

Microbiological identification of genes that have resistance to antibiotics in *Staphylococcus aureus*

Identificação Microbiológica de genes de resistência a antibióticos em *Staphylococcus aureus*

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Abstract

Introduction: the hospital environment favors the acquisition of antimicrobial resistance, being important the aseptic environment and the control of carriers for reducing these infections. Species of *Staphylococcus aureus* resistant to antibiotics became a cosmopolitan problem due to its degree of virulence and the association with various diseases. These opportunistic microorganisms are usually found in the nasal cavities which develop rapidly in the asymptomatic patients and, once reaching the hands, may spread rapidly among those patients who are in a state of immunosuppression. **Objective:** therefore, this study aimed to identify the presence of antibiotic-resistant *Staphylococcus aureus* in the nostrils of health professionals in Campina Grande-PB. **Methodology:** for this purpose, it was collected 50 samples of nasal health professionals who had clinically healthy, with no sign of staphylococcal infection. **Results:** it was found that 28 of the 50 samples, or 56% were positive for *S. aureus*, and 12/28 samples (42.86%) were resistant to oxacillin and is therefore considered MRSA. **Conclusion:** the high levels of *S. aureus* among health professionals may contribute to the spread of this pathogen among immunosuppressed patients and, therefore, are necessary and essential preventive measures such as hand hygiene and nasal decolonization of MRSA carriers from those with mupirocin.

Keywords: Infection. *Staphylococcus aureus*. MRSA Resistance.

Resumo

Introdução: as infecções hospitalares favorecem a obtenção de resistência aos antimicrobianos, deste modo, a assepsia do meio ambiente e o controle dos portadores, constituem um meio para redução dessas infecções. Espécies de *Staphylococcus aureus* resistente a antimicrobianos tornaram-se um problema cosmopolita, devido ao seu grau de virulência e associação a várias doenças. Estes microorganismos oportunistas encontram-se nas fossas nasais, principal reservatório, desenvolvendo rápida resistência naqueles portadores assintomáticos e, uma vez atingindo as mãos, podem disseminar rapidamente dentre aqueles pacientes que se encontram em estado de imunossupressão. **Objetivo:** portanto, este trabalho teve como objetivo identificar a presença de *Staphylococcus aureus* resistente a antibióticos nas fossas nasais de profissionais da saúde no município de Campina Grande-PB. **Metodologia:** para isto, foram realizadas coletas de 50 amostras nasais de profissionais da saúde que se apresentavam clinicamente saudáveis. **Resultados:** das 50 amostras, 28/50 (56%) foram positivos para *S. aureus*. E destas, 12/28 (42,86%) resistentes à meticilina ou oxacilina (MRSA). Conclusão: o alto nível de *S. aureus* entre os profissionais podem contribuir para a disseminação deste patógeno entre os pacientes imunossuprimidos. Portanto são necessárias medidas profiláticas tais como a higiene das mãos e descolonização das fossas nasais para MRSA utilizando mupirocin.

Palavras-chave: Infecção. *Staphylococcus aureus*. Resistência a MRSA

INTRODUCTION

It is known that the hospital environment offers an intimate relationship with nosocomial infections that can cause the spread of infectious agents (ANDRADE; ANGERAMI; PADOVANI, 2000). According to the health ministry, hospital infection is one infection acquired after admission of the patient and whose manifestation occurs during or after its discharge, as long as it relates

to their hospitalization (BRASIL. MINISTÉRIO DA SAÚDE, 1998).

Among the factors that might cause a hospital infection it can be mentioned: the patient himself, the microorganisms determinants of such infections and hospital environment. One way to reduce this growing form of contamination is the aseptic hospital environment as a way to keep it biologically safe for both patients and for professionals who work at the hospital.

