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Major Article

The occurrence and dissemination of methicillin and vancomycin-resistant *Staphylococcus* in samples from patients and health professionals of a university hospital in Recife, State of Pernambuco, Brazil

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ABSTRACT

Introduction: Methicillin-resistant *Staphylococcus aureus* (MRSA) strains have been responsible for many nosocomial outbreaks. Within hospitals, colonized employees often act as reservoirs for the spread of this organism. This study collected clinical samples of 91 patients admitted to the intensive care unit (ICU), hemodialysis/nephrology service and surgical clinic, and biological samples from the nasal cavities of 120 professionals working in those environments, of a University Hospital in Recife, in the State of Pernambuco, Brazil. The main objective of this study was to determine the occurrence and dissemination of methicillin- and vancomycin-resistant *Staphylococcus* spp. **Methods:** The isolates obtained were tested for susceptibility to oxacillin and vancomycin and detection of the *mec*A gene. In addition, the isolates were evaluated for the presence of clones by ribotyping-polymerase chain reaction (PCR). **Results:** MRSA occurrence, as detected by the presence of the *mec*A gene, was more prevalent among nursing technicians; 48.1% (13/27) and 40.7% (11/27) of the isolates were from health professionals of the surgical clinic. In patients, the most frequent occurrence of *mec*A-positive isolates was among the samples from catheter tips (33.3%; 3/9), obtained mostly from the hemodialysis/nephrology service. Eight vancomycin-resistant strains were found among the MRSA isolates through vancomycin screening. Based on the amplification patterns, 17 ribotypes were identified, with some distributed between patients and professionals. **Conclusions:** Despite the great diversity of clones, which makes it difficult to trace the source of the infection, knowledge of the molecular and phenotypic profiles of *Staphylococcus* samples can contribute towards guiding therapeutic approaches in the treatment and control of nosocomial infections.

Keywords: MRSA. Vancomycin. Patients. Health professionals. Ribotyping-PCR.

INTRODUCTION

Staphylococcus remains one of the most common pathogens in systemic infections in communities and hospitals. With the advent of resistance to methicillin in the 1960s, *Staphylococcus* began to receive special attention, especially with regard to controlling the spread of this microorganism. Since then, the therapeutic options have become increasingly restricted[1,2].

In most cases, this methicillin resistance is determined by the presence of the *mec*A gene, located in the chromosome and responsible for the synthesis of PBP2a or PBP2', a penicillin-binding protein (PBP), which regulates bacterial cell wall synthesis in the presence of beta-lactam antibiotics[2,3]. This gene is widely distributed among *Staphylococcus aureus* and between species of coagulase-negative *Staphylococcus,* and its detection by molecular methods is considered the *gold standard* for a qualitative assessment of resistance to methicillin[2,4,5].

Health professionals are carriers of this bacterium due to their vulnerability in their everyday activities, and some studies suggest that they act as disseminators[6-8]. The nasal cavity is the most frequent site of MRSA colonization[9,10]; identification of colonization is considered as a preventive strategy that enables reductions of the incidence of infections with this microorganism[9]. Studies have shown that MRSA colonization very often precedes infection. Ellis et al.[11] reported that 38% of participants colonized with Community-Acquired Methicillin-resistant *Staphylococcus aureus* (CA-MRSA) developed a skin and soft tissue infection over a period of 8-10 weeks[11]. The colonization of the relatives of children with staphylococcal infections in a children's hospital in Detroit, Michigan was also previously reported[12].

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The identification of MRSA carriers is a step towards establishing a control policy, thereby helping to identify the measures needed to reduce the colonization pressure[9]. The knowledge of the molecular epidemiology of the diseases caused by these bacteria may assist in developing more efficient strategies for reducing the infection, as genetic relationships among the different clones can be inferred, the gene flow route can be detected and the spread of infection can be traced from the molecular profiles [9,13].

Appropriate systems for typing are needed to determine the genetic variability of the isolates, thus providing an effective epidemiological control. The ribotyping-polymerase chain reaction (PCR) is often referenced and used because of its high taxonomic and epidemiological value. This technique is a valuable tool for identifying and differentiating isolates of the *Staphylococcus* genus[13-15].

