagulase-negative *Staphylococcus* (CoNS) is firstly mediated by the production of penicillin-binding proteins (PBP2a), besides the normally produced proteins, PBP1, PBP2, PBP3 and PBP4, but with extremely low affinity for beta-lactam antibiotics, what hinders bacterial cell wall assembly<sup>(22)</sup>. The *mecA* gene, which encodes PBP2a, is highly conserved among methicillin-resistant *S. aureus* and *Staphylococcus epidermidis*, and is contained in a mobile genetic element, the staphylococcal cassette chromosome *mec* (SCC*mec*)<sup>(23)</sup>. There are some genes, called *factor essential for methicillin resistance* (*fem*), which help the *mecA* gene to express beta-lactam resistance. In 2011, a novel *mecA* homologue, termed *mecC*, located at type-XI SCC*mec*, was described in MRSA strains isolated in human beings and cattle<sup>(24)</sup>. Detection of the

*mecC* gene in MRSA, as well as in other *Staphylococcus* species, has been performed in several countries<sup>(25)</sup>.

The high prevalence of *S. aureus* strains and the consequent employment of vancomycin in Brazilian hospitals, added to the lack of control on antimicrobial use and the inadequate conditions of public health institutions, predispose to the emergence of strains of intermediate susceptibility (VISA), or resistant to vancomycin (VRSA)<sup>(26)</sup>. The reduced susceptibility to vancomycin in *S. aureus* (VISA) emerged in 1996, in Japan, where in the following year the first strain with heteroresistance to vancomycin (hVISA) was isolated, with a minimal inhibitory concentration (MIC)  $\leq 2$   $\mu\text{g/ml}$  to vancomycin, but with subpopulations of MIC  $\geq 4$   $\mu\text{g/ml}$  of approximately  $10^5$  to  $10^6$  cells. Because they are present at a much reduced number, they are not detected in the inocula used in the methodology recommended by the Clinical and Laboratory Standards Institute (CLSI)<sup>(27, 28)</sup>.

In the subsequent years, isolated VISA strains were reported in the United States, France and Korea<sup>(29, 30)</sup>. In Brazil, the first report of multiple VRSA strains isolated at a hospital was the result of a study in 140 isolates from hospitalized patients exposed to vancomycin. Five of these isolates presented vancomycin MIC of 8  $\mu\text{g/ml}$ , four of the VRSA strains were characterized as belonging to the Brazilian endemic clone, and all the five strains were negative for *vanA*, *vanB* and *vanC* genes by polymerase chain reaction (PCR)<sup>(31, 32)</sup>.

In 2002, in the city of Michigan, United States, the first VRSA was isolated<sup>(33, 34)</sup>. This microorganism presented the *vanA* gene, suggesting the transfer of genetic material from *Enterococcus* spp., as the patient presented infection by vancomycin-resistant *Enterococcus* (VRE). In the latest years, many cases of VRSA have been reported, principally in hospital settings<sup>(27)</sup>.

## OBJECTIVES

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This study aimed to investigate the prevalence and the susceptibility profile to antimicrobials in strains of negative-coagulase *S. aureus* and *Staphylococcus* sp. isolated from health professionals' hands and nostrils and from medical devices (stethoscope, sphygmomanometer, and Doppler machine) used at a pregnant women's health center in the city of Rio de Janeiro. In order to do so, we adopted phenotypic and molecular approaches to determine resistance profiles and classify phenotypes. From the results, we set out to point the risks associated with colonization by health professionals' resistant strains, and suggest corrective measures to prevent harm to the patients seen by these professionals.

