



Gaceta Médica Boliviana
ISSN: 1012-2966
ISSN: 2227-3662
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Bolivia

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Gaceta Médica Boliviana, vol. 43, no. 1, 2020

Universidad Mayor de San Simón, Bolivia

Available in: <https://www.redalyc.org/articulo.oa?id=445674690018>

DOI: <https://doi.org/10.47993/gmb.v43i1.19>

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Antibiotic susceptibility of Staphylococcus aureus from nasal isolates in students in northern Peru

Susceptibilidad antibiótica de Staphylococcus aureus de
aislados nasales en estudiantes del norte de Perú

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Gaceta Médica Boliviana, vol. 43, no. 1,
2020

Universidad Mayor de San Simón,
Bolivia

Received: 08 February 2020

Accepted: 11 April 2020

DOI: <https://doi.org/10.47993/gmb.v43i1.19>

Redalyc: <https://www.redalyc.org/articulo.oa?id=445674690018>

Abstract: **Objectives:** To determine the antibiotic susceptibility profile of Staphylococcus aureus in Biology, Nursing and Medicine students of the Universidad Nacional Pedro Ruiz Gallo-Lambayeque.

Methods: Samples were collected by nasal swabs. Primary isolation on Salt Mannitol Agar was performed for the recovery and identification of Staphylococcus aureus from October 2015- March 2016. Bacterial susceptibility testing was performed by antimicrobial susceptibility testing by the diffusion disc method.

Results: 43 positive cultures were isolated for Staphylococcus aureus, representing 28.6%; 107 samples were negative, representing 71.4%. It was determined that 90.6% of the strains were resistant to Oxacillin, 81.3% to Sulfamethoxazole - Trimethoprim, 95.3% to Penicillin, 34.8% to Cefoxitin, and all strains were 100% sensitive to Imipenem and Vancomycin. The cross-reaction evaluation showed that 2.6% of the strains were resistant to Cefazidime, 12.8% to Azithromycin, 15.4% to Cefotaxime, and 20.9% to Gentamicin; on the other hand, they were 100% sensitive to Amikacin, Ciprofloxacin, Ceftriaxone and Cefuroxime.

Conclusions: 28.6% of the total study population tested positive for Staphylococcus aureus, 90.6% of the strains were resistant to Oxacillin, and all strains were sensitive to Imipenem. The Professional School of Biological Sciences had the highest frequency of Staphylococcus aureus isolates.

Keywords: methicillin-resistant staphylococcus aureus, asymptomatic Infections, student health services.

Resumen: **Objetivos:** determinar el perfil de susceptibilidad antibiótica de Staphylococcus aureus, en estudiantes de Biología, Enfermería y Medicina de la Universidad Nacional Pedro Ruiz Gallo-Lambayeque.

Métodos: Las muestras fueron recolectadas mediante hisopados nasales. Se realizó el aislamiento primario en Agar Manitol Salado, para la recuperación e identificación de Staphylococcus aureus de octubre 2015- marzo 2016. La prueba de susceptibilidad bacteriana se realizó la prueba de sensibilidad antimicrobiana por el método de disco de difusión.

Resultados: se aislaron 43 cultivos positivos para Staphylococcus aureus, lo cual representó el 28,6%; siendo negativas 107 muestras, representando el 71,4%. Se determinó que, el 90,6% de las cepas fueron resistentes a Oxacilina, el 81,3 % a Sulfametoxazol - Trimetoprima, el 95,3 % a Penicilina, el 34,8% a Cefoxitina, siendo todas las cepas 100% sensibles a Imipenem y Vancomicina. La evaluación de la reacción cruzada dio como resultado que el 2.6% de las cepas presentaron resistencia a Cefazidima, 12,8% a Azitromicina, el 15,4 % a Cefotaxima, y el 20,9% a Gentamicina;

por el contrario fueron 100% sensibles a Amikacina, Ciprofloxacino, Ceftriaxona y Cefuroxima.

Conclusiones: El 28,6% del total de la población en estudio dio positiva para *Staphylococcus aureus*, el 90,6 % de las cepas de fueron resistentes a Oxacilina, siendo todas las cepas sensibles a Imipenem. La Escuela profesional de Ciencias Biológicas fue quien presentó mayor frecuencia de aislamientos de *Staphylococcus aureus*.

Palabras clave: *staphylococcus aureus* resistente a metilicina, infecciones asintomáticas, servicios de salud para estudiantes.