It also to give prominence the possibility of contamination through the health care professional as disseminator of the micro-organism, as well as utensils

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and equipment. Thus, one should not overlook that the hygiene of the professionals are important to exclude the possibility of contamination of hospital items that may colonize patients, given that these are immunocompromised. *Staphylococcus aureus* is a major pathogen due to the degree of virulence and its association with different diseases that can lead to death (VIEIRA, 2010). So the colonization of *S. aureus* and subsequent infection in hospitalized patients is a major public health problem due to the possibility of acquiring resistance to multiple antibiotics. Rightfully so, risk factors related to the acquisition of resistant organisms include hospitalization and treatment with antibiotics (UENO; JORGE, 2001).

It is believed that the increase in antimicrobial resistance of infectious agents occurs due to inappropriate use of antimicrobials. Considering that in the healthy individual, *Staphylococcus aureus* is usually a commensal of the nostril, skin and intestine, it can often be incorporated into local sterile after a trauma, skin abrasions and mucous membranes or during surgical procedures (MENEGOTTO; PICOLI, 2007).

One cannot fail to consider that the strains usually isolated from asymptomatic carriers have shown high rates of mutagenicity and antimicrobial resistance (HESHIKI et al., 2002). Furthermore, although this bacterium has been one of the first to be controlled with the advent of antibiotics, it has a tremendous ability to adapt and acquire resistance, causing one of the most important species in frames of hospital infections (SANTOS et al., 2007). These bacteria have particular significance when it becomes resistant to methicillin which is an antibiotic used in the treatment of staphylococcal infections, because it is a marker for resistance to all beta-lactams (including cephalosporins and carbapenems) (MENDES, 2010), which previously were effective for treatment of staphylococcal infections. This resistance is associated with chromosomal genes that encode changes in receptor that has action to beta-lactam antibiotics, penicillin binding proteins (PBP's), leading to the production of new PBP's that have little affinity for beta-lactams (TAVARES, 2000).

It is well known the involvement of *Staphylococcus aureus* in infections. Since it is commonly found in the skin and nasal cavities of healthy individuals, these bacteria can result from simple infections such as pimples or cellulitis. In others cases, it can cause more serious infections such as pneumonia and endocarditis (SANTOS et al., 2007).

It is commonly transmitted from person to person (cross-infection), through indirect contact (aerosols, secretions and food, among others) or by direct contact, with the transfer depending on the presence of the microorganism source (professionals or patients) and microorganisms rater released (SANTOS; DARINI, 2002). Thus, it is important to detect and control of asymptomatic carriers of *Staphylococcus aureus* which have great importance because it is the health professionals who are in direct contact with patients, which could favor its transmission. The microbiological and molecular identification of *Staphylococcus aureus* resistant

to antimicrobials, provides tools for improved diagnosis, allowing to determine the prevalence of these staphylococci as etiological agents of infections and enable the development of strategies to control the spread of these resistant strains (COELHO et al., 2007).

It is known that the carriers (hospital staff and medical staff) are not able to develop the disease under normal conditions due to their immune system to be able to fight the infectious agent. In contrast, patients who are debilitated and post-operative patients, HIV patients, as well as other conditions that cause immunodeficiency are susceptible to developing infections by this bacterium. Thus, the identification and control of carriers becomes an important measure, because they are health professionals, since they may provide a risk to contamination of others and equipment.

Therefore, this study aimed to determine the presence of *Staphylococcus aureus* resistant to antibiotics in the nostrils of health professionals from a hospital in Campina Grande, given prominence to some important aspects for hospital infections, in order to stimulate reflections and sensitize health professionals of hand washing and hygiene when handling utensils.

METODOLOGIA

Sampling

The study is an applied and quantitative research that was carried out in a hospital in the city of Campina Grande, PB. The professionals were chosen at random, including doctors, nurses, psychologists, dentists, nursing technicians, physiotherapists wick working with patients in Intensive Care Units (ICUs). These professionals were selected according to the following inclusion criteria: be acting professionally during the study period, in one of the ICUs of the hospital selected, between 24 and 30 hours per week and being in aggreed to join in the study by signing the document called *Termo de Consentimento Livre e Esclarecido*. It was excluded those ones who showed any sign of infection of the up surface of nostril and were using antibiotics about three months before the date of collection.

