Original Article

Phenotypic and genotypic profile of Staphylococcus aureus isolated in nursing students, 2008*

Perfil fenotípico e genotípico de Staphylococcus aureus isolados de estudantes de enfermagem, 2008

Perfil fenotípico y genotípico del Staphylococcus aureus aislados de estudiantes de enfermería, 2008

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ABSTRACT

Objective: To investigate the prevalence of nasal entrainment, phenotypic and genotypic profile of *Staphylococcus aureus*, as isolated from nursing students. **Methods:** A cross-sectional population of 101 students enrolled in the first three grades of the undergraduate nursing course in 2008. *Staphylococcus aureus* was isolated from biological material obtained from the swab through the nasal vestibules. Susceptibility to oxacillin and vancomycin was determined by minimum inhibitory concentration test. The mecA gene was identified by testing the polymerase chain reaction. **Results:** There was a 90.1% positive finding of *Staphylococcus aureus*. The frequency of oxacillin resistance was 9.8%; all samples were sensitive to vancomycin. The eight strains resistant to oxacillin carried the mecA gene. **Conclusion:** The prevalence of *Staphylococcus aureus* was high. Oxacillin resistance was significant, but all strains were sensitive to vancomycin. Isolates resistant to oxacillin carried the mecA gene.

Keywords: *Staphylococcus aureus*; Drug resistance; Colonization

RESUMO

Objetivo: Verificar a prevalência de carreamento nasal, perfil fenotípico e genotípico de *S. aureus* isolados de estudantes de enfermagem. **Métodos:** Estudo transversal, com população composta por 101 alunos, cursando as três primeiras séries do curso de graduação em Enfermagem no ano de 2008. *S. aureus* foi isolado de material biológico obtido dos vestíbulos nasais através de swab. A susceptibilidade à oxacilina e vancomicina foi determinada pelo teste de concentração inibitória mínima. A presença do gene *MecA* foi determinada pelo teste de reação em cadeia da polimerase. **Resultados:** Verificou-se 90,1% de positividade para *S. aureus.* A frequência de resistência à oxacilina foi de 9,8% e todas as amostras foram sensíveis à vancomicina. A oito amostras resistentes À oxacilina apresentaram o gene *MecA*. **Conclusão:** A prevalência foi elevada. A resistência à oxacilna foi expressiva e todas as amostras foram sensíveis à vancomicina. As amostras resistentes à oxacilina carreavam o gene *MecA.*

Descritores: *Staphylococcus aureus*; Resistência; Colonização

RESUMEN

Objetivo: Verificar la prevalencia de transporte nasal, perfil fenotípico y genotípico de *S. aureus* aislados de estudiantes de enfermería. **Métodos:** Estudio transversal, con población compuesta por 101 alumnos, cursando las tres primeras series del Pregrado en Enfermería en el año 2008. El *S. aureus* fue aislado del material biológico obtenido de los vestíbulos nasales a través de swab. La susceptibilidad a la oxacilina y vancomicina fue determinada por el test de concentración inhibitoria mínima. La presencia del gen *MecA* fue determinada por el test de reacción en cadena de la polimerasa. **Resultados:** Se verificó el 90,1% de positividad para el *S. aureus.* La frecuencia de resistencia a la oxacilina fue de 9,8% y todas las muestras fueron sensibles a la vancomicina. Las ocho muestras resistentes a la oxacilina presentaron el gen *MecA*. **Conclusión:** La prevalencia fue elevada. La resistencia a la oxacilina fue expresiva y todas las muestras fueron sensibles a la vancomicina. Las muestras resistentes a la oxacilina transportaban el gen *MecA.*

Descriptores: *Staphylococcus aureus*; Resistência; Colonización

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INTRODUCTION

Staphylococcus aureus (*S. aureus*) is an important pathogen involved in the etiology of human infections, and it is found as normal flora in nasal passages, groins, and armpits[1].

