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Incidence of Nasal Carriers of *Staphylococcus aureus* Among Undergraduate Nursing Students in the City of Belford Roxo, Brazil

Vanusa Guimarães Dutra[1], Jaqueline Santos de Andrade Martins[2], Antonio Neres Norberg[2,3*], Aluízio Antonio de Santa Helena[2], José Tadeu Madeira de Oliveira[2,4], Marcos Dornelas Ribeiro[1], Luiz Otávio Cerqueira Gouveia[1]
[1]Brazilian Army Biology Institute – IBEx, Rio de Janeiro, Brazil
[2]UNIABEU University Center, Rio de Janeiro, Brazil
[3]Souza Marques Medicine School – FTESM, São Carlos Metropolitan School – FAMESC, Rio de Janeiro, Brazil
[4]Benjamin Constant Institute, Rio de Janeiro, Brazil

Original Research Article

***Corresponding author**
Antonio Neres Norberg

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Abstract: *Staphylococcus aureus* is a common bacterium that affects humans and causes several syndromes and infections with fast evolution which can endanger the patient's life. Investigate the incidence of nasal carriers of *S. aureus* among graduation nursing students who attends to the hospital for at least one year. 122 samples from the nasal cavities were analyzed. The identification of the bacteria was performed by conventional bacteriological methods. The antimicrobial susceptibility tests were performed using the BioMerieux Vitek system in the Bacteriology Laboratory of the Brazilian Army Biology Institute - IBEx. Of the 122 examined students, 51 were carriers of *S. aureus*, corresponding to a prevalence of 41.8%. The results demonstrated that nursing students who attend the hospital environment are more susceptible to carry *S. aureus* antibiotic-resistant strains. We suggest periodic medical exams to these students for appropriated antibiotic therapy and adopt precautionary measures in order to avoid the interpersonal transmission of the bacteria in the relationship with health professionals and patients at the hospital environment.

Keywords: *Staphylococcus aureus*, BioMerieux Vitek, antibiotic therapy.

INTRODUCTION

Staphylococcus aureus is a common pathogen that affects humans and causes several syndromes ranging from mild infections of the skin and tissues to fast evolving infections that endanger the patient's life. Among the most worrying manifestations there are necrotizing pneumonia, severe sepsis and necrotizing fasciitis [1]. This bacterium is a frequent agent of infections in children. The colonization of the human nasal mucosa by *S. aureus* establishes a carrier state, which is considered a potential source of infection and constitutes a high risk factor for the development of invasive infections [2, 3].

Recent studies show that *S. aureus* may develop in some occasions an effective mechanism to defend itself against the immune response of the host and consequently are able to survive in the tissues through a mechanism of escape to the immune system, thus originating an invasive infection [4]. Bacteria of the genus *Staphylococcus* are described as Gram-positive, immobile, non-spore-forming cocci rounded-shape cells measuring around 1 micron in diameter. Through optical microscopy appear as single cells, in pairs or clustered like a bunch of grapes. Most species are aerobic or facultative anaerobes. These bacteria multiply fast in most artificial culture media at a temperature of 37°C, but produces better pigment formation at temperatures between 20°C and 25°C. When cultured in solid media, the colonies have rounded shapes and bright raised rough appearances with gold-yellow colouring [5]. Most species of the genus are benign or have commensal symbiosis with the host, but some species cause suppurations, abscesses and several pyogenic infections in various sites of the organism, which can cause fatal sepsis [6]. Some species of *Staphylococcus* are able to produce hemolysis, coagulate the plasma and produce enzymes and extracellular toxins. A thermostable enteric toxin produced by *S. aureus* is responsible for food contamination and causes food poisoning [7]. This bacterium is frequent in the nasopharynx of healthy adults (around 25%) and has a higher incidence among hospitalized patients, health professionals and people with eczematous diseases. It is a microorganism with easy dispersion, being responsible for several hospital infections. Staphylococcal infections, usually superficial and mild in most patients, may become serious in newborns, surgical patients, carcinomatous, diabetic, and immunocompromised. Multidrug-resistant

strains (MRSA) are more common in hospital settings [8, 9].

