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PREVALENCE AND PROFILE OF SENSITIVITY OF *STAPHYLOCOCCUS AUREUS* ISOLATED FROM PATIENTS AND STAFF NURSING

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ABSTRACT
To determine the prevalence and susceptibility profile of strains of *Staphylococcus aureus* isolated from patients and members of the nursing staff. This is a descriptive, exploratory, in which samples were isolated from the 84 patients' and 22 employees' nasal vestibules and hands of an Intensive Care Unit (ICU) of a general hospital. The prevalence of *Staphyloccccus aureus* among patients was 54.76% and 59.04% among employees. Eighty one samples were isolated from 61 patients and 20 professionals. Among patients, 39 (63.93%) were resistant to oxacillin by the test of minimum inhibitory concentration and 42 (68.85%) by disk diffusion method. The *mecA* gene was found in 79.49%. Among professionals, 80% were resistant to oxacillin by both methods, and of these, all expressed the *mecA* gene. All samples were sensitive to vancomycin. We recommend the implementation of preventive measures for multiresistant sample as hand hygiene and nasal decontamination in the procedures of risk.

Keywords: *Staphylococcus aureus*. Intensive care units. Methicillin resistance.

INTRODUCTION

Staphylococcus aureus has occupied a prominent place in the etiology of nosocomial infections, among others, due to its high versatility in acquiring antimicrobial resistance [1,2,3]. From the 40s of the twentieth century, they emerged the earliest records of outbreaks of S. aureus resistant to penicillin in the hospital and in the 60s this resistance extended to β-lactam penicillin recognizing, then, at the end of that decade, the strains of Staphylococcus aureus resistant to methicillin (MRSA) as responsible for a pandemic [1.2].

The presence of multi-resistant microorganisms (MR) among them *S. aureus*, is among the major adverse events related to the care of patients in intensive care units (ICUs) [4]. Multi-resistance is defined as the condition in which a microorganism has resistance equal to or more than three classes of antimicrobial agents routinely used for its treatment [5].

One way of resistance is the intrinsic to oxacillin mediated by the *mecA* gene. The confirmation of this genetic resistance is possible by employing the technique of Polymerase Chain Reaction (PCR) [6,7,8]. However, other mechanisms may be involved, such as changes in membrane permeability, enzymatic degradation of antimicrobials and change in the active site [9].

The susceptibility can be determined by different methods including the method of diffusion in agar by the system of disk and dilution, in solid or liquid, which provide quantitative values of cut points that allow identifying the value of the Minimum Inhibitory Concentration - (MIC) [9]. This value is the lowest concentration of antimicrobial agent that prevents visible growth of a microorganism in sensitivity tests by the broth or agar dilution method [10].

In antimicrobial susceptibility tests for *S. aureus* the inclusion of oxacillin is recommended because it is a marker of resistance to other antimicrobial agents [11].

Current concerns in Brazil focus on early identification of MRSA targeting the monitoring of vancomycin resistance by strains of *S. aureus*. Vancomycin is one of the latest developed drugs for the treatment of MRSA infections [12].

In the 90s of the twentieth century, initially in Japan, the emergence of strains of *S. aureus*

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happens with reduced susceptibility to vancomycin and in 2002, in the United States, the first described case of resistance to *S. aureus* to vancomycin occurred [13]. *S. aureus* with intermediate resistance to vancomycin were also identified in Brazil in 2000, at a hospital in Rio de Janeiro [13].

In this context, the study of the identification of carriers of *S. aureus* and evaluation of the sensitivity profile of this micro-organism has gained prominence in programs to prevent nosocomial infection. This is because humans are the main reservoir of *S. aureus* and cross-transmission route takes through the nostrils onto the hands of health professionals and subsequently they reach the patient. Another possibility for spreading the micro-organism is the endogenous pathway, from the colonization of the nasopharynx [14,15].

Thus, the determination of the prevalence of MRSA entrainment is an important epidemiological strategy to propose measures to prevent, control, and also one of the most widely used criteria for the choice of therapy [8,15].

Based on these, this study aimed to determine the prevalence of carriers of *S. aureus* between patients and members of the nursing staff of the ICU of a general hospital and to identify the susceptibility profile of the samples.

METHODOLOGY

This is a quantitative, analytical exploratory descriptive study carried out in the period from March to June 2010, in the ICU of a hospital in the northern of Paraná state (Brazil), a center of reference for the care of patients who require intensive care in the area of the 18th Regional de Saúde - Health Regional; there are ten beds and approximately 66 admissions per month.