Nasal carriage of *S. aureus* is the main responsible for the colonization of the skin and it becomes a reason for concern when we consider the prevalence of these micro-organisms in the population and among hospital workers[2-3]. Identifying nasal carriers of *S. aureus* is important to understand the epidemiology of the infections[4], to assess the risk for acquisition and transmission[5-6].

The prevalence of asymptomatic patients with *S. aureus* among individuals in the university community is 40 %[7], with an expressive percentage of multi-drug resistant strains, especially among those people that are part of the hospital staff[3]. The colonization of workers in a hospital environment can range from 30% to 70%, and it can reach up to 90%, depending on the environment conditions of the patients cared for, the use of antimicrobial drugs, and the hospital structure. In adults with no connection to the hospital environment, the variation should be limited to 20% and 50 %[8].

Nursing students at Universidade Estadual de Maringá start their education in extra hospital environments, and then they have practical training in hospitals and health centers. This environmental change during academic education could lead to a change in the frequency of nasal carriage of *S. Aureus* in these individuals, as well as a change in the phenotypic and genotypic profile of the carried strains.

Over the years, nosocomial *S aureus* has become resistant to most drugs usually employed in the clinical practice, and currently a significant percentage of these strains of community origin has also become multi-drug resistant[9]. Oxacillin is an important marker of resistance to other antimicrobial agents and several studies have demonstrated the increase in the resistance frequency to this drum among *S. Aureus* strains, both from community or hospital source[9-10].

Currently, in Brazil, the use of vancomycin is one of the last lines of treatment for staphylococcal infection, and studies demonstrating the isolation of resistant strains are still scarce. However, this resistance is widespread in several countries as reported by some studies[3,9,11] and the epidemiological surveillance is important to detect resistance in our environment.

As of the 90's, reports about the infections associated to oxacillin and vancomycin resistance have increased. The Clinical and Laboratory Standards Institute (CLSI) recommends that these resistances are detected through the Minimum Inhibitory Concentration (MIC) test[12]. The MIC is a standard method for quantitative assessment of the bacterial resistance to antimicrobials[9].

The production of semisynthetic penicillin in 1959 led to the creation of methicillin that has a beta-lactam ring that interacts with the penicillin binding proteins of the membrane, preventing the complete formation of the peptidoglycan layer of bacterial cell wall. The gene *mecA,* responsible for the resistance to methicillin, changes these membrane proteins, encoding a new binding protein called PBP2a with low affinity for beta-lactam antibiotics, and two regulatory genes *mecI* and *mecRI,* forming the complex staphylococcal chromosome *mec* (*SCCmec*). The gene that encodes the bacterial resistance to drugs can be found in the chromosome or in plasmids, and the plasmid DNA is more easily transmitted by conjugation[13]. Identifying the type of resistance, and the way it is transmitted is an important instrument in the prevention and control of staphylococcal infection[1,13].

The objective of the present study was to check the prevalence of *S. aureus* nasal carriage among nursing students at a State University in Paraná and the genotypic and phenotypic profile of isolated samples.

METHODS

Cross-sectional study carried out from May and December 2008; the population involved was formed by 101 nursing students at Universidade Estadual de Maringá (UEM), distributed as follows: 40 students in the 1st year of the undergraduate course, 27 in the 2nd year, and 24 in the 3rd year.

All nursing students that agreed to take part in the research and that, at the time of collection, did not present clinical signs and symptoms of infection took part in the study. All norms from the Resolution 196/96 of the National Health Council[14], of the Ministry of Health that monitors the studies with human being and were approved by the Standing Committee on Ethics in Research involving humans (COPEP) from UEM under the Opinion # 536/2008.

Students were explained about the objectives of the studies and questions were settled before giving the Statement Consent (TCLE) and filling in a structured questionnaire that was numbered to make data tabulation easier.