## MATERIALS AND METHODS

### Study site and sample collection

The study was performed at two outpatient maternity clinics of two basic health centers in the region of Jacarepaguá, in the municipality of Rio de Janeiro. With the aid of dry sterile swabs, 79 samples were collected from the following medical appliances: stethoscope earpieces ( $n = 12$ , 24.49%), stethoscope diaphragm ( $n = 4$ , 8.16%), sphygmomanometer cuff ( $n = 4$ , 8.16%), sphygmomanometer bulb ( $n = 4$ , 8.16%), Doppler device ( $n = 9$ , 18.37%); and from health professionals: nostrils ( $n = 7$ , 14.29%) and hands ( $n = 9$ , 18.37%), from May 2009 to January 2010. After characterization, strains were stored at the collection of reference microorganisms on health surveillance (CMRVS) of Instituto Nacional de Controle de Qualidade em Saúde (INCQS) of Fundação Oswaldo Cruz (Fiocruz).

### Approval by the research ethics committee

All health care workers that participated in the study filled and signed the free informed consent. The study was approved on October 15, 2009, by the research ethics committee (CEP) of the municipal health and civil defense office, under protocol n° 141/09, certificate of presentation for ethical consideration (CAAE): 0160.0.314.000-09. All the precepts contained in Resolution n° 196 of 1996 of the Ministry of Health<sup>(35)</sup>, which guides researches with human beings, were respected.

### Phenotypic characterization

Just after collection, the 79 samples were inoculated in test tubes containing brain-heart infusion (BHI) broth (Merck) and transported in a closed container at room temperature to the laboratory of reference microorganisms of INCQS/Fiocruz, as well as incubated at 37°C for 24 hours. Later on, 0.1-ml aliquots were streaked onto mannitol salt agar (MSA) (Merck) and incubated at 37°C for 24 hours. Among the cultures that fermented mannitol, two colonies were selected that underwent Gram stain, and catalase, deoxyribonuclease (DNase)<sup>(36)</sup> and free coagulase biochemical tests, with the addition of 0.5 ml of 24-hour-old culture in BHI broth to a tube containing 0.5 ml of rabbit plasma with ethylenediaminetetraacetic acid (EDTA) (Becton Dickinson), incubated at 37°C in a thermostatic bath for 24 hours, taking readings each hour during five hours to verify clot formation. The absence of clot, in this period, led to the incubation of tubes for up to 24 hours. Reference *S. aureus* INCQS 00039 (ATCC 6538)

(positive control) and *S. epidermidis* INCQS 00016 (ATCC 12228) (negative control) strains were used as controls.

### Susceptibility to antimicrobials

Antimicrobial susceptibility tests were conducted by the modified Kirby-Bauer disk diffusion method (in agar), from a bacterial culture with a turbidity equivalent to a 0.5 McFarland standard in Mueller Hinton (MH) broth<sup>(37)</sup>. The reference strain *S. aureus* INCQS 00015 (ATCC 25923) was used as control according to CLSI<sup>(38)</sup>. The following antimicrobial agents were assessed: erythromycin (15 µg), clindamycin (2 µg), oxacillin (1 µg), vancomycin (30 µg), rifampicin (5 µg), chloramphenicol (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg) and cefoxitin (30 µg) (Cefar, São Paulo-SP, Brasil). The results of vancomycin and teicoplanin susceptibility tests were interpreted in line with the table Sensifar and Multifar – Cefar®, according to the criteria recommended in the technical note of Agência Nacional de Vigilância Sanitária (Anvisa) n° 01/2010.

Vancomycin and teicoplanin MICs were determined by the Etest® (bioMérieux) system, using the reference strain *S. aureus* INCQS 000381 (ATCC 29213). After a culture was grown with microbial turbidity corresponding to a 0.5 McFarland standard in MH broth, the specimen was seeded with a swab over the surface of Petri plates containing MH agar, to which Etest® strips were applied, and incubated at 35°C for 24 hours.

### Molecular characterization

Genomic deoxyribonucleic acid (DNA) extraction and purification were performed from aliquots of 500 µl of each one of the 49 cultures, which were transferred to Eppendorf tubes, and centrifuged for 10 min at 5,000 g. The sediment was used for genomic DNA extraction with the DNeasy Blood & Tissue kit (Qiagen GmGH, Hildeithalln, Germany), according to the instructions by the manufacturer. Purified DNA samples were then stored at 20°C for further use.

