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EXPERIMENTAL WORK

Antimicrobial Susceptibility of *Staphylococcus aureus* isolated in Nursing and Medical students

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Keywords

Staphylococcus aureus; Methicillin-Resistant *Staphylococcus aureus*; Oxacillin; Mupirocin; Vancomycin.

Abstract

This study aims to evaluate the occurrence of asymptomatic carriers and the antimicrobial susceptibility profile to mupirocin, oxacillin and vancomycin of *Staphylococcus aureus* isolated in undergraduate students from the first and last period of the Nursing and Medicine courses at EMESCAM. The methods used were nasal swab specimens collected in 147 academics, seeded in hypertonic mannitol agar and biochemical tests were performed to identify S. aureus. Antimicrobial susceptibility testing was performed using the disc-diffusion methodology and the susceptibility of vancomycin was confirmed by the Vitek 2® automated method. The results indicated that of the 147 samples collected, 61 (41.5%) were positive for S. aureus. Among Nursing students, 11 participants (34.4%) were carriers of S. aureus and no MRSA strain was found. Among medical students, 50 (43.5%) were positive, with 6.6% of MRSA lines. No resistance to mupirocin or vancomycin was found in the studied samples.

The research concluded that there was no association between colonization by S. aureus and periods of Nursing and Medicine courses, which indicated an increase in the estimated prevalence. The colonization by MRSA found among students of the first and last periods of Medicine and the profile of antimicrobial susceptibility suggest the possibility of the strains found to be CA-MRSA.

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INTRODUCTION

Staphylococcus aureus (S. aureus) is a Gram positive coccus, colonizer of the skin, perineum and mucosa, such as oral and nasopharyngeal[1]. As the result of its high virulence, it has the potential to trigger several clinical manifestations such as skin and soft tissue infections, infective endocarditis and bacteremia[2]. Regardless of this, there is a high prevalence of asymptomatic carriers, which are a source of dissemination and, in the case of health workers, a means of transporting nosocomial lines[3]. In spite of the fact that it is asymptomatic, some factors may facilitate the installation of an infection, such as depression of the individual's immune response, for example, patients with retrovirus and undergoing more aggressive treatment or invasive medical procedures that open access routes to the microorganismo[4,5].

Bacteria of the genus *Staphylococcus* are ubiquitous[6] and suited to surviving in a hospital environment. They are capable to remain for years in objects such as clothing, textiles and plastics typically used[7], and also in disposable containers of sterile materials such as gauzes and gloves. In such manner, contamination and consequent colonization may occur both after the contact with a carrier as well as with a contaminated object[8].

Some studies related to decolonization of health professionals report great difficulty in eradicating nasal colonization, while subungual decolonization is relatively simple. These data point out that there is a greater possibility of subungual colonization being transient. Nonetheless, nasal colonization has a more stable characteristic[9,10].

In the United States, colonization can reach 89.4 million people, of whom 0.8% are methicillin-resistant *Staphylococcus aureus* (MRSA)[11]. In some Brazilian studies, the rate of S. aureus carriers is around 37%, with 3% MRSA[12].

Alexander Flemming discovered penicillin in the 1920s. This discovery was a milestone in the history of the treatment of infectious diseases. In 1941, the first clinical trial was carried out with this antibiotic, a beta-lactam, determining effective treatment against staphylococcal infections. Nevertheless, a few years later, due to the indiscriminate use, the first strains resistant to the antibiotic were selected. These bacteria were able to hydrolyze the beta-lactam ring of penicillin by means of the enzyme beta-lactamase, becoming it inactive. With the purpose of solving this problem, in the same year a semi-synthetic betalactam was developed, methicillin, which, despite having only intravenous presentation and being related to several cases of interstitial nephritis, had its use recommended. In 1961, the first case of resistance was reported in England and these strains became known as MRSA[13,14]. In Brazil, methicillin was subsequently replaced by a congener, oxacillin.