Despite the relevance of these microorganisms as important pathogens associated with health care-related infections, studies that provide data regarding the colonization of health care workers, especially multi-professional assessments in Pernambuco, Brazil, associating them with the spread of such infections, are still scarce. Hence, this study aims to describe the occurrence and spread of methicillin- and vancomycin-resistant *Staphylococcus spp.* in samples from the nasal cavities of health professionals and clinical samples from patients admitted to the intensive care unit (ICU), hemodialysis/nephrology service and surgical clinic of a university hospital in the City of Recife by analyzing epidemiological/bacterial MRSA marker data to assist in promoting control actions at the hospital.

METHODS

Collection of biological material

The study included all patients and all health professionals from the sectors of the general ICU, surgical clinic and hemodialysis/nephrology service of the *Hospital das Clínicas*, Federal University of Pernambuco (UFPE), Brazil during the period from April to August 2011 based on the list of patients and staff in each sector.

Samples from the nasal cavities of health professionals were collected using sterile swabs after each individual signed a Statement of Free and Informed Consent (SFIC) and completed a questionnaire on information about their professional activities. A previous study showed that this site was most likely to be colonized[8]. These samples were introduced into brain heart infusion (BHI) and transported to the Bacteriology Laboratory, Department of Tropical Medicine/UFPE.

The patient samples were collected according to the standard procedures used in the hospital for catheter tips, drains, blood, abscesses, surgical wounds, tracheal secretions and so forth, and the isolates were obtained after culture in the Bacteriology Laboratory of the *Hospital das Clínicas* of the Federal University of Pernambuco. Patients who did not have any areas with characteristics of bacterial infection were submitted to the collection of nasal swabs after signing an SFIC.

Isolation and identification of *Staphylococcus*

The isolates of *Staphylococcus* spp., identified by the Bacteriology Laboratory of the hospital, from the various biological samples collected from patients and the nasal swab samples from health professionals were placed in BHI broth and then inoculated into 5% sheep blood agar and incubated for 24-48 h at 37°C. Colonies with macroscopic characteristics of the genus *Staphylococcus* were Gram stained, and when confirmed by morphology and staining, were submitted for identification using deoxyribonuclease (DNase), catalase and coagulase tests and mannitol fermentation. The remaining samples of secretions from patients were seeded according to the standard protocol in 5% sheep blood agar and MacConkey agar. However, only the colonies with characteristics of the genus *Staphylococcus* were identified[16].

Cefoxitin and oxacillin susceptibility

After identification, susceptibility testing of *Staphylococcus* was performed using the disk-diffusion method on Mueller-Hinton agar[17] with 1μg oxacillin and 30μg cefoxitin. The Clinical and Laboratory Standards Institute (CLSI) 2013 interpretive breakpoints were considered.

Screening for oxacillin resistance

The isolates selected were those that showed resistance or had intermediate profiles to oxacillin and/or cefoxitin in a disk-diffusion test. Colonies from 5% blood agar plates were resuspended in BHI to obtain a turbidity equivalent to 0.5 on the McFarland scale. A 1µL platinum wire loop was dipped in the suspension, and the bacteria were seeded in a 10-15mm area on plates with Mueller-Hinton agar medium containing NaCl (4% v/v; 0.68mol/L) and 6μg/mL oxacillin. These plates were incubated at 35°C for 24h and then read, considering that >1 colony = resistance[17]. For quality control, the standard MRSA strain ATCC 33591 was used for the positive control (MRSA), and the standard Methicillin-sensitive *S. aureus* (MSSA) strain ATCC 29213 was used for the negative control.

Screening for vancomycin resistance

The isolates that possessed the *mec*A gene, assayed by PCR, were subjected to screening for vancomycin resistance. Colonies from 5% blood agar plates were resuspended in BHI to obtain a turbidity equivalent to 2.0 on the McFarland scale. A 1µL platinum wire loop was dipped in the suspension, and the bacteria were seeded in an area with a 10-15mm diameter on plates with BHI agar medium containing 6μg/mL vancomycin (Oxoid). The plates were incubated at 35°C for 24h and 48h and then read, considering that >1 colony = resistance[18-20]. For quality control, the standard strains *Enterococcus faecalis* ATCC 29212 – sensitive and *Enterococcus faecalis* ATCC 51299 – resistant were used.