Species of the genus *Staphylococcus* are found in the environment and in animal derived products such as milk, cheese and meat. This microorganism often colonizes the skin and its glands and also membranous sites of mammals and birds. It is often found in the mouth, mammary glands and gastrointestinal tract, urinary tract and respiratory tract of humans. Among healthy people, three models of interaction with *S. aureus* may occur: persistent carrier, intermittent carrier, and non-carrier. Considering pathogenicity, the genus is classified into two categories: coagulase-positive and coagulase-negative. Among coagulase-positive strains, *S. aureus* is the species related to both community and hospital human infections [3].

The evolutionary history of antibiotic-resistance acquisition of *S. aureus* resistance had an important moment in the 1940s when 82% sepsis mortality rates were reported by this bacterium in the United States of America. In the same period benzylpenicillin was introduced in the treatment of staphylococcal infections with great success until the middle of the 1950s, when it was observed that some strains of *S. aureus* isolated from blood cultures in hospitals showed high levels of resistance to penicillin. This mechanism of resistance involves the acquisition of a plasmid capable of degrading the antibiotic. With the discovery and introduction of methicillin in 1959, the treatment of penicillin-resistant *S. aureus* was resolved, but the first cases of methicillin-resistant bacteria were reported in Europe in the 1960s [10-12]. The progressive spread of this strain reduced the therapeutic value of the antibiotics used until now and only a few strains remain sensitive to penicillin [13, 14].

Infections caused by *S. aureus* occur more frequently in people still colonized with *S. aureus*, and people who are long-time carriers have an increased risk of subsequent infection [15]. Based on this information, it is essential to investigate the prevalence and the antibiotic sensitivity profile of isolated samples from health professionals in direct contact with patients, particularly nursing professionals, in order to ensure a positive impact in care, as well as developing knowledge about high-risk groups [16].

Considering the importance of this bacterium, this research has the objective to investigate the incidence and sensitivity to antimicrobials of strains of *S. aureus* isolated from nasal cavities of nursing undergraduate students who perform technical activities in hospital environment.

MATERIALS AND METHODS

This research has a cross-sectional, descriptive and observational design, with a representative sample, performed with volunteer undergraduate nursing students of both genders. The group was formed by 122 undergraduate students with technical training in nursing in permanent activities in the hospital environment. The adopted inclusion criteria to the research universe was: to perform uninterrupted technical activity for a period of at least 12 months in hospitals, not to have been submitted to antibiotic therapy in the last 6 months and to be volunteer to participate in the research. The collections of nasal secretions were performed using sterile swabs. Each sample was packed in tubes containing Stuart's transport medium until processing. The material was cultured in agar-blood media and agar-hypertonic-mannitol media and incubated at 37^{o}C for 24 hours. The identification was made by the stain characteristics, the observation of Gram positive cocci, catalase tests, hemolysis in blood agar, fermentation of mannitol test, coagulase test and deoxyribonuclease test. Antibiotic susceptibility tests were performed through the BioMerieux-Vitek system at the Laboratory of Bacteriology of the Brazilian Army Biology Institute - IBEx for the following antibiotics: benzylpenicillin, oxacillin, gentamicin, ciprofloxacin, moxifloxacin, norfloxacin, erythromycin, clindamycin, linezolid, teicoplanin, vancomycin, tigecycline, fusidic acid, rifampicin, sulfamethoxazole-trimethoprim.

RESULTS

Of the 122 samples, 51 (41.8%) were positive for *Staphylococcus aureus*, which strains presented different antibiotic sensibility profile (Table-1).

DISCUSSION

The research on *Staphylococcus aureus* strains isolated from students attending the hospital environment was developed following the protocols of Koneman and Stephen [17] and Levinson [5], who used the morphofunctional characterization of the bacterial cells and classic tests in the process of diagnosis and identification of genus and species of the bacterium. *S. aureus* was already known as a pathogen before the antibiotic era, being one of the most frequent bacteria causing nosocomial infections, a fact that indicates the importance of rigorous prophylaxis measures. In the epidemiological and pathogenic prospect of these infections, healthy carriers are considered important links in the transmission chain, and there is a high variation regarding prevalence and incidence among them. Studies by Castro *et al*., show that this bacterium is currently disturbing because of the acquired methicillin resistance, and for this reason the bacterium has become a community and nosocomial epidemiological problem. Infections with multidrug resistant strains are mainly acquired in hospitals due to the inadequate use of antibiotics, which has dramatically increased the resistance of bacteria to treatment by these drugs [18], a fact confirmed by our

research among health professionals attending the hospital environment.