We tested the members of the nursing staff and the patients who were hospitalized during this period. The professionals included were those who consented to participate and had been working in the unit during the study period, totaling 22 individuals, i.e., all the nursing staff in the sector at that moment. There was not exclusion among members of the nursing staff. For inclusion of 84 patients in the study we requested the consent for those who were aware and for those unable to answer it, the consent form was read and explained to the responsible relative. We excluded those with trauma at the collection site, only one patient.

The material of participants' nasal vestibules and hands was collected using sterile swabs and transferred to a tube containing *Trypticase Soy Broth* (TSB) plus 6.5% NaCl. After 24 hours of growth in bacterial conservatory in an oven at 37 °C, it was plated on Petri plaques (90 x 15 mm) containing salty mannitol agar (Becton Dicksenson and Company, BD Diagnostic Systems, USA) plus 6.5 % NaCl [16].

After 24-48h of incubation at 37 °C, colonies suspected of being *S. aureus*, opaque, convex, creamy with color ranging from white to various shades of yellow, were subjected to Gram staining and to reading in immersion microscopy [16]. Those identified as Gram positive cocci were grouped in the form of bunches of grapes and transferred to tubes containing TSB supplemented with 6.5% NaCl.

After 6-12 hours of incubation, the samples were tested for coagulase production by the tube method [17], being used lyophilized rabbit plasma (Plasma clot-LB, Laborclin Products for Laboratory Ltda., Pinhais, Paraná, Brazil) with the readings achievements, in the times of 30 min, 4h, 12h and 24h. At all stages of seeding we used control samples - American Type Culture Collection (ATCC 25923) – for identification. After, the samples were stored in a medium of TSB supplemented with 20% glycerol and frozen at - 20 °C [17].

Susceptibility to antimicrobials commonly used in routine clinical, from isolated ones, was determined by disk diffusion method [9]. Thus, the antimicrobials used were: Oxacillin 1 μg, Cefoxitin 30 μg, Gentamicin 10 μg, Telithromycin 15 μg, Tetracycline 30 μg, Linezolid 30 μg, Penicillin G 10 μg, Azithromycin 15 μg, Clindamycin 2 μg, Trimethoprim/sulfamethoxazole 75 μg, Doxycycline 30 μg, Rifampicina 5 μg, Teicoplanin 30 μg, Ofloxacin 5 μg.

The samples resistant to oxacillin were evaluated by means of the agar dilution method for determination of minimum inhibitory concentration [10]. This method was included a vancomycin resistance test, because its use by disk diffusion method is not recommended. The presence of the *mecA* gene was assessed by polymerase chain reaction [18].

Data were tabulated in Microsoft Excel® version 2003 and described in tables of contingency. Some associations of interest were verified using the chi-square test, adopting a significance level of 5% (p <0,05).

Among the tested sites, the results were classified in five ways, according to the recovery of *S. aureus* on the hands, nasal vestibule, just on hands and only in the nasal vestibule.

The development of the study was in accordance with the ethical guidelines and was approved by the Ethics and Research (COPEP) of Universidade Estadual de Maringá (UEM) under the Opinion No. 395/2010.

RESULTS AND DISCUSSION

The statistical test used to compare the two percentages did not show difference in the occurrence of entrainment of *S.aureus* among members of nursing staff and patients.

Table 1 - Occurrence rate of entrainment of *S. aureus* among members of the nursing staff and patients in the Intensive Care Unit of a general hospital in northern of Paraná, 2010.

Variable	Nursing staff members	Patients	Total	Amount *p*
Entrainment of *S.aureus*				0,7160
Yes	13 (59,09%)	46 (54,76%)	59	
No	09 (40,91%)	38 (45,24%)	47	
Total	22 (100%)	84 (100%)	106	

The frequency of isolation of *S. aureus*, which are multiresistant to antimicrobials and commonly used in clinical routine, has increased considerably in recent decades, what allows comparison with other services.

The prevalence of entrainment of multidrug-resistant *S. aureus* among members of the nursing staff is diverse. For the group of professionals in this study, the prevalence of entrainment was similar to the rate of entrainment (60.9%) of 486 professionals surveyed in a hospital in the city of Curitiba [19]. However, other studies showed conflicting results. At the University Hospital in the city of Goiânia, where 268 professionals were involved, 227 (84.7%) were silting S. aureus [19]. On the other hand, at the Hospital Escola Público de Santo André the rate was 47.6% [19].