S. aureus were isolated based on the biological materials of nasal vestibules, using sterile *swab* that was later stored in tubes with tryptic soy broth (TSB) with 6.5% sodium chloride (NaCl). The initial bacterial culture was in an incubator at 37 ° C overnight.

Next, the growing broth was sown in the surface of the Petri dish (90X15mm), containing Mannitol Salt Agar (MSA), (Becton Dicksenson and Company, BD Diagnostic Systems, USA), with or without oxacillin, in

a 4μg/ml concentration and incubated again for 24-48 hours at 37° C. At this seeding stage, control samples of *S. aureus* of the *American Type Culture Collection* (ATCC) have been used, one sample was sensitive to oxacillin (ATCC 25923) and one was resistant (ATCC 43300).

Colonies that were suspected to be *S. aureus* were tested with Gram stain[15] and those identified as Gram-positive cocci grouped in clusters were transferred to TSB, adding 6.5% of NaCl. After 6 hours of incubation, Coagulase test was performed in tube employing lyophilized rabbit plasma (LB Plasma-clot, Laborclin laboratory products Ltda, Pinhais Paraná, Brazil). S. aureus was identified after a clot was formed and the readings were carried out in 30 min., 4 hours, and 24 hours[16].

After identification, *S. aureus* samples were tested for Minimum Inhibitory Concentration (MIC) in solid medium for oxacillin and vancomycin, and the Disk Diffusion Test was used for the following antimicrobials: penicillin G, oxacillin, cefoxitin, erythromycin, clindamycin, trimethoprim sulfamethoxazole, vancomycin, telithromycin, linezolid, tetracycline, doxycycline, rifampin, gentamicin, ofloxacin and teicoplanin, according to the CLSI protocol[12].

For the MIC, different concentrations of antimicrobials have been used, starting with 0.06 μg/ml up to 256 μg/ml for oxacillin, and 0.06 μg/ml up to 16 μg/ml for vancomycin. The control sample ATCC 29213 has been used as negative control for both antimicrobials, and ATCC 33591 was employed for positive control to oxacillin.

Bacterial DNA was extracted through the methodology proposed by researchers[17], with some changes. The samples, after plated on Miller Hinton agar, were transferred to eppendorf containing Tris-EDTA (TE) and centrifuged at 8000 rpm. The supernatant was discarded and the pellet suspended in a 600 μl solution of Cetyl Trimethylammonium Bromide (CTAB) and 40 μl chloroform isoamyl alcohol (CIA), and then they were heated in a water bath at 65oC for 30 min. Later, 800 μl of CIA was added and centrifuged at 12000 rpm for 5 min. About 600 μl of the supernatant was transferred to an eppendorf tube; the same volume of cold isopropanol was added and left on a freezer at -20oC overnight for DNA precipitation. Next, the samples were centrifuged at 14000 rpm at 4oC for 20 minutes, eliminating the supernatant. We have added 200 μl of ethanol 70% to the pellet, centrifuged at 14000 rpm, and then the supernatant was discarded. After drying, the DNA obtained was diluted in 200 μl of TE and stored in a freezer at -20o C.

The *S. aureus* samples identified as oxacillin resistant were assessed by the technique of polymerase chain reaction (PCR) to identify the gene *Mec A,* according to the methodology proposed by Vannuffel(18), with some changes. For the reaction with 25 μl, reagents used were, 12.1 μl of water Milli Q sterile, 2.5 μl of Tampon 10X, 0.4 μl of Taq polymerase 5U / L, 1.5 μl of 200 M dNTP, 1.5 μl magnesium chloride, 1.5 μl of each primer at 50 pmol, and 3.0 μl DNA. The reactions were performed with thermal cycler with the following cycling: 95 ° C for 10 minutes, 30 cycles: 95 ° C for 30 seconds, 52 ° C for 30 seconds. 72 ° C for 1 minute. and a final cycle at 72 ° C for 10 minutes. The *S. aureus* ATCC 33591 strain was employed as positive control in the PCR reaction. Electrophoresis of amplified products was performed in agarose gel 1.5% stained with ethidium bromide for 1 hour at 90 volts. The detection of a fragment with 154 base pairs (BP) confirmed the presence of the gene *MecA.*

RESULTS

Among the 101 volunteers of the sample, there was a 90.1% (91/101) of positivity to *S. aureus*. The age of individuals ranged from 17 and 33 years, with 59.3% between 17 and 20 years.