### Detection of *coa*, *femA*, *mecA*, *vanA* and *vanB* genes

The PCR mixture had a final volume of 25 µl of the Master Mix M7505 kit (Promega) added with 20 pmol of each oligonucleotide, following instructions by the manufacturer. The used primers were synthesized by Invitrogen (Carlsbad, CA); amplifications were conducted in PTC-200 Peltier Thermal Cycler (MJ Research) and Eppendorf EP Master Cycler (**Table 1**). All PCR reactions were performed at least three times for assessment of reproducibility.

TABLE 1 – Genes, primers, and PCR conditions

Target gene	Primer	Sequence 5' ---- 3'	Program	Size (pb)	Reference
<i>coa</i>	<i>CoaG2</i>	GAGACCAAGATTCAACAAG	94°C-2' 94°C-30" 65°C-2' 35× 72°C-4' 72°C-7'	900	Guler <i>et al.</i> (2005) <sup>(39)</sup>
	<i>CoaG3</i>	AAGAAAACCACTCACATCA	95°C-5' 94°C-2' 57°C-2' 35× 72°C-1' 72°C-7'		
<i>femA</i>	<i>FemAF</i>	TCACGCAACTGTTGGCCACT	95°C-5' 94°C-2' 57°C-2' 35× 72°C-1' 72°C-7'	700	This study
	<i>FemAR</i>	CCATTGCACTGCATAACTTCCGC	95°C-5' 94°C-2' 57°C-2' 30× 72°C-1' 72°C-7'		
<i>mecA</i>	<i>mecAF</i>	GATCTGTACTGGGTTAATCA	95°C-5' 94°C-2' 57°C-2' 30× 72°C-1' 72°C-7'	500	This study
	<i>mecAR</i>	CATATGACGCTATCCATT3	95°C-5' 94°C-1' 57°C-2' 30× 72°C-1' 72°C-7'		
<i>vanA</i>	<i>VanA1</i>	GGGAAAACGACAATTGC	95°C-2' 94°C-1' 54°C-1' 30× 72°C-1' 72°C-10'	732	Dutka-Malen <i>et al.</i> (1995) <sup>(40)</sup>
	<i>VanA2</i>	GTACAATGTGGCCGTTA	95°C-2' 94°C-1' 54°C-1' 30× 72°C-1' 72°C-10'		
<i>vanB</i>	<i>VanB1</i>	ATGGGAAGCCGATAGTC	95°C-2' 94°C-1' 54°C-1' 30× 72°C-1' 72°C-10'	635	Dutka-Malen <i>et al.</i> (1995) <sup>(40)</sup>
	<i>VanB2</i>	GATTTTCGTTCTCGA CC	95°C-2' 94°C-1' 54°C-1' 30× 72°C-1' 72°C-10'		

PCR: polymerase chain reaction.

The following strains were used for PCR control: *S. aureus* (MRSA) INCQS 00306 (ATCC 33591) and *S. epidermidis* INCQS 00016 (ATCC 12228). Electrophoresis was conducted in a horizontal Electrophoresis Cell (Bioamerica) apparatus containing 0.5× Tris/Borate/EDTA (TBE) buffer, for 60 minutes at 60 v with a Power Pac 300 (Bio-Rad). Images were digitized with the video documentation system ImageQuant 300®, GE.

### Sequencing and identity analysis

The *vanB* gene PCR product was purified with a QIAquick® PCR Purification kit (Qiagen), according to the manufacturer's manual. The purified product underwent sequencing using the Big Dye Terminator kit for capillary electrophoresis in a ABI 3730 DNA Analyzer (Applied Biosystems, Foster City CA, USA) (Platform PDTIS/Fiocruz). The chromatogram was converted to Fasta format using the Sequencher 3.0 (Gene Codes Corporation, Ann Arbor, MI) software. Sequence similarity analysis was performed by BLASTn program (<http://www.ncbi.nlm.nih.gov/BLAST/>), at GenBank (National Center for Biotechnology Information [NCBI]).