Table-1: Sensitivity / resistance pattern of *Staphylococcus aureus* isolated from nasal cavities from 122 nursing students.

Tested antibiotics	sensible strains	% sensible strains	resistant strains	% resistant strains
Benzylpenicillin	4	7,8	47	92,2
Oxacillin	33	64,7	18	35,3
Gentamicin	46	90,2	5	9,8
Ciprofloxacin	39	76,5	12	23,5
Moxifloxacin	35	68,6	16	31,4
Norfloxacin	31	60,8	20	39,2
Erytromycin	28	54,9	23	45,1
Clindamycina	31	60,8	20	39,2
Linezolid	42	82,3	9	17,7
Teicoplanin	44	86,3	7	13,7
Vancomycin	45	88,2	6	11,8
Tigecycline	51	100	0	0
Fusidic acid	51	100	0	0
Rifampicina	51	100	0	0
Sulfamethoxazole-trimethoprim	45	88,2	6	11,8

Ledezma and Sanabria commented that *S. aureus* and other bacteria genera constantly change their characteristics face to antibiotic susceptibility. Thus, in order to promote an adequate treatment for the persistent carrier, exams must be performed to determine the sensitivity of the strain to antibacterials. These researchers cultivated 720 samples of superficial secretions and 605 were positive for *S. aureus*. Antibiotic sensitivity tests revealed that 52% of the strains found were resistant to oxacillin. They concluded that a progressive increase in resistance of this bacterium to antibiotics is expected [12]. We agree with the considerations of these authors, and the results of our research point to a similar conclusion as we recommends exams of sensibility before the antibiotic therapy in order to avoid the propagation of resistant strains.

Environment, peculiar living and habits conditions of each population group influences the prevalence of *S. aureus* colonization and the antibiotic resistance profile of the strains that colonize the nasal cavity.

According to Abraão, ethnic origin represents one of the key factors regarding the risk of colonization and infection by *S. aureus* and indigenous communities are more susceptible to to such risks. These populations are carriers of strains of *S. aureus* with distinct characteristics, which are not usually found in non-indigenous populations. Particular characteristics among different populations, such as living in conditions of overcrowding, impaired health care and poor hygiene conditions may be more relevant for the pathogenesis of some clinical forms of *S. aureus* infections [19]. Odorizzi *et al*. found a prevalence rate of 12.3%, with no record of multidrug resistant strains, and all the samples studied showed sensitivity to most of the antibiotics tested, except for nalidixic acid, which demonstrated 100% resistance [20].

The prevalence of *S. aureus* among food handlers in market number 4 in Asuncion, Paraguay was determined by Achón *et al*. These researchers collected material from the nasal cavity of a group of 105 food handlers in order to evaluate the sensitivity of *S. aureus* to antimicrobials. Of the 105 examined people, 33.3% were carriers, and sensitivity tests revealed that 88.6% of the strains were oxacillin sensitive, with 5.7% intermediate sensitivity and 5.7% resistance. Our studies found 41.8% of nasal carriers, in which 64.7% were sensitive to oxacillin. The lower sensitivity rate among nursing students is probably due the frequent contact with patients with resistant strains and contamination in the hospital environment [21].

The prevalence of nasal carriers of *S. aureus* in children with physical disabilities was studied by Queste *et al*. These researchers collected 80 nasal samples from children and 18 were positive to *S. aureus*, equivalent to a prevalence of 22.5%. These authors considered that continuous surveillance is necessary and measures should be adopted to reduce the associated infections [2].

Bermejo *et al*. conducted a bacteriological investigation on superficial wound secretions of patients treated in clinics of the city of Buenos Aires, Argentina. Cultures were positive for *S. aureus*. Antibiotic sensitivity tests revealed a 74.7% oxacillin resistance rate. These authors expressed their concern about the significant increase in resistance acquired by *S. aureus* strains to antibiotics, especially for methicillin. We agree with the authors and share the worry about the progress of the resistance of this bacteria face to a wide range of antimicrobials [22]. In our research, the found

strains were 100% sensitive only to 3 of the 15 tested antibiotics (tigecycline, fusidic acid and rifampicin).