All samples were sensitive to vancomycin, and the MIC ranged from 0.5μg/ml and 2μg/ml. As for oxacillin, eight samples were resistant and among them, seven presented a MIC in relation to 2 μg/ml vancomycin. Data from Table 1 presented accumulative distribution of MIC for oxacillin in the different years of the undergraduate course.

As for the antibiogram, an increase in bacterial resistance was observed over the years of the undergraduate course, which can be observed in the data of Table 2. The samples resistant to oxacillin

Table 1 – Accumulative distribution of the minimal inhibitory concentration for oxacillin, observed among the different years of the Nursing Undergraduate Program at Universidade Estadual de Maringá. Maringá - PR, 2008.

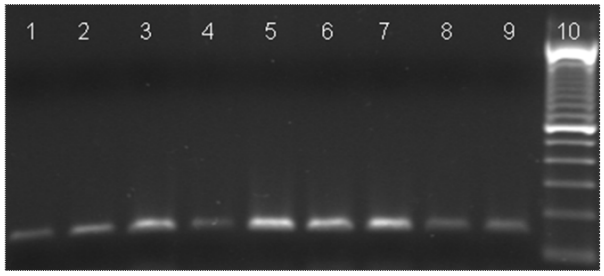
μg/ml	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	CIM_{50}	CIM_{90}	Total
1st year	10	18	5	3	2	0	0	0	1	0	0	1	0.25	1	40
2nd year	5	15	1	4	1	1	0	0	0	0	0	0	0.25	1	27
3rd year	0	11	6	2	0	1	1	0	0	3	0	0	0.50	64	24
Total	15	44	12	9	3	2	1	0	1	3	0	1	0.25	2	91

Table 2 – Distribution of the antimicrobial resistance in the different years of the nursing undergraduate course at Universidade Estadual de Maringá. Maringá - PR, 2008.

Antimicrobial	1st year	%	2nd year	%	3rd year	%	Total	
								%
Penicillin	33/40	82.2	26/27	92.6	24/24	91.7	80/91	87.9
Oxacillin	4/40	10.0	2/27	7.4	6/24	25.0	12/91	13.2
Cefoxitin	25/40	62.2	8/27	29.6	6/24	25.0	39/91	42.9
Erythromycin	26/40	65.0	26/27	96.3	23/24	95.8	75/91	82.4
Clindamycin	15/40	37.5	21/27	77.8	19/24	79.2	55/91	60.4
Trimethoprim-sulfamethoxazole	24/40	60.0	23/27	85.2	18/24	75.0	65/91	71.4
Vancomycin	-	-	-	-	-	-	-	-
Telithromycin	37/40	92.5	26/27	96.3	23/24	95.8	86/91	94.5
Linezolid	-		-	-	-	-	-	-
Tetracycline	22/40	55.0	20/27	74.1	18/24	75.0	60/91	65.9
Doxycycline	16/40	40.0	15/27	55.6	12/24	50.0	43/91	47.3
Rifampicin	17/40	42.5	13/27	48.1	11/24	45.8	41/91	45.1
Gentamicin	4/40	10.0	15/27	55.6	13/24	54.2	32/91	35.2
Ofloxacin	25/40	62.5	18/27	66.7	13/24	54.2	56/91	61.5
Teicoplanin	2/40	2.0	0	0	0	0	2/91	2.2

presented 100% (8/8) resistance to penicillin, to trimethoprim-sulfamethoxazole, and tetracycline, 87.5% (7/8) resistance to erythromycin and telithromycin, and 75% (6/8) resistance to clindamycin and doxycycline.