## RESULTS

### Biochemical identification and antimicrobial susceptibility

The 79 samples collected from the two analyzed health units resulted in the isolation of 49 strains of *Staphylococcus* spp. (62%): 33 (67.34%) from medical devices, and 16 (32.66%) from health professionals. The 49 isolates presented morphology and staining characteristics of Gram-positive cocci, produced catalase and fermented mannitol. Forty-one isolates (83.67%) produced DNase enzyme; 35 (71.42%), coagulase enzyme; and the other 14 (28.57%) were negative for coagulase production. Regarding antimicrobial susceptibility, the 49 isolates presented 19 resistance profiles (**Table 2**). According to the presented phenotypes, the isolates were identified as *S. aureus* ( $n = 35$ , 71.42%) and CoNS ( $n = 14$ , 28.57%). Among these 14 isolates, six (42.85%) were methicillin-resistant coagulase-negative *Staphylococcus* (MRCoNS). Among the 35 *S. aureus*, 11 (31.42%) were MRSA; among these, one was also vancomycin resistant, and was identified as VRSA, and 24 (68.57%) were susceptible to methicillin (MSSA).

TABLE 2 – Antimicrobial resistance profiles of *Staphylococcus* spp. isolates

Profiles	Resistance phenotype	N° of isolates
1	Sensitive to all	15
2	ERY	7
3	RIF	1
4	CHL	2
5	CLI	1
6	ERY and OXA	3 <sup>c</sup>
7	ERY and GEN	1
8	ERY and RIF	1
9	OXA and CFO	1 <sup>c</sup>
10	ERY, OXA and CFO	7 <sup>b</sup>
11	ERY, CHL and CLI	1
12	ERY, GEN and CFO	2
13	ERY, OXA, GEN and CFO	1 <sup>a</sup>
14	ERY, CHL, CLI and RIF	1
15	ERY, CLI, OXA, RIF and CFO	1 <sup>a</sup>
16	ERY, CLI, OXA, CIP and CFO	1 <sup>c</sup>
17	ERI, CLO, CLI, OXA, RIF and CFO	1 <sup>a</sup>
18	ERI, CLI, OXA, VAN, RIF, CFO and TEI	1 <sup>a</sup>
19	ERI, CLI, OXA, GEN, CIP, RIF and CFO	1 <sup>a</sup>
Total		49

<sup>a</sup>All MRSA strains; <sup>b</sup>five MRSA strains and one MRCoN; <sup>c</sup>all MRCoN strains; ERY: erythromycin; RIF: rifampicin; CHL: chloramphenicol; CLI: clindamycin; GEN: gentamicin; OXA: oxacillin; CFO: ceftiofloxacin; CIP: ciprofloxacin; VAN: vancomycin; TEI: teicoplanin; MRSA: methicillin-resistant *Staphylococcus aureus*; MRCoN: methicillin-resistant coagulase-negative *Staphylococcus*.

The strains that presented resistance to five or more antibiotic classes were considered multiresistant. One single isolate, from the hand of a physician, presented resistance to vancomycin, with CIM  $\geq$  256  $\mu$ g/ml, and to five other analyzed antibiotics: oxacillin, erythromycin, clindamycin, rifampicin and ceftiofloxacin; resistance to teicoplanin was also verified, with CIM  $\geq$  256  $\mu$ g/ml. This isolate was stored at CMRVS, under access number P3425.

### Identification of *femA*, *coa*, *mecA*, *vanA* and *vanB* genes

Among the 49 isolates, 35 (71.4%) presented a single 900-bp fragment compatible with *femA* gene. PCR of the *coa* gene resulted in the amplification of a single fragment of 650-900 bp in 37 (75.51%) of the analyzed strains. A 500-bp fragment corresponding to the *mecA* gene was detected in 15 (30.6%) strains; among these,

one isolate (6.66%) revealed the presence of a 635-bp fragment corresponding to the *vanB* gene. The presence of a *vanA* gene specific fragment was not verified. The sequence of the *vanB* gene presented 98% identity with sequences of this gene at GeneBank, where it was stored under KP 731622 access number.