Galvão investigated the incidence of methicillin-resistant *S. aureus* among patients in hemodialysis treatment at a clinic in the city of Natal, Brazil. Of a total of 375 patients, 90 (24%) carried the *S. aureus* in their nasal cavities. In 9 of them (2.4%) the strains were resistant to methicillin. This author considered that colonization by methicillin-resistant *Staphylococcus aureus* is of concern mainly in hemodialysis patients because they are more susceptible to microbial infections [23]. We agree with the author, and we emphasize that the hospital environment to which these patients can access may become a major focus of infection by bacteria resistant to antibiotics.

Investigations in several countries reveal that there is a wide difference in resistance to methicillin profile among *S. aureus* strains isolated from nasal carriers of health services professionals. Thus, Na'was and Fakhoury found almost two decades ago 5.1% of methicillin-resistant strains among attendants in four hospitals in Jordan [24]. A research conducted by Gaspar *et al*. reported a 15.3% rate of MRSA in health care workers in São Carlos Hospital in Madrid, Spain [25]. The prevalence found in our research among nursing students attending the hospital environment was 41.8%, considered one of the highest. This high rate can be explained by the high incidence of *S. aureus* infections in the hospitals frequented by the group examined in our research, with the possibility of transmission of microbial agents from the infected patients to the professionals in that environment.

Legese *et al*., investigated healthy *S. aureus* nasal carriers among health professionals of two hospitals in Tigray, Northeast Ethiopia. The total prevalence for *S. aureus* was 12% [26], while among nursing students in the city of Belford Roxo was 41.8%, a much higher rate.

The researchers Camilo *et al*., considered that *Staphylococcus aureus* is one of the most prevalent pathogenic bacteria found in healthcare units. These authors evaluated the prevalence of *S. aureus* as a contaminant in the hands and nasal mucosa of nursing professionals of a hospital in the city of Cascavel, Province of Paraná, Brazil. To perform the research 200 samples of nasal secretion and the surface of the hands were collected from nursing professionals. The material was sent to the hospital laboratory and processed through bacteriological methods. The result showed that 56% of the participants were colonized by *S. aureus* and the antibiotic sensitivity tests revealed that 92% of the samples were resistant to methicillin. The authors emphasized the importance of investigating the prevalence and resistance of this bacterium and the need for surveillance studies regarding the dissemination of microorganisms in hospital environment [27]. The rate of nasal carriers of *S. aureus* in healthcare professionals in the city of Cascavel was higher than that found in our research (41.8%), but indicates how healthcare professionals may be hosts of potential microorganisms that cause nosocomial infections.

Nasal carriers of *S. aureus* among healthcare professionals of the hospital Al Shifa in Gaza, Palestine, had a high rate of colonization by this microorganism. Aila *et al.,* demonstrated that among 200 hospital workers, 62 (31%) were carriers of *S. aureus* and, among the nasal carriers, 51 (82.3%) were colonized with methicillin-resistant strains. According to the authors, this index is alarming and highlights the need for infection control measures to prevent the spread of this strain to vulnerable patients [28]. We believe that this control must be exercised from the first moments of the technical training of nursing students, as well as constant supervision on preventive measures to avoid interpersonal transmission of *S. aureus* and other pathogenic agents.

Dulon *et al*., considered that the chance of nursing professionals to be colonized with MRSA is almost twice than that of the physicians, and three times higher than that of other health professionals [29]. It happens because the nursing activities have more direct contact with the patients [27, 30]. Cruz affirms that the long working period of these professionals can lead to mistakes and distraction besides a greater time exposure to an unhealthy environment [31].

Martínez *et al*., identified nasal carriers of *S. aureus* among healthcare professionals of Pepe Portilla Provincial Pediatric Hospital in the city of Pinar del Río, Cuba, in the year of 2014. Of the 230 nasal cavity samples, 51 (22.2%) were positive for the bacterium. Nursing professionals corresponded to 43.1% of all nasal carriers, followed by medical personnel, with 23.5%. The strains showed multidrug resistant profiles, but presented 100% sensitivity to linezolid and vancomycin [32]. The incidence rates of nasal carriers of *S. aureus* among nursing professionals in Cuba and the nursing students in Belford Roxo are close, but we emphasize that among the strains found in our research, 17.7% were resistant to linezolid, characterizing a situation more worrying about the pattern of resistance among nursing students in Belford Roxo.

Guducuoglu *et al*., conducted an investigation of asymptomatic carriers of *S. aureus* in a School-Hospital in Turkey. A total of 327 people were recruited from the hospital staff to participate on the research, and the results demonstrated that 58 (17.7%) were nasal carriers of *S. aureus* [33]. The incidence found by these researchers is lower than those found in our research, which scored 41.8%.