Beta-lactams bind to proteins which participate in cell wall synthesis, known as penicillin binding proteins (PBPs), which prevent cell wall formation and result in bacterial lysis. The mechanism of action of resistance to methicillin is related to the development of an additional PBP (PBP 2a), with functional action and without affinity for beta-lactam antibiotics. The coding of the new PBPs is related to the acquisition, mainly of the mecA gene, which is part

of a mobile genetic element detected in strains isolated from MRSA. This gene is an integral part of a genomic element called the staphylococcal chromosomal cassette *mec* (SCCmec) [16].

Infections caused by hospital-associated methicillin-resistant *Staphylococcus aureus* (HA-MRSA) are restricted to this environment. The strains that cause infection in individuals with no risk factor for nosocomial infection are known as community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA). Unlike HA-MRSA that presents the mobile genetic element SCC*mec* of types I, II and III, CA-MRSA presents the type IV and type V SCC*mec*. The type IV and V chromosome cassettes are smaller than the other types and do not have coupled genes which encode resistance to other non-beta-lactam antibiotics. Thus, CA-MRSA is generally susceptible to most non-beta-lactam antibiotics[17]. However, the community clone has already been identified in the hospital environment[18,19].

A significant increase in MRSA infections in populations without risk factors occurred in the mid-1990s. A study by the Centers for Disease Control and Prevention (CDC) estimated that in 2005 approximately 1,300,000 infections were caused by CA-MRSA in the United States of America, of which 7% were considered invasive, leading to an incidence of 31.8 cases per 100,000 inhabitants[20].

Vancomycin is a glycopeptide antimicrobial agent recommended as a treatment for MRSA isolates, sensitive to the minimum inhibitory concentration (MIC) susceptibility profile established by the *Clinical and Laboratory Standards Institute* (CLSI) [21].

Mupirocin is a topical antimicrobial agent, initially isolated from *Pseudomonas fluorescens*. It is used in the treatment of skin infections and postoperative wounds, as well as in the decolonization of the nasal mucosa by *S. aureus* strains[22].

Taking into account the high incidence of S. aureus carriers, the various mechanisms of virulence of this pathogen and the progressive submission to the specific influences of the hospital environment in which health professionals are inserted, it is important to investigate the estimated prevalence of asymptomatic carriers of this bacterium, among Nursing and Medical School undergraduate students of the Santa Casa de Misericórdia School of Science (EMESCAM). Hence, this research intends to contribute to clinical, educational and administrative strategies, ensuring good practices of infection control during the provision of assistance to patients and reduction of cases of infections by this agent.

OBJECTIVES

General

To evaluate the estimated prevalence and antimicrobial susceptibility profile of *S. aureus* strains isolated from the nasal cavity of undergraduate students from the first and last periods of the Nursing and Medical School at EMESCAM.

Especifics

To analyze the isolated strains with resptect to the profile of antimicrobial susceptibility to oxacillin, mupirocin and vancomycin.

METHODOLOGY

Study Type

This is an observational, transversal, explanatory and quantitative study, based on data collected by samples of the right nasal vestibule from Nursing and Medical undergraduate students from EMESCAM.

Location and Period

This study was carried out with samples obtained from EMESCAM and Santa

Casa de Misericórdia Hospital of Vitória (HSCMV), taking into account the nature of the activity performed by the students. There was also the contribution of a private microbiological analysis laboratory. The work was carried out from September 2014 to November 2015.

Sample

A total of 147 students enrolled in the first and last periods of EMESCAM's Nursing and Medical courses were subjects to the present study.

Inclusion Criteria

The inclusion criterion consisted in selecting only the first and last period students from EMESCAM's Nursing and Medicine courses through the signing of the Informed Consent Term (ICT).

Exclusion Criteria

Students in the first and last period of EMESCAM Nursing and Medical majors who previously presented some pathology related to *S. aureus* and / or were using antimicrobials which could alter the susceptibility profile.