It was included 50 members of the ICU professionals. Of these, 13 were nurses, 25 were nursing technicians, 3 doctors, 1 psychologist, 7 physiotherapists, 1 dentist. This selection considered their potential backers of multiresistant microorganisms and disseminators of cross infection in the hospital.

Collection and processing of clinical material

Samples were collected from the nostrils of the selected individuals. For each of them, two samples were collected with sterile swabs, considered the secretion of the right nasal cavity (SND) and secretion of the left nasal cavity (SNE). These sample were properly packed in an insulated box and then processed in Microbiological Analysis Laboratory of Maurício de Nassau faculty during the period of October-November 2012.

All clinical samples collected were immersed in BHI broth (Himedia – Himedia Laboratories Pvt. Ltd. Vandhani Ind. Est. Mumbai, Índia) and incubated in a bacteriological (DeLeo Equipamentos Laboratoriais, Porto Alegre, Rio Grande do Sul, Brazil) incubator at 37 ° C for 24 hours. Subsequently, these samples were removed from the incubator and were sowed in the culture medium blood agar and Mannitol Salt (BD – Becton, Dickinson and Company Sparks, MD 21152 USA. 38800 Le Pont de Claix, France). Afterwards they were incubated at 37 ° C for 24 hours. After this period of time, colonies suspected of belonging to the species *Staphylococcus aureus* were tested with catalase (positive), stained by the Gram method (Gram-positive cocci arranged in the form of curls) and the *Staphylococcus aureus* was determined by Sthafclin test (Laborclin Produtos para Laboratório Ltda. Pinhais, Paraná, Brazil)

The antimicrobial susceptibility profile of the strains isolated from health professionals was determined by the disk diffusion method (Kirby-Bauer), which was performed according to the Clinical and Laboratory Standards Institute (CLSI) (PERFORMANCE..., 2012). From strains identified as *Staphylococcus aureus*, it was prepared a direct colony suspension, in which was selected about 3 to 5 colonies that were transferred to a tube containing 4 mL of sterile physiological solution as proposed by Oplustil, Zocoli, e Tobouti (2010). Then, after the solution has become turbidity, it was compared with the 0.5 McFarland scale (OPLUSTIL; ZOCOLI; TOBOUTI, 2010)

Approximately 15 to 20 minutes after the preparation of the suspension, inoculation was performed using a sterile swab, removing the excess liquid by compression on the tube wall.

Thus, seeding was carried out on the plate with the culture medium Mueller-Hinton agar (BD – Becton, Dickinson and Company Sparks, MD 21152 USA. 38800 Le Pont de Claix, France) in 4 different directions in order to obtain uniform growth of the colonies. Soon after, the discs containing antibiotics were placed, with the aid of tweezers as aseptic conditions and following a distance of 150 mm from the edges of the plates and the distance between the discs of 24 mm (OPLUSTIL; ZOCOLI; TOBOUTI, 2010). After their application, the discs were pressed lightly in order to be in contact with the agar and held incubation of the plates for a period of 18 to 24 hours at a temperature of 35 ° C. Then, it was performed the reading of the diameter of inhibition zones of growth. These discs were previously removed from the freezer (Continental – Copacabana RDV37.) 1 hour before their use, so that they could reach room temperature.

During the procedure, it was tested the following antimicrobial discs: Erythromycin – 15ug (ERI), Gentamicin – 10ug (GEN), Clindamycin – 2UG (CLI), Ciprofloxacin – 5ug (CIP), Amoxicillin / Clavulanate – 20/10ug (AMC),

Cephalothin – 30ug, Imipenem – 10ug (IPM), Oxacillin – 30ug (OXA) and trimethoprim-sulfamethoxazole – 1.25/23.75ug (SUT). These antimicrobials were selected for this study because they are the most used in clinical practice in health care and are according to the criteria of standardization recommended by Clinical and Laboratory Standards Institute (CLSI) (PERFORMANCE..., 2012).