DNA preparation

Total deoxyribonucleic acid (DNA) was extracted from individual colonies after growth in BHI broth for 24h at 37°C, following the protocol described by Freitas et al.[21].

Identification by PCR of the *mec*A gene

PCR was performed utilizing the primers described by Petinaki et al.[22]. The amplification reaction mixture was prepared in a total volume of 25µL containing 50mM KCl, 10mM Tris-HCl, 1.5mM $MgCl_2$, 200mM dNTP (Promega), 20pmol of each primer, 20ng of genomic DNA, and 1U Taq DNA polymerase (Promega). The reactions were performed in a thermocycler (Biometra), programmed initially for 30 thermal cycles, with denaturation of 1 min at 94°C, annealing of 1 min at 50°C and extension of 1 min at 72°C, followed by a final step of 10min at 72°C. The negative control contained all of the components of the reaction mixture except DNA. ATCC 33591 *S. aureus* (MRSA) was used for the positive control. The amplification product was submitted to 1% agarose gel electrophoresis with ethidium bromide staining and was visualized with an ultraviolet (UV) transilluminator and then digitized (Kodak Digital Science).

Ribotyping-PCR

The isolates that were positive for the *mec*A gene by PCR were subjected to ribotyping-PCR to assess the genetic relationship of the isolates following the protocol described by Cuny et al.[23]. For the 16S-23S ribosomal ribonucleic acid (rRNA) spacer region amplifications, the primers rRNA1 (5'- TTG TAC ACA CCG CCC GTC A-3') and rRNA2 (5'- GGT ACC TTA GAT GTT TCA GTT C-3') were used. The amplification reaction mixture was prepared in a total volume of 25µL containing 50mM KCl, 10mM Tris-HCl, 1.5mM $MgCl_2$, 200mM dNTP (Promega), 20pmol of each primer, 20ng of genomic DNA, and 1U Taq DNA polymerase (Promega). The reactions were performed in a thermocycler (Biometra), programmed initially for 30 thermal cycles, with denaturation of 1min at 94°C, annealing of 1 min at 55°C and extension of 1min at 72°C, followed by a final step of 7min at 72°C. The amplification product was submitted to 2% agarose gel electrophoresis with ethidium bromide staining (2µg/mL) and visualized with a UV transilluminator and then digitized (Kodak Digital Science). A 100bp Ladder (Invitrogen) was used as a molecular weight standard to estimate the sizes of the amplified fragments.

Statistical analysis

The clinical and microbiological data were statistically analyzed using Epi Info (version 6.04.) according to the frequency distribution. The dendrogram was constructed using Darwin 5.0.158 software (Cirad - Department: Systèmes Biologiques (BIOS), Research Unit: Genetic improvement of vegetatively propagated crops, Team: BioMathematics, Avenue Agropolis - TA A75/02, 34398 Montpellier Cedex 5 – France).

Ethical considerations

This study was approved by the Ethics Committee on Research of the Federal University of Pernambuco (CEP/CCS/UFPE - *Comitê de Ética em Pesquisa/Centro de Ciências da Saúde/Universidade Federal de Pernambuco*), CAAE number 0490.0.172.000-11.

RESULTS

Samples were collected from 91 patients **(Table 1)** and 120 health professionals, including physicians, nurses, nursing technicians, physiotherapists, nutritionists and psychologists **(Table 2)** from the sectors of the ICU, surgical clinic and the hemodialysis/nephrology service of the *Hospital das Clínicas* of the Federal University of Pernambuco in the period from April to August 2011.

A sample was obtained from each patient and, after identification tests, 30 bacteria of the genus *Staphylococcus* were isolated. The most frequent sample type from patients was blood culture (37.4%), followed by catheter tip (11%). In all, 14 negative cultures were obtained **(Table 1)**.

Among the isolates from patients that were classified as the genus *Staphylococcus*, 11 were identified as coagulase-negative *Staphylococcus* and 19 as *S. aureus*. Using the oxacillin and/or cefoxitin disk-diffusion tests, 21 *Staphylococcus* spp. with resistance profiles were selected and submitted for detection of the *mec*A gene by PCR. Nine positive isolates were detected after this step. The *Staphylococcus* spp. isolates from the health professionals were also subjected to this test, with 63 resistant isolates being selected **(Table 3)**; of these, 27 isolates encoded the *mec*A gene, with a total of 36 MRSA isolates **(Table 4)**.