### Characterization of *Staphylococcus* spp.

After phenotypical and molecular identification, the 49 isolates were identified as MSSA ( $n = 27$ ), MRSA ( $n = 10$ ) – one of these isolates was also identified as VRSA –, CoNS ( $n = 7$ ) and MRCoNS ( $n = 5$ ). Among these isolates, 35 produced coagulase (*S. aureus*) and 14 did not (CoNS), by the conventional method of free coagulase. However, the *coa* gene was amplified in 37 isolates, which were then identified as *S. aureus*. Among the 49 strains, 15 presented the *mecA* gene. Thirty-seven isolates were characterized as *S. aureus*; among them, 10 presented the *mecA* gene, and were identified as MRSA; another five were considered MRCoNS.

## DISCUSSION

*Staphylococcus aureus* is part of the skin microbiota of up to a third of the general population; the nasal vestibules (35%) and the perianal region (30%) are the main reservoirs, followed by the axillary and interdigital regions (5%-10%), where dissemination can occur, causing infections<sup>(41)</sup>. Therefore, infections in healthcare settings caused by multiresistant *S. aureus* have become quite relevant in the latest decades, being responsible for high indices of morbidity and mortality<sup>(42-44)</sup>.

In health centers, the main reservoirs of *S. aureus* are the infected patients, although physicians, nurses and other staff members may be reservoirs and elements of propagation and maintenance. In this perspective, it is worth emphasizing that prevention of *S. aureus* infection depends principally on the mechanisms for controlling environment and healthy carriers<sup>(76)</sup>. Oxacillin or methicillin-resistant (MRSA) isolates are among the major pathogens causing infections in the world, leading to the emergence of and disseminating increasingly virulent and multiresistant strains<sup>(46)</sup>.

In this study, the use of a polyphasic identification approach allowed the detection of *Staphylococcus* spp., presenting MRSA, VRSA, MSSA, CoNS, and MRCoNS phenotypes, in isolates from both equipment and health professionals. For example, presence of coagulase (71.42%) and detection of the *coa* gene (75.51%) made us suggest that although the coagulase test in tubes is considered the standard method for differentiation of *Staphylococcus* spp., it

may present false negative results, as demonstrated in our study, in which coagulase-negative strains revealed the presence of the *coa* gene by PCR<sup>(80)</sup>.

Detection of the *coa* gene has been adopted in species differentiation and typing because it is considered accurate and often more sensitive than detection of coagulase by biochemical assays<sup>(48, 49)</sup>. Besides being a factor of virulence, the gene encoding coagulase synthesis is present in several allelic forms, what makes it possible for isolates to be classified into different variants. Like the *spa* (protein A) gene, the *coa* gene has a polymorphic region used for differentiation of *S. aureus* isolates with the analysis of polymorphism of length of restriction fragments, a method used in epidemiologic studies<sup>(49-51)</sup>.

In this study, the presence of the *femA* gene in 35 *S. aureus* isolates was confirmed. It is present exclusively in this species and is used for the selective detection of this microorganism<sup>(52-54)</sup>, although homologues of this gene have been characterized into CoNS species, such as *S. epidermidis*, *S. simulans*, *S. hominis* and *S. saprophyticus*<sup>(55)</sup>. The absence of the *femA* gene from two coagulase-negative isolates that presented the *coa* gene may be due to variations in the annealing regions of primers, making the amplification of *femA* impossible<sup>(56)</sup>.

Our results demonstrated that among the 17 oxacillin-resistant (ORSA) isolates, 11 (64.7%) expressed coagulase; and 10 (58.8%), the *mecA* gene, being identified as MRSA. The oxacillin-resistant strain, which did not present the *mecA* gene, leads us to suggest the presence of another mechanism of oxacillin resistance, as, for instance, hyperproduction of beta-lactamase, modification of the PBP binding site or presence of the *mecC* gene<sup>(24, 57-60)</sup>.