Statistical Analysis

At first, the data were analyzed by means of descriptive statistics techniques, with numerical, tabular and graphical synthesis of the information collected. All inferential statistics were calculated using significance level of 5%. The association between qualitative variables was performed by chi-square test or Fisher's exact test, if the expected values were less than 5. Data analysis was analyzed on SPSS software version 23.

Ethical Issues

The project was filed in the Brazil Platform and approved on 05/16/2014 by the Research Ethics Committee (CEP) with EMESCAM Human Subjects, according to the following number of the certificate 038353/2014 and CAAE 31116213.4.0000.5065.

The researchers declare that there was no conflict of interest in carrying out this research, which complied with all the terms contained in the Resolution 466/12 of the National Health Council / Ministry of Health and all other complementary resolutions.

The research group was committed to ensuring privacy and confidentiality of information obtained and used for the development of this research. The results obtained in the development of this work were used only to reach the stablished objectives of this research, and were not used for other researches without the consent of the CEP.

Collection Of Biological Material And Incubation

The materials used in the collection were appropriatel identified with the student's code, date, major and period of each volunteer. The clinical specimen was collected from the mucosa of the right nasal vestibule using sterile swab and subsequently embedded in *Stuart*®[23] transport medium. The samples were transported to the Laboratory of Microbiology of EMESCAM, followed by sowing on hypertonic mannitol agar and incubation at 37ºC for 24-48 hours[23].

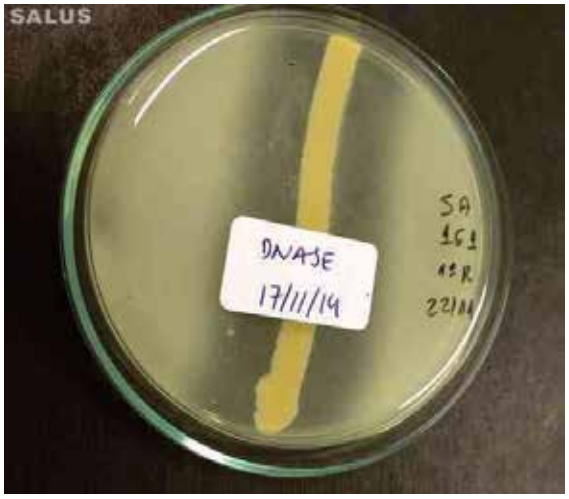
Analysis of Bacteriological Reading and Identification

After sowing on hypertonic mannitol agar and adequate incubation, the presence of colonies with characteristic growth and turning color of the culture medium to yellow were evaluated, characterizing the presence of S. aureus23 (Figure 1). Gram stain and proof of DNA assimilation (DNAse enzyme production) 23 were performed as confirmatory tests for presumptive identification of S. aureus (Figure 2).

Figure 1 – Characteristic colonies of the species *Staphylococcus aureus* in agar culture medium mannitol

Source: Own Authorship.

Figure 2 – DNA assimilation test (DNAse enzyme production)

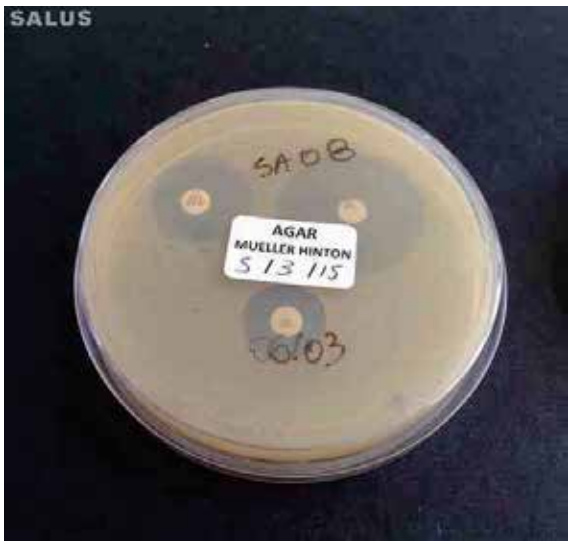


Source: Own Authorship.