Of the isolates subjected to oxacillin screening, 61.9% were resistant; of these, 75% of the isolates had the *mec*A gene **(Table 3)**. The greatest occurrence of *mec*A- positive isolates in the samples from patients was among the isolates from catheter tips (33.3%) **(Table 1)**. Despite similar percentages, the sector that was the most frequent source for positive isolates in this group was the hemodialysis/nephrology service (44.4%) **(Table 4)**. On conducting the vancomycin screening, eight isolates were determined to be resistant **(Table 4)**.

In this study, there was no statistically significant difference observed among health professionals when the prevalence of MRSA in females was compared to males. Considering age groups, individuals between 20 and 28 years old were the most colonized by MRSA, these microorganisms being most prevalent among nursing technicians (48.1% among the positive isolates). Considering hospital sectors, the surgical clinic accounted for the highest incidence of positive isolates (40.7% among health professionals).

The prevalence of MRSA was high (77.8%) among professionals who simultaneously used a medical coat, gloves and a mask only in specific situations of contact with fluids or secretions of patients. The group that reported that they most often used a medical coat, gloves and a mask together from the personal protective equipment (PPE) available was also the one most colonized by MRSA (29.6%). The occurrence of MRSA was also highest among the professionals who performed their activities during the day (24/100) compared with those who performed their duties during the night (3/20).

The occurrence was also higher among those who had between 1 and 5 years of professional experience and had been working in that sector for less than 1 year. The isolation

frequencies of resistant strains were similar among those who worked only in the hospital under study and those who worked in another hospital **(Table 2)**.

In the ribotyping-PCR reactions, two to five fragments of approximately 500-900bp in size were observed. Based on the amplification patterns, the 36 isolates were classified into 17 ribotypes, designated in this study as R1 to R17 (**Figure 1**). Six isolates of *S. aureus* (four patients and two professionals) were distributed in three ribotypes (R1, R7, R15). The coagulase-negative *Staphylococcus* (CoNS) isolates from five patients were distributed in four different ribotypes (R2, R4, R10, R15), and 25 isolates from health professionals were distributed in 15 professional ribotypes, with a prevalence of ribotypes R10 (six isolates), R7 and R13 (three isolates each) and R4 and R14 (two isolates each). The ribotypes R3, R5, R6, R8, R9, R11, R12, R16 and R17 each occurred in only one isolate **(Figure 1)**. The profiles R1, R2, R4, R7 and R10 were observed in isolates from both patients and health professionals.

TABLE 1 - Distribution of *Staphylococcus* spp. isolates from the patients by sample related the hospital sectors.

	ICU			hemodyalisis and nephrology services			Surgical clinics			
Sample type	*Staphylococcus* isolates number	other microorganisms	Negative cultures	*Staphylococcus* isolates number	Other microorganisms	Negative cultures	*Staphylococcus* isolates number	Other microorganisms	Negative cultures	Total
Blood	2	5	1	1	5	9	5 (2)	6	1	35
Catheter tip	2 (1)	0	0	3 (2)	1	0	3	1	0	10
Peritoneal fluid	0	0	0	1 (1)	0	1	0	0	0	2
Pleural fluid	0	0	1	0	0	0	0	0	0	1
Bile bladder fluid	0	0	0	0	0	0	0	0	1	1
Tracheal aspirates	2 (1)	5	0	0	0	0	1	1	0	9
Surgical wound	1 (1)	2	0	0	0	0	0	2	0	5
Skin secretion	0	0	0	0	1	0	3	0	0	4
Left axilla secretion	0	0	0	0	0	0	1	0	0	1
Nasal swab	0	0	0	5 (1)	0	0	0	0	0	5
Urine	0	1	0	0	1	0	0	2	0	4
Oropharyngeal secretions	0	0	0	0	1	0	0	0	0	1
Drain secretion	0	0	0	0	0	0	0	2	0	2
Catheter secretion	0	0	0	0	3	0	0	1	0	4
Sacral sores	0	1	0	0	0	0	0	3	0	4
Abdominal secretion	0	0	0	0	0	0	0	1	0	1
Tissue fragment	0	0	0	0	0	0	0	1	0	1
Inguinal tumor	0	0	0	0	0	0	0	1	0	1
Total	7 (3)	14	2	10 (4)	12	10	13 (2)	21	2	91

ICU: intensive care unit. **Note:** the parenthesis data are the number of *mec*A postive isolates.