In Brazil, MRSA dissemination has been object of studies aimed at verifying the frequency of resistance and its implications in the health system<sup>(20, 21, 41, 61)</sup>. Two studies in Rio Grande do Sul demonstrated the presence of 32.7% MRSA in hospitalized patients, and 20.6% in saliva of 13 cleaning workers at the hospital<sup>(61, 62)</sup>. In Pernambuco, the prevalence rate was 13% in ICU inpatients, while in Bahia, this rate was 28%<sup>(2, 20)</sup>. An investigation of nasal swabs of hospitalized newborn at a maternity hospital in Rio de Janeiro verified a frequency of 47% of this pathogen<sup>(63)</sup>. The presence of MRSA was also detected in hands and buccal cavity of 73% of dentists, 52% of other health professionals and 54% of patients at dental clinics of basic health centers in the city<sup>(11)</sup>.

In recent decades, vancomycin has been the drug of choice for the treatment of MRSA infections. However, the excessive use of this antibiotic has led to increased resistance in *Enterococcus*, CoNS and MRSA strains. VISA isolates were initially detected in Japan, United States, France, South Africa and South Korea, from single patients

or groups of patients in a same hospital, demonstrating the transfer capacity of this organism and drawing attention to the importance of improving control measures for nosocomial infections<sup>(64-67)</sup>.

In Brazil, the presence of vancomycin tolerance in ORSA strains was determined in 49.1% of the 395 hospital isolates in São Paulo, what certainly increases the risk of failures in treatments with vancomycin, besides increasing the risk of emergence of VISA<sup>(31)</sup>. In Brazil, HAIs by these microorganisms were initially reported in São Paulo and Rio de Janeiro. Later, an outbreak of VISA was described at a hospital in São Paulo, with isolation of four strains at a burn center<sup>(31, 68, 69)</sup>. The first report of multiple VRSA isolates at a Brazilian hospital was the result of a study in 140 isolates of inpatients exposed to vancomycin<sup>(32)</sup>. At the present study, one of the most surprising pieces of data was the detection of an isolate – VRSA – colonizing the hand of a physician at the health care center. This asymptomatic colonization is highly clinically significant, as the individual colonized in the nostrils may contaminate his own hands and become a vehicle for this pathogen via the mechanism of contact infection. Consequently, the pathogen dissemination can occur in the health care setting, where there is circulation of individuals and patients who are more susceptible to exogenous infection<sup>(32)</sup>.

A study involving a thousand healthy individual also demonstrated 22.5% and 16.6% of individuals colonized by *Staphylococcus aureus* and MRSA, respectively, in nostrils, forearm and hands<sup>(70)</sup>. Concern over VRSA colonization and transmission is not caused exclusively by nasal carriers, but also by medical devices as vehicles of this transmission. Thus, this colonization is considered a public health problem, and it is of interest to investigate whether health professionals are also nasal carriers of MRSA<sup>(71)</sup>.

Studies about the epidemiological role of hands in the transmission of infection among health care workers have recognized their potential as source of eventual hospital infections, as well as the possible relationship between isolates from different anatomical locations of the same individual, mainly between nasal cavities and hand<sup>(1)</sup>. Besides, hands are also considered one of the main sources of cross-transmission of nosocomial infections among patients, equipment and/or contaminated surfaces, attributed to inadequate hygiene processes<sup>(72-74)</sup>.

Awareness of the risks of infection transmission, limitations of disinfection methods, and difficulties of processing inherent in the nature of each medical device is fundamental for the adequate measures to be taken<sup>(75)</sup>. Although hand hygiene has been the most important and recognized measure for prevention and control of infections, mainly by *S. aureus*, strong resistance to it is still observed before or after handling of patients in health services. Therefore, putting hand hygiene in practice has been a complex and

difficult task, as professionals need to be aware of its importance in health care settings for safety and quality of care<sup>(76,77)</sup>.