The samples resistant to oxacillin by the MIC method underwent PCR technique, because they all presented the gene *Mec A,* as showed by the data in Picture 1.



Channel 10: molecular weight marker 100pb DNA ladder; Channel 9: positive control; Channels 1, 2, 3, 4, 5, 6,7 and 8: S. aureus isolated from nursing students at the Nursing Graduate Program UEM in 2008.

Picture 1 – Results of the Polymerase Chain Reaction test to determine *MecA* gene in *S. aureus* samples isolated from nursing graduate students, 2008.

DISCUSSION

Nursing students present a characteristic profile with mostly young females. Nursing students in public universities with ages ranging from 17 to 19 years represent 48% of the students[19].

S. aureus is present in the skin and mucosal of human beings, especially in the nasal cavity. Individuals that carry S. *aureus* and do not present symptoms are usually known as "healthy carriers" and they are considered as one of the main sources of transmission of the infection, both nosocomial and community(2,6,20). In the case of asymptomatic carriers, especially students during the hospital academic practice, it is difficult to apply measures for control and prevention because of the physical proximity between the care provider and the patient[2].

The prevalence of nasal carriage of S. *aureus* among the 101 nursing students investigated in the present study was 90.1% (91/101), a high prevalence. Individuals that work in a hospital environment present a 30% and 70% prevalence, and they can reach up to 90 %[8]. The use of TSB supplemented with NaCl for the initial growth increases the isolation of S. aureus, as already demonstrated by other studies[4,21-23].

The distribution of the frequency of nasal carriage of *S. aureus*, according to the periodicity of the course, showed that in the first year 100% (40/40) were carriers; in the 2nd year, 84.4% (27/32) and in the 3rd year, 82.8% (24/29). The carriage rate among students in the 1st year of the course compared to the other should be smaller since, in addition to being from the community, they do not have training in hospitals and basic health care units, a result that was not confirmed.

Among the 91 isolated *S. aureus* samples, 8 (8.8%) were resistant to oxacillin, and the bacterial growth was between 4 μg/m and 256 μg/. This percentage is expressive for asymptomatic individuals, because oxacillin is an important resistance marker for other antimicrobials, such as aminoglycosides, macrolides, chloramphenicol, tetracycline and fluoroquinolones[24]. Important oxacillin resistance rates among asymptomatic individuals of the community have also been observed by some authors[1,25].

All samples assessed in the present study were sensitive to vancomycin. Vancomycin intermediate *S. aureus* (VISA) appeared in 1996, in Japan and, in 2002, in the United States of America the first vancomycin resistant S. Aureus (VRSA) was identified[3,9]. In Brazil, in 2000,

the first resistant strain to vancomycin was found in a Burn Center in Rio de Janeiro[3] however, this spread has not occurred in our environment and it was restricted to isolate cases.

The prevention measures for acquisition of S. aureus infection include nasal decontamination. A topic bacterial agent has been used to eradicate nasal cavities, armpits, groins and in the hands of patients and health workers. Through this measure, we could reduce the infection rates among patients that will undergo surgeries[26].

CONCLUSION

The presence of S. aureus strains resistant to oxacillin in healthy or asymptomatic people is a threat and an important source of micro-organism; when we consider that these students will be in the work environment very briefly.

The objective of the present study was to verify the prevalence of *S. aureus* nasal carriage among nursing students, contributing to the epidemiology of this micro-organism, and giving professionals the opportunity to prevent transmission and to reflect upon the nasal decontamination technique.

The results have demonstrated a high prevalence of S. aureus among students and an expressive prevalence of oxacillin. Nursing students' knowledge on the bacterial resistance provides patients, the service and professionals, the necessary information to build prevention strategies.

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