TABLE 2 - Distribution of variables related to the presence of MRSA in health professionals from the Hospital of UFPE, 2011.

	Total		MRSA		Non MRSA	
Variables	n	%	n	%	n	%
Gender						
male	20	16.7	5	18.5	15	16.1
female	100	83.3	22	81.5	78	83.9
Age (years)						
20-28	43	35.8	9	33.3	34	36.6
29-33	26	21.7	4	14.8	22	23.7
34-44	27	22.5	7	25.9	20	21.5
45-60	24	20.0	7	25.9	17	18.3
Professional activity						
nurse	43	35.8	7	25.9	36	38.7
nurse technician	45	37.5	13	48.1	32	34.4
physician	26	21.7	6	22.2	20	21.5
physiotherapist	3	2.5	1	3.7	2	2.1
nutritionist	2	1.7	0	0	2	2.1
psychologist	1	0.8	0	0	1	1.1
Sector						
ICU	23	19.2	7	25.9	16	17.2
surgical clinic	50	41.7	11	40.7	39	41.9
hemodialysis and nephrology service	47	39.2	9	33.3	38	40.9
Use of PPE						
always	27	22.5	5	18.5	22	23.7
sometimes	87	72.5	21	77.8	66	71.0
never	6	5.0	1	3.7	5	5.4
PPE used most often						
medical coat+gloves+mask	32	26.7	8	29.6	24	25.8
medical coat	8	6.7	1	3.7	7	7.5
gloves	5	4.2	2	7.4	3	3.2
gloves+mask	9	7.5	2	7.4	7	7.5
gloves+medical coat	21	17.5	7	25.9	14	15.0
medical coat+gloves+mask +cap+glasses	3	2.5	0	0	3	3.2
medical coat+gloves+mask+cap	29	24.2	3	11.1	26	28.0
medical coat+gloves+mask+glasses	3	2.5	1	3.7	2	2.1
gloves+mask+cap	3	2.5	2	7.4	1	1.1
medical coat+cap	3	2.5	1	3.7	2	2.1
gloves+medical coat+cap	2	1.7	0	0	2	2.1
mask+medical coat	1	0.8	0	0	1	1.1
mask+medical coat+cap	1	0.8	0	0	1	1.1

Continues...

TABLE 2 - Continuation.

Variables	Total n	Total %	MRSA n	MRSA %	Non MRSA n	Non MRSA %
Shift						
Diurnal	100	83.3	24	88.9	76	81.7
Nocturnal	20	16.7	3	11.1	17	18.3
Length of time in the profession (years)						
<1	25	20.8	4	14.8	21	22.6
1-5	30	25.0	9	33.3	21	22.6
5-10	22	18.3	4	14.8	18	19.4
10-15	13	10.8	2	7.4	11	11.8
15-20	9	7.5	3	11.1	6	6.4
>20	21	17.5	5	18.5	16	17.2
Length of time in the sector (years)						
<1	54	45.0	11	40.7	43	46.2
1-3	25	20.8	7	25.9	18	19.3
3-7	12	10.0	3	11.1	9	9.7
7-11	12	10.0	2	7.4	10	10.7
11-15	5	4.2	0	0	5	5.4
15-20	8	6.7	4	14.8	4	4.3
>20	4	3.3	0	0	4	4.3
Number of hospitals worked in						
1	57	47.5	11	40.7	46	49.5
2	49	40.8	11	40.7	38	40.9
3	10	8.3	3	11.1	7	7.5
>3	4	3.3	2	7.4	2	2.1

MRSA: Methicillin-resistant *Staphylococcus aureus*; **UFPE**: *Universidade Federal de Pernambuco*; **ICU:** intensive care unit; **PPE:** personal protective equipment. **Note:** The variable "Use of Personal Protective Equipment (PPE)" for this study was defined as the frequency of use of all personal protective equipment (Medical coat+gloves+mask) during activities. Categorized as: always (in all situations of patient contact); sometimes (only in situations that manipulate biological fluids, such as blood and secretions, and/or when the patient has infectious disease symptoms); or never (do not use all PPEs in any situation).