In the group of patients, the prevalence of S. aureus was found to be 54.76%, larger than a sample recorded in the ICU of the Hospital Oswaldo Cruz 37.7% [20].

Table 2. Distribution of the occurrence of *S. aureus* entrainment according to the body parts tested in an ICU of a general hospital in northern of Paraná, 2010.

Variables		Nursing staff members	Patients	Amount *p*
a.	**Hands**			0,6938
Yes		08 (61,54%)	31 (67,39%)	
No		05 (38,46%)	15 (32,61%)	
b.	**Nasal vestibles**			0,0569
Yes		12 (92,31%)	30 (65,22%)	
No		01 (07,69%)	16 (34,78%)	
c.	**Both**			0,1621
Yes		07 (53,85%)	15 (32,61%)	
No		06 (46,25%)	31 (67,39%)	
d.	**Only hands (a-c)**			0,0569
Yes		01 (07,69%)	16 (34,78%)	
No		12 (92,31%)	30 (65,22%)	
e.	**Only nostrils (b-c)**			0,6938
Yes		05 (38,46%)	15 (32,61%)	
No		08 (61,54%)	31 (67,39%)	

There was a percentage of occurrence of *Staphylococcus aureus* in the patients' hands 67.39% and 61.54% in the hands of the Nursing staff members, there is not statistical difference (p = 0.6938). When we analyzed the occurrence only in the hands, i. e., excluding participants

who had occurred at both parts, the group of patients had a percentage of 34.78% and the group of staff members 7.69%, although not statistically . However, it is noteworthy that the value of p approached 5% (p = 0.0569).

The results of the occurrence of *S. aureus* in the nasal vestibules show that the group of staff members presented the percentage of 92.31% and 65.22% of the patients group.

Regarding the concomitant presence of *S. aureus* on the hands and nasal vestibules between participants, the percentage of occurrence among the group of team members was 53.85% and 32.61% of the patients group.

It was observed that from the total number of patients of this study, more than half (65.22%) had samples of *S. aureus* isolated from the nasal vestibule, this condition may be a factor that facilitates the spread of micro-organisms and endogenous infection. However, the highest percentage (67.39%) of patients had samples of the micro-organism isolated from hands.

Patients who were hospitalized on beds previously occupied by patients colonized with *S. aureus* occasionally acquire this micro-organism, whether by the hand of professionals, who handle contaminated materials, or by direct contact with micro-organisms that stay in environment [21].

The lack of adherence to standard precaution measures by members of the nursing team, added to the stay in the hospital and to the patient contact, makes these professionals are subject to colonization by micro-organisms, placing them in the condition of patients and disseminators of infection [21], contributing to the occurrence of serious and often irreversible outbreak.

Regarding to members of the nursing staff who had *S. aureus*, 92.31% presented it in the nasal vestibules and 61.54% on hands. Although the literature has indicated that the professionals' hands are potential sources of transmission of *S. aureus* during the provision of care, the ecological niche of the *S. aureus* strains are the anterior nostrils, in which can be disseminated.

Although the nasal conduction of *S. aureus* is asymptomatic, it has great clinical and epidemiological importance, since it is a risk factor for endogenous infections, and the individual with nasal *S. aureus* can also act as a source of contamination for other individuals. Approximately 80% of invasive infections due to S. aureus strains are caused by spread from the nasopharynx of carriers [22].

Thus, especially in hospitals, the asymptomatic nasal host may be a patient, a visitor or a health professional that contributes to the transmission of bacteria by spreading air [23].

This research showed that the percentage of nursing staff members and patients were identified as carriers of *S. aureus* and may be playing an important role in the epidemiology of staphylococcal infection.

Table 3 - Distribution of antimicrobial resistance of *S. aureus* samples isolated from patients and members of the nursing team to antimicrobials commonly used in clinical routine in an intensive care unit of a general hospital in northern of Paraná, 2010.

Antibiotics	Samples from patients (n = 61)		Samples from the nursing staff members (n = 20)	
	N	%	n	%
Penicilin G	60	98	20	100
Cefoxitin	34	56	16	80
Oxacillin	42	69	16	80
Azithromycin	49	80	17	85
Clindamicina	45	74	17	85
Trimethoprim/Sulfamethoxazole	41	67	08	40
Telithromycin	41	67	16	80
Linezolid	07	11	04	20
Tetracycline	23	38	08	40
Doxycycline	10	16	04	20
Rifampicin	14	23	02	10
Gentamicin	41	67	15	75
Teicoplamin	0	0	0	0
Ciprofloxacin	35	57	15	75

Subtittle: n = numbers of resistant samples, % percentual.