Evaluation of the Antimicrobial Susceptibility Profile

The antimicrobial susceptibility assessment of *S. aureus* was carried out by the antimicrobial susceptibility test (TSA) by the diffusion disc test method[24] with cefoxitin, mupirocin and vancomycin disks (Figure 3). In the study, cefoxitin discs were used to predict resistance to oxacillin, as it is a very sensitive and specific marker of resistance mediated by mecA25[26]. Oxacillin may also be used for this purpose, but it has a weak association with the presence of *MecA* gene. The same method is used for the diffusion disc test, but only cefoxitin is tested for methicillin resistance. Furthermore, cefoxitin disc tests are at least comparable in accuracy to oxacillin disc tests, but the former is generaly easier to read, because of the greater halo and the possibility of being read using reflected light, and not transmitted, as in the case of the oxacillin disk. Hence, according to the CLSI, which introduced cefoxitin cutoff points for staphylococci in 2005, for *S. aureus* the cefoxitin disc should be preferred to that of oxacillin[27]. With regard to susceptibility to mupirocin, the analysis was performed based on the criteria inserted in CLSI document M100-S20 for screening, due to high levels of mupirocin resistance[28].

Figura 3 – Antimicrobial susceptibility test with cefoxitin, mupirocin and vancomycin disks



Source: Own Authorship.

The interpretation of the TSA result was based on data obtained in CLSI document M100-S25[24].

The susceptibility of vancomycin to MRSA strains was analyzed by the TSA methodology by diffusion disc test and corroborated in a private laboratory using the *Vitek*® 2 automated system (bioMérieux). This system is able to generate TSA results quickly and provides the CIM result, accompanied by interpretation (susceptible, intermediate

or resistant) for most fast-growing aerobic bacteria in a period of 4 to 18 hours[29].

RESULTS

Sampling

A total of 147 samples were collected from 147 individuals.

Three students were excluded from the study in the screening phase because they fulfilled exclusion criteria, more specifically in antimicrobial use, being a student of the first period of Nursing major and two students of the first period of Medical major.

Isolates Of *S. Aureus*

Of the 147 academics who participated in the study, 61 (41.5%) were identified as asymptomatic carriers of *S. aureus*.

3.2.1 Isolates of S. aureus in Nursing course

There was participation of 32 Nursing students, and 11 (34.4%) were found positive for colonization by *S. aureus*. From the first period, 21 students participated and six (28.6%) were identified as asymptomatic carriers. In the last period, 11 students participated in the study and five (45.5%) were colonized (Table 1).

Table 1 – Asymptomatic carriers of *Staphylococcus aureus.*

	Participants	Asymptomatic Carriers	
		Score	%
First Nursing Semester	21	06	28,6%
Last Nursing Semester	11	05	45,5%
First Medicine Semester	62	29	46,8%
Last Medicine Semester	53	21	39,6%

Source: Own Authorship.

Isolates of S. aureus from the Medical major

A total of 115 medical student samples were collected, of which 50 (43.5%) were associated to asymptomatic carriers. From the first semester, 62 students participated and 29 (46.8%) were found positive for *S. aureus* colonization. In the last semester, 53 students participated and 21 (39.6%) were found to be colonized (Table 1).

Profile of Antimicrobial Susceptibility

With respect to the evaluation of antimicrobial susceptibility against mupirocin and vancomycin, all strains were considered sensitive (Table 2).

Table 2 – Results of antimicrobial susceptibility test

Período (N)	Resistência à mupirocina	Resistência à vancomicina	MRSA	
			Contagem	%
Primeiro Período de Enfermagem (N)	00	00	00	00%
Último Período de Enfermagem (N)	00	00	00	00%
Primeiro Período de Medicina (N)	00	00	02	6,9%
Último Período de Medicina (N)	00	00	02	9,5%

Source: Own Authorship. MRSA: methicilin resistant *Staphylococcus aureus*. N: Number of isolated S. aureus.