The main prevention measures to control the spread of multiresistant microorganisms include: laboratory data-based surveillance, isolation of infected or colonized patients, use of barrier precautions (gloves and caps), hand washing and antiseptics, and cleaning the environment near the patient<sup>(78)</sup>. Inherent in all control measures of HAIs, education of health care workers is very important to the correct performance of patient care duties<sup>(79)</sup>. The factors associated with poor adherence to hand hygiene are, principally, heavy workloads, glove use, and conduction of activities involving cross transmission during specialized techniques<sup>(80)</sup>.

The implementation of control and prevention measures must include continuous education, monitoring of adherence to hand hygiene practices, besides feedback of data, installation and maintenance of equipment, rational use of antibiotics, and recommendations based on caution in invasive procedures<sup>(81)</sup>.

## CONCLUSION

Our results showed higher frequency of MSSA and MRCoNS among *S. aureus* and CoNS isolates, respectively, colonizing

equipment and health care workers. However, the already described SSCmec transfer from MRCoNS to MSSA<sup>(82)</sup> could alter these results, increasing the frequency of methicillin-resistant strains.

As far as we know, this is the first report about isolation of a VRSA strain presenting the vancomycin resistance gene *vanB*, with CIM  $\geq 256$   $\mu\text{g/ml}$  to vancomycin and teicoplanin, from the hands of a health care worker in Brazil.

As a result, we demonstrate the increased dissemination of resistance, and conclude that the adoption of procedures for hand antiseptics and disinfection of medical items is essential to prevent dissemination of these pathogens among patients, health professionals, and individuals of the community.

## ACKNOWLEDGEMENTS

We thank Secretaria Municipal de Saúde e Defesa Civil of the state of Rio de Janeiro, the direction and health professionals of the municipal health centers that participated in this study. We also thank the financial support of Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (474204/2012-0), Fundação Carlos Chagas de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) (E-26/112.657/2012), and INCQS-Fiocruz.

## RESUMO

**Introdução:** A contaminação cruzada por *Staphylococcus aureus* entre pacientes, profissionais e materiais de uso médico em unidades de saúde é uma preocupação constante, o que leva pesquisadores a estudar a prevalência desse patógeno em portadores assintomáticos. **Objetivos:** Investigamos a colonização e o perfil de suscetibilidade aos antimicrobianos de *Staphylococcus spp.* em superfícies de artigos médicos e em profissionais de duas unidades básicas de saúde no município do Rio de Janeiro. **Materials e métodos:** Foram coletadas 79 amostras que resultaram em 49 isolados, submetidos à caracterização fenotípica e molecular por meio da reação em cadeia da polimerase (PCR) dos genes *coa*, *femA* e *mecA*. **Resultados:** De acordo com os fenótipos apresentados, os isolados foram identificados como *S. aureus* ( $n = 35$ ; 71,42%) e *Staphylococcus coagulase negativa* (CoNS) ( $n = 14$ ; 28,57%). Destes 14 isolados, 42,85% foram *Staphylococcus coagulase negativa* resistentes a metilina (MRCoNS). Dos 35 *S. aureus*, 31,42% foram resistentes a metilina (MRSA). Uma cepa foi resistente a vancomicina e identificada como *S. aureus* resistente a vancomicina (VRSA) após a detecção do gene *vanB*. Sessenta e oito por cento foram suscetíveis a metilina (MSSA). Os genes *coa*, *femA* e *mecA* foram amplificados em 75,51%; 71,42% e 30,61% dos isolados, respectivamente. Após amplificação do gene *mecA*, 20,41% foram classificados como MRSA e 10,20% como MRCoNS. **Conclusão:** Nossos resultados mostraram frequência maior de MSSA e MRCoNS entre *S. aureus* e CoNS, respectivamente, colonizando equipamentos e profissionais de saúde. No entanto, a já descrita transferência do cassete cromossômico estafilocócico *mec* (SSCmec) de MRCoNS para MSSA poderia alterar esses resultados, aumentando a frequência de cepas MRSA.

**Unitermos:** *Staphylococcus aureus*; MRSA; VRSA.

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