TABLE 3 - Results of oxacillin screening distributed by isolation font, showing the number of *mec*A positive isolates.

	Isolation font				
	patients		health care workers		
Test results	*S. aureus*	SCoN	*S. aureus*	SCoN	Total
Resistant	6 (4)	9 (4)	3	34 (19)	52 (27)
Sensitive	5	1 (1)	8 (2)	18 (6)	32 (9)
Total	11 (4)	10 (5)	11 (2)	55 (25)	84 (36)

***S.:** Staphylococcus;* **SCoN:** coagulase-negative *Staphylococcus*. **Note:** the parenthesis data are the number of *mec*A postive isolates.

TABLE 4 - Distribution of *mec*A positive isolates by isolation sector and isolates by isolation font.

Sector	Isolation font		Number of isolates by species	Total
ICU	patients	*S. aureus*	1	10
		SCoN	2	
	health care workers	*S. aureus*	1	
		SCoN	6 (1)	
Surgical clinic	patients	*S. aureus*	2 (1)	13
		SCoN	0	
	health care workers	*S. aureus*	0	
		SCoN	11 (2)	
Hemodialysis and nephrology service	patients	*S. aureus*	1	13
		SCoN	3	
	health care workers	*S. aureus*	1	
		SCoN	8 (4)	
Total			36 (8)	36

ICU: intensive care unit; ***S.:*** *Staphylococcus;* **SCoN:** coagulase-negative *Staphylococcus.* **Note:** the data in parentheses are the numbers of resistant strains in the screening of vancomycin distributed by isolation font.

DISCUSSION

The prevalences of MRSA were 10% (9/91) for patients and 22.5% (27/120) for health professionals; both groups carried primarily coagulase-negative *Staphylococcus,* with prevalences of 5.5% and 20.8%, respectively. These percentages may be considered low, as the prevalence of isolation of MRSA strains ranges from 40 to 80%[2,9,24] in Brazilian hospitals, and the data from the Antimicrobial Surveillance Program (SENTRY) show that MRSA corresponds to 31% of the causes of nosocomial and community infections and is considered the most common among the most prevalent pathogens[25]. However, the fact that professionals have a higher percentage of MRSA points to contamination in the hospital itself. The SENTRY survey conducted in Brazilian hospitals showed that the resistance of **CoNS** in blood cultures is 80%[25]; and in our study, this was the most frequent type of sample.

Among ICU patients from another university hospital in Recife, a prevalence of *S. aureus* colonization of 37.7% was reported, of which MRSA accounted for 13%[26], similar to the prevalence of 10% (3/30) for MRS in the ICU that was measured in this study. As to the health-care team, in a study conducted in a university hospital in Londrina, colonization by *S. aureus* among the medical staff was close to that normally detected in the community: 17.7%, of whom 1.2% were MRSA carriers[27], which is lower than the MRSA colonization percentage of 23.1% (6/26) detected in this study. In a study at another university hospital in Recife, the colonization of health workers reached 25.7%, but the percentage of MRSA was considered below the limits described in the literature (only three nurses among the 202 professionals from whom samples were collected had positive samples[10]) and was below the percentage that we detected with respect to MRSA. In another study conducted in the same hospital as our study, the colonization of nursing staff accounted for 25.8% of positive samples and; once again, the percentage of MRSA was considered below the limits described in the literature (3.3% of the samples[8]).

A study in a public hospital in the interior of the State of São Paulo suggested that nurses and nursing technicians are the professional class that is most colonized by MRSA, citing prevalences of 7.1% among nurses[28] and 10.8% among nursing technicians.

The age group of MRSA occurrence among health professionals was the same as that described in other studies conducted in the same hospital in previous years[8,10,29]. The frequency of MRSA infection was highest in the 20- to 28-year-old age group. These studies suggest that this incidence may be due to the need for improvements in professional practice, such as washing hands before and after procedures and avoiding contacting nostrils with hands. This same deficiency in professional practice could also explain the higher prevalence in the group who had been health-care professionals for between 1 and 5 years.