Nosocomial infections lengthen the length of hospitalisation by 4 days on average, and about one third of all hospital-acquired infections are considered preventable. However, the classification of an infection as nosocomial does not mean that there is a causal relationship between a medical intervention and the occurrence of the infection ¹. In Germany the mortality attributable to nosocomial multi-drug resistant organisms (MDRO) is between 6,000 and 15,000 patients, around 30,000 to 35,000 patients develop infections. The problems of nosocomial infections and increasing antimicrobial resistance are highly relevant and should not be underestimated ². One of the organisms present in the community is *Staphylococcus aureus*, an opportunistic pathogen that can colonise skin and mucous membranes, such as the nostrils, and remain quiescent and asymptomatic without decreasing its infective capacity, thus giving rise to the asymptomatic carrier state, in which it transmits from host to host and spreads in the environment causing infections in different areas of the body ^{3,4}.

Staphylococcus aureus capable of producing resistance to most antibiotics used in empirical treatment ⁵. It presents several resistance mechanisms, including the *mecA* gene, which disables methicillin and beta-lactam antibiotics such as quinolones, lincosamides and carbapenems such as imipenem and meropenem, increasing morbidity, mortality and treatment costs ^{6,7}. *Methicillin-resistant Staphylococcus aureus* (MRSA) is a major cause of hospital-acquired infections that are increasingly difficult to combat due to emerging resistance to all current classes of antibiotics ⁸. MRSA colonisation of the nares, whether present in the community or acquired during hospitalisation, increases the risk of MRSA infection. Identification of MRSA colonisation on admission may point to a high-risk population that could benefit from interventions to decrease the risk of subsequent MRSA infection ⁹. Replacement of hospital strains by community-associated strains has been observed; most risk factors of patients infected with community and hospital strains were similar, suggesting that, so far, community strains behave like their traditional hospital-associated counterparts ¹⁰.

The increase in the incidence of MRSA infection has been associated with the recognition of new MRSA clones known as community-associated MRSA (CA-MRSA). CA-MRSA strains differ from hospital MRSA strains; they infect a different group of patients, cause different clinical syndromes, differ in antimicrobial susceptibility patterns, spread

rapidly among healthy people in the community, and often also cause infections in healthcare settings¹¹. *Staphylococcus aureus* resistance is a problem if not adequately treated and prevented from spreading, the objective of the study was to evaluate the antimicrobial susceptibility of *Staphylococcus aureus* from nasopharyngeal isolates of Biology, Nursing and Human Medicine students of the Universidad Nacional Pedro Ruiz Gallo who have nosocomial contact.

Materials and methods

The present research work is an exploratory cross-sectional study, carried out in the Microbiology and Parasitology laboratories of the Faculty of Biological Sciences of the Universidad Nacional Pedro Ruiz Gallo. The sample size was determined on the basis of a pilot sample of 15 students, five students from each faculty evaluated, of which 30 % tested positive for *Staphylococcus aureus*. The total number of students who participated was 150, distributed among 50 students from the Faculty of Biological Sciences in the last cycles, 50 nursing students who already attend medical centres and 50 fourth year medical students from the Universidad Nacional Pedro Ruiz Gallo. Nasal swab samples of students from three professional schools of the National University ?Pedro Ruiz Gallo?, during the period October 2015-March 2016 of the National University Pedro Ruiz Gallo. Only those students who agreed to participate in the study after signing the informed consent form were included, who subsequently underwent nasal swabbing to obtain the sample. The study was accepted by the ethics committee of the Universidad Nacional Pedro Ruiz Gallo.

Bacterial isolation

Samples were obtained by nasal swabbing by inserting a swab into the nostril of the carrier, rotating three times clockwise and three times counterclockwise. They were immediately seeded on Salted Mannitol Agar plates by exhaustion and streaking for primary isolation; incubated at 35-37 °C for 24 hours, under aerobic conditions.

At the end of incubation, the characteristics of the suspected *Staphylococcus aureus* colonies were determined, and microbiological identification was carried out according to the Bacteriological Procedures Manual on Intrahospital Infections-INS¹².

For phenotypic identification, conventional biochemical assays were performed and antimicrobial susceptibility was assessed using the Kirby-Bauer method (agar diffusion method).

Determination of antibiotic susceptibility by antibiogram

It consisted of using a plate with Müller Hinton agar, where a bacterial suspension adjusted to the 0.5 standard of the Mc Farland scale was inoculated. The antibiotics used to evaluate *Staphylococcus aureus* were: Oxacillin, Vancomycin, Penicillin, Imipenem, Ampicillin/Sulbactam and Cefoxitin. Isolates with intermediate rank were considered as

resistant, taking into account the sensitivity or resistance parameters of the Performance Standards for Antimicrobial Susceptibility Testing ¹³.