We analyzed 81 samples, 20 were isolated from staff members and 61 from the patients. The disk diffusion test showed that the samples of *S. aureus* showed 100% of resistance to penicillin in the group of nursing staff members and 98% among patients, and high sensitivity to teicoplanin, linezolid, and rifampin. It was found also that the percentage of samples showed multiple resistance to antimicrobials, including oxacillin, for samples isolated from patients and staff members, respectively, they showed resistance rates of 69% and 80%, and gentamicin at 67 % and 75%.

There were similarity in the high microbial resistance between the the results obtained for oxacillin and cefoxitin among members of the nursing team, but this did not occur in patients.

It is noteworthy that since the beginning of the clinical use of penicillin, *S. aureus* started to develop resistance to the beta-lactam, by the beta-lactamase production which is capable of hydrolyzing the beta-lactam ring of the drug, making it inactive [11]. In 1944, only 6% of *S. aureus* were resistant to penicillin, whereas in 1950 this resistance has already reached the rate of 50% [12].

In 1960 scientists discovered a drug of the penicillin group called methicillin which was the first semi-synthetic penicillin not susceptible to the action of beta-lactamase. However, in the early 1970s, strains of *S. aureus* resistant to methicillin and oxacillin appeared [11].

It is noteworthy that the increase of infections by *Staphylococcus aureus* resistant to oxacillin (Orsa) was accompanied by the growth of resistance to various antibiotics, such as aminoglycosides, quinolones, macrolides and tetracyclines [24], however the current concern focuses on early identification of Orsa with vancomycin resistance, which is one of the latest developed drugs for the treatment of infections with Orsa [12]. The results of this study showed that *S. aureus*, in its totality, was sensitive to vancomycin.

The susceptibility to oxacillin, analyzed through the CIM test, showed that 81 samples of isolated *S. aureus*, 55 (68%) were resistant, with CIM ranging from 4 mg/mL to 64 mg/mL. As for vancomycin, it was not found a resistant sample.

Even through the CIM test it was observed that 61samples from patients, 39 (63.93%) were resistant to methicillin and 42 (68.85%) were also resistant by disk diffusion method. Among the 20 samples of the nursing staff members, 16 (80%) were resistant to oxacillin by both methods.

It was found that three samples of patients were resistant to CIM and susceptible by disk diffusion and six samples were sensitive to CIM and resistant to the disk method, demonstrating the need to compare the two methods and to correlate them according to that the CLSI recommends.

The multi-resistance, including the resistance to methicillin, as observed in this study may cause difficulties with respect to the therapy used to treat infections caused by this micro-organism [14].

All samples identified as oxacillin resistant by CIM method were evaluated for the presence of the mecA gene. It was observed that 31 (79%) of 39 isolated samples from patients and 16 (100%) of the 16 from the nursing staff members carried the gene.

Antimicrobial resistance in *S. aureus* can be encoded chromosomally or plasmid mediated. The hyper-lactamase is part of the mechanisms of resistance to oxacillin, presence of a penicillin binding protein (PBP) modified called PBP2a and changes in binding capacity of PBPs [13]. This altered protein is encoded by a chromosomal gene called mecA, which is responsible for the intrinsic resistance of staphylococci to oxacillin, and all beta-lactam antibiotics [6,7,11]. There is still the resistance mediated by other mechanisms, such as alterations in membrane permeability, alteration of the site of antimicrobial action, efflux pump and enzymatic mechanism [9]. These findings justify that given sample can be sturdy and do not adduce the mecA gene.

The result of Orsa prevalence in the patients (69%) agrees with studies at other institutions in Brazil have shown the amounts from 40 to 80% of ICU patients [25]. However, the percentage of oxacillin resistance for members of the nursing staff (80%) differs of the profile of isolated samples from professionals in a hospital in Curitiba, in which the prevalence of Orsa was 48% among workers [26].

It was verified a high percentage of multi-resistant samples from the nursing staff members

and patients, which may explain the difficulty therapy. The recovery Orsa of these sources implies that, even when adopting the use of antibiotics, they have not found a way to eliminate the multi-resistant strains that remain viable.

Therapeutic options for the treatment of infections caused by Orsa have been reduced every day, what is limited to the use of broad-spectrum antibiotics, which can promote microbial resistance, confirming the ineffectiveness of these therapeutic drugs, as noted by other authors [24].