In this study, a higher incidence of methicillin-resistant samples was observed only by phenotypic methods, such as oxacillin screening, compared to genetic screening, which suggests the presence of other resistance mechanisms independent of the *mec*A gene[22,30,31].

Among the MRSA samples, eight isolates resistant to vancomycin were detected by the screening method, and only one corresponded to an isolate coming from a patient blood

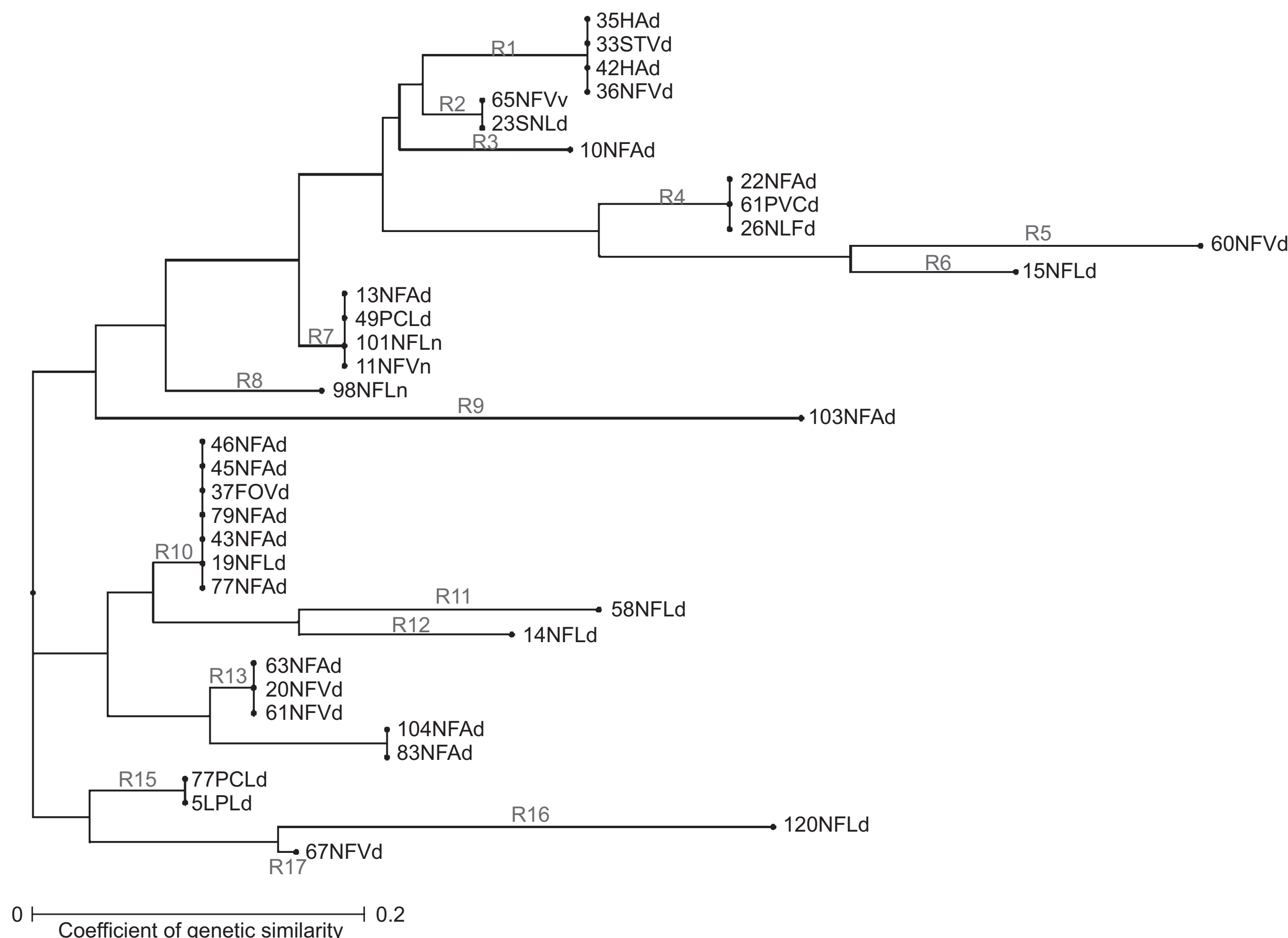

FIGURE 1 - Z estimated by ribotyping-PCR for 36 methicillin-resistant *Staphylococcus* spp. isolates from patients and health care workers from a university hospital in Recife, State of Pernambuco, Brazil. Note: The letter ***R*** indicates ribotype. In the description of the isolates, the number indicates the isolate number, the first two letters indicate the type of sample, the third letter indicates the hospital sector and the last letter indicates the collection period. Sample types: **LP:** peritoneal fluid; **PC:** catheter tip; **SN:** nasal swab from patient; **ST:** tracheal aspirates, **H:** blood; **FO:** surgical wound; **NF:** nasal swab from health professional. Sectors: **A:** Surgical clinic; **I:** Hemodialysis/Nephrology Service. **V:** ICU. Collection period: **d:** day (7h to 19h) and **n:** night (19h to 7h). **PCR:** polymerase chain reaction; **ICU**: intensive care unit.

culture (isolate 35HAd, **Figure 1**). The remaining samples were from professionals, two of which were classified as the same ribotype (isolates 101NFLn and 110NFVn, **Figure 1**), suggesting that they are the same strain. Studies indicate that resistance to vancomycin should be determined by more sensitive techniques, such as plate screening, E-test, microdilution and genotype detection[17-20]. In Brazil, intermediate resistance to vancomycin in patients has been described, but few studies have reported colonization of health professionals by these strains[32]. The first case of transferable vancomycin resistance in a community-associated MRSA strain was reported in a Brazilian hospital, indicating that the presence of MRSA containing the *van*A gene could be a future public problem[33].

Some studies that use ribotyping-PCR to assess genetic similarity also present ample polymorphisms, as in our study, considering the number of ribotypes observed, thus indicating dispersion in the hospital sectors[34,35], but these studies do not make comparisons of dispersion between classes of patients and professionals.