Determination of cross-reactivity of Oxacillin-resistant *Staphylococcus aureus* strains with other antibiotics.

After assessing the antibiotic resistance of *Staphylococcus aureus*, a second antibiogram was performed on those strains that were resistant to Oxacillin, to determine cross-reactivity with other antibiotics. The antibiotics used were; Ceftazidime, Azithromycin, Cefotaxime, Gentamicin, Amikacin, Ciprofloxacin, Ceftriaxone and Cefuroxime; Oxacillin (was placed in the centre of the agar plate).

Factors associated with *Staphylococcus aureus* carriers

The following epidemiological factors were assessed by means of a survey: overcrowding (if the person shared a bedroom with more than 3 other people), passive or active smoking (if the person smoked or lived with at least one smoker), constant exposure to dust (if the person's locality or home is very exposed to dust), people with chronic respiratory diseases at home (asthma, allergic rhinitis, etc.) and precariousness of housing. Clinical factors such as: recurrent pharyngeal infections, history of acute respiratory infections (colds and flu), history of allergies and self-medication.

Statistical analysis of data

With the results obtained, a database was created using Microsoft Excel 2013. Frequency distribution tables were drawn up to organise the data into joint classes, with the aim of representing the data obtained in percentages, and on the basis of these, to carry out the descriptive analysis and draw graphs to facilitate understanding of the results. The statistical software InfoStat was used.

Results

A total of 150 nasal swab samples were analysed, 43 (28.6 %) positive cultures for *Staphylococcus aureus* were isolated; 107 (71.4 %) negative cultures for *Staphylococcus aureus* were negative.

Regarding the frequency of *Staphylococcus aureus*, it was determined that, of the 43 strains obtained, the professional school of Biological Sciences had the highest number of isolates 18 (12 %), followed by the professional school of Human Medicine 14 (9.3 %), with Nursing being the professional school with the fewest isolates 11 (7.3 %).

Susceptibility profile of *Staphylococcus aureus* isolates

From the isolation of the 43 strains of *Staphylococcus aureus*, the antibiogram was performed in order to evaluate the susceptibility of the microorganism under study, determining that 90.6% of the strains were resistant to Oxacillin, 81.3% to Sulfamethoxazole - Trimethoprim, 95.3% to Penicillin, 34.8% to Cefoxitin, and all strains were 100% sensitive to Imipenem and Vancomycin. Isolates with an intermediate range in the antibiogram readings were considered resistant (Table 1)

	Resistant		Sensible	
		%		%
Cefoxitin	15	34.8	28	65.1
Imipenem	-	-	43	100
Oxacillin	39	90.6	4	9.3
Penicillin	41	95.3	2	4.7
Sulfam. + Trim.	35	81.3	8	18.6
Vancomycin	-	-	43	100

Table 1

Resistance profile of *Staphylococcus aureus* from nasal isolates from students of the Universidad Nacional Pedro Ruiz Gallo.

Cross-reaction of *Staphylococcus aureus* strains resistant to Oxacillin with other antibiotics.

A second antibiogram was performed on the strains that were resistant to Oxacillin, to determine cross-reactivity with other antibiotics, especially cephalosporins. Thirty-nine strains were tested, of which 2.6% were resistant to Cefazidime, 12.8% to Azithromycin, 15.4% to Cefotaxime, and 20.9% to Gentamicin; in contrast, they were 100% sensitive to Amikacin, Ciprofloxacin, Ceftriaxone and Cefuroxime (Table 2).

	Resistant		Sensible	
		%		%
Oxacillin	39	100	00	00.00
Amikacina	-	-	39	100
Azithromycin	5	12.8	34	87.2
Cefazidime	1	2.6	38	97.4
Ciprofloxacin	-	0.00	39	100
Ceftriaxona	-	-	39	100
Cefotaxima	6	15.4	33	84.6
Cefuroxime	-	-	39	100
Gentamicin	9	20.9	30	79.1

Table 2.

Cross-reaction of *Staphylococcus aureus* strains resistant to Oxacillin.

Clinical factors associated with nasal isolates from students at the Universidad Nacional Pedro Ruiz Gallo

All participants in the present study were surveyed in order to detect possible clinical factors associated with bacterial colonisation in the study, which included: Recurrent pharyngeal infections (FI), History of acute respiratory infections (AIR), History of allergies (AA); Self-medication habits (HA).