The analyses of the results were performed quantitatively by means of descriptive statistics. The research followed the resolution 196/196 of the Ministry of Health and was approved by the Ethics Committee of the Universidade Estadual da Paraíba – UEPB which number of the authorization is n° 0255.0.133.000-12.

RESULTS

The study population had an age range between 25 and 53 years old, in which 86% were female and only 14% male. It was found that from the 50 samples, 28 were positive for the presence of *S. aureus* in the nostril. Thus, Table 1 shows the frequency of *Staphylococcus aureus* and coagulase-negative *Staphylococcus* (SCN) in nasal swab samples, in which 56% of the isolated strains were *Staphylococcus aureus*.

Table 1 – Frequency of colonization by *Staphylococcus aureus* and coagulase-negative *Staphylococcus* (SCN) in samples of nasal swabs of health professionals.

	Number of Sample	Percentage (%)
<i>S. aureus</i>	28	56
SCN	22	44
Total	50	100

Among the 28 samples of *Staphylococcus aureus*, analyzed by disk diffusion method (Kirby-Bauer), twelve showed themselves resistant to oxacillin representing the samples of MRSA (Table 2). Therefore, it is possible to conclude that a significant percentage of samples are resistant to the antibiotic oxacillin.

Table 2 – Sensitivity submitted by *Staphylococcus aureus* to oxacillin.

Antibiotic	Sensitive		Resistant	
	Nº	%	Nº	%
Oxacillin	16	57,14	12	42,86

The sensitivity submitted by 28 strains of *S. aureus* may be observed in table 3. The antibiotics tested against the strains were: β -lactams (amoxicillin/clavulanic acid, cephalothin, oxacillin), carbapenem (imipenem), lincosamidas (clindamycin), macrolides (erythromycin), aminoglycosides (gentamicin), quinolones (ciprofloxacin) and acid inhibitors Folic (trimetoprina-sulfamethoxazole).

Table 3 – Sensitivity submitted by *Staphylococcus aureus* to antimicrobials.

Antibiotics	Sensitive		Intermediate		Resistant	
	Nº	%	Nº	%	Nº	%
Amoxicillin / Clavulanic acid	17	60,71	–	–	11	39,29
Cephalothin	21	75	2	7,14	5	17,86
Imipenem	23	82,14	–	–	5	17,86
Oxacillin	16	57,14	–	–	12	42,86
Clindamycin	17	60,71	–	–	11	39,29
Erythromycin	8	28,57	5	17,86	15	53,57
Gentamicin	23	82,14	2	7,14	3	10,71
Ciprofloxacin	22	78,57	2	7,14	4	14,28
Sulfamethoxazole – trimetoprina	23	82,14	1	3,57	4	14,28

DISCUSSION

The main reservoir of staphylococci in man are the nostrils and the high incidence in the population makes it difficult to eradicate (RADDI; LEITE; MENDONÇA, 1988). Studies suggest that the epidemiology of MRSA is changing, since the microorganisms are no longer confined to a hospital environment, but also appearing in healthy individuals in the community (CA-MRSA) (VALADAS, 2010). The results of the studies accomplished by some researchers, cited below, show a variation in the proportions of nasal carriers of *Staphylococcus aureus*. In the present investigation, it can be observed a rate of 56% in individuals carrying *S. aureus* in the nostrils between health professionals that work in ICUs. This rate was higher than those ones observed by Garcêz (2011), that observed only 19% of the patients from ICU, considering 62 samples, the presence of *Staphylococcus aureus* in their nasal samples.

The isolation of *S. aureus* shows up with a high frequency of nasal samples, especially for it belongs to the normal microbiota. The evaluation of the colonization by *S. aureus* in carriers that live in the Center Children's Hospital of the Faculty of Medicine of Ribeirão Preto – University of São Paulo, had an occurrence of 55 samples positive for *S. aureus*, of the 345 samples collected from the nasal cavity and from the right hand and left hand. That occurrence represents a rate of 58.2% (32 samples) of patients who were found in the nasal cavity so a similar percentage that was found in this present study (SANTOS; DARINI, 2002).