The high resistance to beta-lactam antibiotics, aminoglycosides and macrolides can be justified because of the isolated samples from patients and nursing staff of ICU, the sector which concentrates the major risk factors for acquisition of multi-resistant micro-organisms, among which we can include the severity of the clinical case, length of stay, use of invasive procedures and indiscriminate antibiotic [20].

CONCLUSION

The results of full sensitivity to vancomycin and teicoplanin were expected, because in Brazil there are few studies that have found resistance to these antibiotics, or that has observed the correlation between oxacillin resistance to vancomycin and teicoplanin.

The percentage of resistant samples to methicillin, vancomycin and teicoplanin is consistent with other studies in Brazil [13]. And almost all oxacillin resistance found in samples of *S. aureus* has genetic origin, confirmed by the presence of the mecA gene.

The knowledge of the epidemiological profile and sensitivity to ICU can contribute in improving the quality of care, considering that nursing actions are aimed at reducing the spread of multi-resistant microorganisms.

Considering the high percentage of nasal carriers of *S. aureus* and the found multi-resistance profile, it is suggested that studies are developed to deploy the nasal use of Mupirocin as prevention and the spread of *S. aureus* control. Other previous studies showed a significant reduction in the isolation of *S. aureus* after the use of Mupirocin, with rates ranging from 75 to 91% [14, 27, 28]. However, the routine use of decolonization in patients with MRSA, but who are not considered as high risk, is not scientifically validated, and the effectiveness of this strategy for a long time is not clear yet, there is a need to evaluate the cost-benefit and the possibility of development of Mupirocin resistance [27, 29].

PREVALÊNCIA E PERFIL DE SENSIBILIDADE DE *STAPHYLOCOCCUS AUREUS* ISOLADOS EM PACIENTES E EQUIPE DE ENFERMAGEM

RESUMO

Determinar a prevalência e o perfil de susceptibilidade de amostras de *Staphylococcus aureus* isoladas em pacientes e membros da equipe de enfermagem. Estudo do tipo exploratório, descritivo analítico onde foram isoladas amostras dos vestíbulos nasais e das mãos de 84 pacientes e 22 funcionários de uma Unidade de Terapia Intensiva (UTI) de um hospital geral. A prevalência de *Staphylococcus aureus* entre os pacientes foi 54,76% e entre os funcionários 59,04%. Foram isoladas 81 amostras, 61 dos pacientes e 20 dos profissionais. Entre os pacientes, 39 (63,93%) foram resistentes à oxacilina pelo teste de concentração inibitória mínima e 42 (68,85%) pelo método de disco difusão. O gene *mecA* foi encontrado em 79,49%. Entre os profissionais, 80% apresentaram resistência à oxacilina pelos dois métodos, e dessas, todas expressaram o gene *mecA*. Todas as amostras foram sensíveis à vancomicina. Recomenda-se a implementação de medidas de prevenção para amostras multirresistentes como a higienização das mãos e descontaminação nasal na iminência de procedimentos de risco.

Palavras-chave: *Staphylococcus aureus.* Unidades de Terapia Intensiva. Resistência à meticilina.

LA PREVALENCIA Y EL PERFIL DE SENSIBILIDAD DE *STAPHYLOCOCCUS AUREUS* AISLADOS EN PACIENTES Y EQUIPO DE ENFERMERÍA

RESUMEN

Determinar la prevalencia y el perfil de susceptibilidad de muestras de *Staphylococcus aureus* aisladas en pacientes y miembros del equipo de enfermería. Estudio del tipo exploratorio, descriptivo analítico, en que fueron aisladas muestras de los vestíbulos nasales y de las manos de 84 pacientes y 22 empleados de una Unidad de Cuidados Intensivos (UCI) de un hospital general. La prevalencia de *Staphylococcus aureus* entre los pacientes fue de 54,76% y 59,04% entre los empleados. Fueron aisladas 81 muestras, 61 de los pacientes y 20 de los

profesionales. Entre los pacientes, 39 (63,93%) fueron resistentes a la oxacilina por la prueba de concentración mínima inhibitoria y 42 (68,85%) por el método de difusión en disco. El gen *mec*A se encontró en 79,49%. Entre los profesionales, 80% presentaron resistencia a la oxacilina por ambos métodos, y de éstas, todas expresaron el gen *mec*A. Todas las muestras fueron sensibles a la vancomicina. Se recomienda la implementación de medidas de prevención para muestras multirresistentes como la higienización de las manos y descontaminación nasal en la inminencia de procedimientos de riesgo.

Palabras clave: *Staphylococcus aureus*. Unidades de Cuidados Intensivos. Resistencia a la meticilina.

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