The data provided by the survey after evaluation of the positive isolates of *Staphylococcus aureus*, showed that 9.3% of the participants had recurrent pharyngeal infections, 41.3% had a history of acute respiratory infections, including colds and flu, 42% suffered from allergies, and 46% had the habit of self-medicating; relating the history with prevalent results, to students with positive isolates and resistant to the antibiotics evaluated. Self-medication was the common clinical factor in all positive *Staphylococcus aureus* isolates.

Epidemiological factors associated with nasal isolates from students at the Universidad Nacional Pedro Ruiz Gallo.

The survey of students from the professional schools evaluated included epidemiological factors that may be associated with the bacterial colonisation under study, considering overcrowding (H), passive smoking (FP), active smoking (FA), constant exposure to dust (EP) and living with people with chronic respiratory diseases (ERC) as predisposing factors.

The results obtained from the survey provided the following information; of the total *Staphylococcus aureus* positive isolates, 2% of participants indicated that they lived in overcrowded conditions, 16% indicated that they were passive smokers, 6.6% active smokers, 44% that they had constant exposure to dust, and 21.3% that they lived with family members with chronic respiratory diseases. The prevalent epidemiological factors were constant exposure to dust and living with patients with chronic respiratory diseases.

Discussion

More local data on CA-MRSA infections are needed so that dermatologists and community physicians can assess the risk of such infections among their patients and avoid inappropriate administration of beta-lactams. No single change in prescribing practices will completely alleviate the selective pressure for the spread of community-acquired MRSA and will not exacerbate resistance in pyogenic streptococci, commonly found along with *S. aureus* in skin and soft tissue infections. The importance of hygiene to prevent the spread of community-acquired MRSA in the community should be emphasised^{14,15}.

Of the samples analysed in our study, 28.6% were found to be positive for the pathogen under study, coinciding with López et al., 2013 who, when evaluating medical students attached to a hospital in Spain, found that 39.3% were colonised by *S. aureus*; 16 likewise Gaona et al, 2009 found that 45.3% of a group of pre-clinical students were carriers of *S. aureus*¹⁷; this disagrees with Villafañe et al., 2013¹⁸, who found that 14.88% of students in the Bacteriology programme were carriers of *S. aureus*. The associated risk factors were the predisposing factors for the personnel evaluated to present the characteristic of being carriers; coinciding in that the positive *S. aureus* isolates were from those students who were more exposed to the hospital environment, as well as those who received antimicrobial therapy in the last three months, also relating the deficiency in hand washing, due to the fact that many students were unaware of the hygiene protocol, and others had not received specific training. The result that differs from the other studies due to the low frequency of nasal carriage is related to the fact that the students evaluated were from minor cycles, without much contact with hospital patients, in addition to complying with biosecurity measures and correct hand washing during the performance of their work.

Although it is true that most research on the frequency and profile of microbial susceptibility is carried out on healthcare personnel and patients hospitalised in critical areas, when the results obtained in the present study were compared, it was determined that they coincide with Sanabria et al, 2008¹⁹, Otth et al., 2007²⁰, Arteaga-Delgado et al., 2016²¹, who investigated *Staphylococcus aureus* carriers in hospital staff, with a percentage of isolates similar to that obtained in the present study; however, studies such as those of González et al, 2005²², Platzer et al., 2008²³ and Paganini et al., 2009²⁴, showed that the isolates obtained for the evaluation of carriers at the intrahospital level is greater than 60%; although research such as that of Aguilar et al. 2009²⁵, in a hospital in our region, determined a low frequency of *Staphylococcus aureus* isolates in the healthcare personnel they evaluated.

With regard to the frequency of *Staphylococcus aureus* isolates in each professional school, Biological Sciences presented the highest number of isolates (12%), followed by the professional school of Human Medicine (9.3%), with Nursing being the professional school with the fewest isolates (7.3%), due to the type of exposure of the students sampled to the hospital environment as they progress through the university cycles, whether in the clinical laboratory area or in the care area; coinciding with Gaona et al, 2009¹⁷ and Villafañe et al., 2013¹⁸, who pointed out that in relation to the advancement in the study cycles, the carrier status is increasing between 2.2% and 3.1% respectively. In the hospital setting, the difference between one area and another will be determined by the direct contact between healthcare staff and patients, with the areas with the highest frequency of carriers of pathogenic bacteria being neonatology, surgery and gynaecology, as detailed by Aguilar et al. 2009²⁵ in their research.