Among 61 asymptomatic *S. aureus* participants identified by this study, four (6,6%) were classified MRSA by the TSA with cefoxitin disk, two (6,9%) participants from the first semester (n=29) and two (9,5%) from the last semester of Medical major (n=21) (Table 2).

Vancomycin susceptibility was also analyzed in two strains of MRSA, one from the first semester and another from the last semestre of Medical major, by the automated system *Vitek*® 2 (bioMérieux), with the two strains showing a MIC of less than 0.5 µg / ml, that is, the strains were considered sensitive. The MIC of two MRSA samples, one from the first semestre and another from the last semestre of Medical major, was not performed for it was not possible to recover the bacteria to perform the procedure.

DISCUSSION

The estimated prevalence of asymptomatic *S. aureus* carriers identified by this study was 41.5%. This value is consistent with that described in the literature, since in studies conducted in Brazil, the reported rate of *S. aureus* carriers is around 37%[12] whereas in international studies, colonization rates range from 19.5 to 38%[33].

It was possible to observe an increase in the prevalence estimated by asymptomatic colonization of S. aureus in the group of students of the last period of Nursing in comparison to the group of students of the first period of Nursing, with a percentage increase of 16.9%. Nonetheless, there was no statistical association between S. aureus colonization and the periods of the Nursing course (p = 0,442). The low representation of Nursing students in this study does not allow inferring in the final contribution of the work.

With reference to medical students, there was no statistical association between *S. aureus* colonization and academic semester, that suggested an increase in the estimated prevalence (p = 0,441). The ralation of the *S. aureus* carrier rate to the time of hospital involvement was not evidenced, since the first semester students had no contact with the hospital yet and presented a 7.2% higher number of carriers when compared to the students of from the last semester. Results obtained in studies published in Ireland, Nigeria and Australia with the purpose of evaluating the students of Nursing and Medical majors during the training course illustrated, on average, a frequency of 30% of patients with *S. aureus* and also indicated that this value did not change with the increase of the student's exposure to the hospital[34], as it was demonstrated in the present study.

In this study, the presence of MRSA in the first semester of medical school was 6.9% of *S. aureus* isolated in this group, and in the last semester of medical school it was 9.5% of S. aureus isolated in this group. Even though the percentage increase of 2.6% from one period to the other, there was no significant difference between the proportions (p = 0.735). The possibility that isolated strains are CA-MRSA is inferred.

No resistance to vancomycin was observed either by TSA or the *Vitek*® 2 automated system (bioMérieux). This data reinforces the possibility that the MRSA strains found in the study are CA-MRSA, since this is susceptible to most non-beta-lactam antimicrobials. This genotypic characteristic is frequently expressed in an antibiogram that shows resistance only to the oxacillin or cefoxitin disc, markers of resistance to beta-lactams[35], as found by this research.

In the the antimicrobial susceptibility evaluation performed by TSA with mupirocin disc there was 100% sensitivity to this antimicrobial, although studies have demonstrated the identification of high resistance levels of *S. aureus* to mupirocin in places such as Nigeria and South Africa,[36] and other studies affirm that the excessive use of this drug may be considered as a risk

factor for bacterial resistance, previously detected[22,36].

This result clarifies the emerging nature of MRSA strains in the community and the potential risk these students play a key role in the epidemiology and pathogenesis of infection, considering that they may be sources of dissemination of *S. aureus* in both community and hospital settings. Nevertheless, in this case, a genetic study would be essential to evaluate the origin of colonization, as the hospital environment is not exempt from having been the cause of colonization, especially in the group of the last semester of medical school.

CONCLUSION

It was noticed in this study is that the rate of colonization by *S. aureus* is in agreement with those found in the literature. There was no statistical association between *S. aureus* colonization and the academic semester of nursing and medical students that could suggest an increase in the estimated prevalence in the cases observed. The MRSA colonization, encountered in the first and last medical school students in this study, and the antimicrobial susceptibility profile suggest the possibility of the MRSA strains found to be CA-MRSA, but for the aetiological confirmation, a genetic study would be necessary. No resistance to mupirocin or vancomycin was found.

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