The material collected from the nasal cavities of 240 medical students in São Paulo showed that only 47 (19.58%) were carriers of *S. aureus* (NEVES, 2007) which differs from the present study that found a higher percentage of patients (56%). Moreover this research accomplished in São Paulo found 26/240 (10.83%) strains resistant to oxacillin (NEVES, 2007) which also disagree with the current study (12/50=24%).

In another study that analyze nasal samples of 88 residents, it was found 17.68% of *Staphylococcus aureus* and 39.23% of SCN (HESHKI et al., 2002). In the analysis of samples of nasal strains acquired in the community, it was reported a rate of 40% positive for *S. aureus* and 60% to SCN, and three strains of *S. aureus* were resistant to oxacillin representing the CA-MRSA. In the case of that cited study, it was also observed a greater tendency to resistance to β -lactam antibiotics such as penicillin (72.5%), and macrolides (45%) and a minor proportion of the tetracycline (17.5%), lincosaminas (15%), aminoglycosides (5%), chloramphenicol (17.5%) and 100% sensitivity to glycopeptides (MENEGOTTO; PICOLI, 2007). This profile is similar to that shown in this study which has found a increased resistance to β -lactams (oxacillin) 42.86% (12/28) and macrolides (erythromycin) 53.57%. Between the others antibiotics it was found a lower percentage of resistance.

Analyzing 104 nasal samples of students of nursing, it was found 27.5% (30 samples) positive for *S. aureus* and 72.5% (79 samples) for SCN (PEREIRA; CUNHA, 2009). These studies differ from the current, once it was found a larger number of strains of *S. aureus* and a minor number of coagulase-negative *S. aureus*.

Currently, one of the biggest public health problems is associated with the worsening of bacterial resistance to antimicrobials, especially hospital infections (SAVI; COL; ONOFRE, 2009), since the indiscriminate use of antibiotics causes changes in the behavior of these micro-organisms and so, extends a range of resistant bacteria, promoting difficulties in establishing control of measures (SOARES et al., 2008).

In all 50 samples analysed, it was observed bacterial growth, being 56% (28/50) of *S. aureus* and 44% (22/50) of SCN. In addition, analyzing the *Staphylococcus coagulase* positive strains, it was realized that all strains showed some resistance to antibiotics. Among these the most prominent are those ones that were obtained resistance to oxacillin which are characterized by *S. aureus* MRSA. Savi, Col e Onofre (2009) suggest that the strains of *S. aureus* resistant to oxacillin (12/28) must be considered to have the *mecA* gene, since all strains of *S. aureus* that are MRSA expresses this gene of resistance. This gene codes for the production of a penicillin-binding protein (PBP), which renders a number of antibiotics ineffective. Thus, having hands as a vehicle for work, health professionals can, through direct contact with the hospitalized patients, providing a staphylococcal infection.

CONCLUSION

After analysis of nasal samples coming from health professionals working in ICUs of the hospital of Campina Grande city, PB, it was concluded that of the 50 samples analyzed, 28 (56%) were positive for *Staphylococcus aureus*, while 22 (44%) of remaining samples were identified as coagulase-negative *Staphylococcus*. Thus, among these clinical isolates of *Staphylococcus aureus* was possible to

examine that they all had some resistance to the antibiotics tested and, in particular, oxacillin in which 12/28 (42.86%) samples positive for *S. aureus*, were found to be resistant, being these strains considered MRSA. The strains of *S. aureus* resistant to oxacillin were considered to have the *mecA* gene, since all strains of *S. aureus* that are MRSA express this gene of resistance. This gene codes for the production of a penicillin-binding protein (PBP) which renders a number of antibiotics ineffective.

Due to the high rate of *S. aureus* MRSA identified, this study proposes the use of prophylactic measures, such as handwashing with soap and water, as this reduces the spread of this pathogen. For the carriers of MRSA, besides the preventive measures it is necessary the decolonization with mupirocin to reduce colonization of their nasal cavity.

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Submetido em: 17/01/2014

Aceito em: 10/08/2015