A four-year cohort study of *S. aureus* nasal carriers among Portuguese nursing students was performed by Conceição *et al.,* At the beginning of the investigation, upon entering university, among 47 undergraduate nursing students 20 (43%) were carriers of methicillin-sensitive *S. aureus* and none had MRSA. After four years of exposure to nursing training, five of these students became carriers of methicillin-resistant *S. aureus*. Of the total number of students, 39 (83%) were considered carriers of this bacterium at the end of 4 years of graduation. The authors considered that the proportion of students who became colonized in the nursing training process demonstrates how this group of professionals can become easily infected by new strains, and emphasized that education regarding infection control should receive more attention in preparation of nursing professionals [34]. The rates found by the Portuguese researchers were considerably higher than those verified in our study, but highlight how hospital frequenters become hosts of bacteria resistant to antibiotics throughout the time of exposure to these pathogens.

Lopes *et al*., investigated *S. aureus* in the saliva and in the nasal secretion of 100 nursing professionals who attend HIV-positive patients. The researchers identified that 43% of these professionals were carriers of the bacteria. The sensibility profile of the strains showed that 14.9% were resistant to oxacillin, 91.9% were resistant to penicillin, 44.6% to erythromycin, 41.9% to clindamycin. The authors emphasized that the nursing professional should know their carrier status of multidrug resistant microorganisms in order to take procedures to prevent the transmission of microorganisms to patients in the hospital environment, especially those immunocompromised [35]. We agree with these authors and consider that this type of control should be periodic and, in addition, performed at the moment of beginning nursing undergraduation, as advised by Conceição *et al*., [34].

Souza investigated the prevalence of *S. aureus* among nursing undergraduate students at the Federal University of Sergipe, Brazil. A prevalence of 39.09% was observed for this bacterium, with a high rate of resistance to azithromycin and erythromycin, and a moderate rate of resistance to tetracycline and ciprofloxacin [36]. Considering that the studied group is similar to that of our research, our results approximate of those found by the author.

Tavakoli investigated the nasal carriage of *S. aureus* carriers among nursing students of the Islamic Azad University of Eghlid Branch, Iran. A total of 206 students were examined, of which 68 (31.4%) were colonized by *S. aureus*. The author recommended the research on all nursing students and treatment of all students and professionals who are nasal carriers of *S. aureus* and have direct contact with patients in the hospital environment [37]. We share the same opinion, emphasizing that these exams should be periodic, since in the hospital environment there is the possibility of frequent contact and nasal colonization with strains that have different resistance patterns to antibiotics.

The incidence of *S. aureus* in the nasal cavity of students of health undergraduation courses in India was studied by Bharathi and Vinodhini. Among 104 students, 20 (19%) were positive for *S. aureus*, and 9 (45%) of them were methicillin resistant strains carriers [38]. This result reveals a lower prevalence than that found in our research. However, as the author, we warns about the high resistance profiles to some types of antibiotics among strains isolated from undergraduate nursing students in Belford Roxo, such as to erythromycin (45.1%), to clindamycin (39.2%) and to norfloxacin (39.2%).

Chambers [39] and Kluitmans *et al*., [40] considered that the worldwide prevalence of asymptomatic carriers of *S. aureus* in the general population is between 25% and 50%, so our results obtained among nursing undergraduates with a rate of 41.8 coincide with the international rates.

CONCLUSIONS

Of the 122 students examined, 51(48,1%) were nasal carriers of *Staphylococcus aureus*. Isolated strains showed a high degree of resistance to some antibiotics, especially erythromycin (45.1%), clindamycin (39.2%) and norfloxacin (39.2%), but showed a 100% sensitivity to tigecycline, fusidic acid and rifampicin. The students who participated in our research attended the hospital environment for at least a year, and it was demonstrated through the resistance profile that these group are more susceptible to carry strains resistant to antibiotics from the contact with this environment. Compared to other population groups, nursing undergraduate students of Belford Roxo presented a high incidence of *Staphylococcus aureus* colonizing the nasal mucosa. We suggest conducting periodic examinations so that the nursing student can know the resistance profile of the carried strain in order to perform the appropriate antibiotic therapy and promote precautionary measures in order to avoid interpersonal transmission of the bacteria in the relation with other healthcare professionals and patients in a hospital environment.

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