In the ribotyping-PCR reactions, few ribotypes (R1, R2, R4, R7 and R10) were distributed among samples from patients and health professionals, suggesting a low spread between these classes. Pulsed-field gel electrophoresis (PFGE) was used to analyze the presence of bacterial clones between patients and professionals, thus indicating the health professionals as a source outbreaks of nosocomial infections[7]. The ribotyping-PCR technique used here has the advantage of being easy to perform and less prone to variations, compared to other methods, because it characterizes a region that is essential for bacterial growth and therefore more stable[13-15]. However, this method cannot confirm

that health professionals are the source of transmission, as they are carriers of several ribotypes not found in patients.

This approach is useful to once again raise the question of the role of health professionals in spreading nosocomial infections. Despite the fact that the contribution of health professionals in the spread of resistant strains has not yet been confirmed[36,37], various studies based on molecular techniques do suggest they are a vehicle of dissemination[7,38,39]. The diversity of ribotypes identified, despite the stability of the internal transcribed spacer (ITS) 16S-23S region, suggests the presence of several clones circulating in the hospital during the study period; thus, it is possible that there may be multiple sources of contamination.

In view of these findings, routine screenings of health care professionals for MRSA colonization is not necessary; likewise, their decolonization, mainly due to the associated cost[36], should only be conducted in situations in which the epidemiological data suggest that they are serving as the transmission source[6,7] – or, as a last resort, to contain transmission when other measures have already been taken in an outbreak[40]. In these situations, the identification of MRSA carriers is a step towards establishing a control policy and helps to identify the measures needed to reduce the colonization pressure[9]. Despite the fact that the low spread of methicillin-resistant isolates between classes has been demonstrated, other factors may also contribute to the spread of the microorganism, such as its capacity to colonize, to multiply itself and to invade the mucosal epithelia cells of the host, along with the capacity to withstand the selective pressure of hospital environments. This situation highlights the importance of monitoring the distribution and routes of the dissemination of MRSA clones in hospitals[41], the emphasis being on identifying isolates resistant to vancomycin in samples of colonization. Thus, measures to contain the spread of infections associated with health care should be further developed and applied.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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