Regarding the antibiotic susceptibility profile, the *Staphylococcus aureus* strains evaluated were 90.6% resistant to oxacillin, 81.3% resistant to Sulfamethoxazole-Trimethoprim and 34.8% to ceftiofur; differing from Gaona et al, 2009, whose strains under study were all sensitive to oxacillin in a first test, in a second evaluation 1.3% of the strains showed resistance to oxacillin, they did not evaluate resistance to SXT or ceftiofur; López, et al., 2013¹⁶ did not evaluate resistance to oxacillin and ceftiofur, but did evaluate resistance to SXT, finding all their isolates sensitive. In reference to hospital isolates, Mamani et al., 2003, found that 32% of *S. aureus* strains were resistant to oxacillin; González et al., 2005, found oxacillin resistance in 9.3% of their isolates.

In *Staphylococcus aureus*, methicillin resistance can be detected by the diffusion technique, with oxacillin and/or ceftiofur discs. Resistance to oxacillin is induced by the *mecA* gene and implies resistance to all beta-lactams, which is why antibiogram evaluation must be careful; in case of heteroresistance, strains are often shown to be sensitive to many beta-lactams, an interpretation that can lead to therapeutic failure, strains with homogeneous resistance to oxacillin show high level of cross-resistance to all beta-lactams including penicillins, cephalosporins, carbapenems

and monobactams. Cefoxitin-resistant *S. aureus* strains also indicate the presence of Susceptibilidad antibiótica de *Staphylococcus aureus* de aislados nasales en estudiantes del norte de Perú, thus resistance to all beta-lactams, cefoxitin sensitivity rules out methicillin heteroresistance ²⁶

The penicillin evaluation showed resistance in 95.3% of the isolated strains, coinciding with Zelaya et al., 2001 ²⁷ who isolated *Staphylococcus aureus* in 61.67% of samples from nasal cavities and 46.67% from health personnel at a hospital in Ica, reporting that all isolates were resistant to penicillin; Sanabria et al, 2003 ²⁸ in Paraguay and Otth et al., 2007 ²⁰ in Chile, in a similar work reported Penicillin resistance in 98% and 86%, respectively, in *S. aureus* isolates from hospitalised patients. The mechanism of penicillin resistance is currently very frequent in *S. aureus*, with the prevalence of resistant strains being around 90%, figures that are variable according to the different institutions and hospitalisation units.

100% of the strains tested showed sensitivity to Vancomycin and Imipenem, coinciding with Gaona et al., 2009 and Villafañe et al., 2013, who obtained absolute sensitivity results with vancomycin, not evaluating resistance with Imipenem in both cases. In clinical isolates, Mamani et al., in a hospital in Lima, and Sanabria et al., in a hospital in Paraguay, both in 2003 obtained 100% vancomycin-sensitive strains; in Ica, Zelaya et al., 2001 determined that 94.60% of *Staphylococcus aureus* strains isolated from nasal cavities were sensitive to imipenem; Aguilar et al. in 2009 in Lambayeque did not find multi-resistance in their research.

When analysing the susceptibility patterns obtained, it could be seen that only 2 strains (4.7%) were resistant to a single antibiotic, 6 strains (13.9%) to two antibiotics, 23 strains (53.4%) to three antibiotics, 11 strains (25.5%) to four antibiotics; finding only 1 strain (2.3%) sensitive to all antibiotics evaluated; showing cases of multiresistance; not agreeing with López, et al, 2012, Gaona et al., 2009 and Villafañe et al., 2013, who in their research with university students did not find multiresistance in the strains they evaluated; in the hospital environment they agree with Mendoza et al, 2000 ²⁹, who found that 50 % of the isolates obtained were *methicillin-resistant Staphylococcus aureus* (MRSA), all of which were multiresistant, disagreeing with Sanabria et al, 2003 in their investigation of nasal carriers from three health centres determined that 21% of the isolated strains were methicillin-resistant, with only one strain resistant to all the antibiotics evaluated, except vancomycin, while Otth et al., 2007 in their study in hospitalised patients, outpatients and carriers, found six patterns of resistance in *S. aureus* strains; no strains resistant to vancomycin were found.

In the evaluations carried out, no specific resistance profile pattern was found among the *Staphylococcus aureus* strains that were analysed, showing that the resistance patterns observed indicate the frequency and consumption of antibiotics used by university students. To perform the analysis of the cross-reaction with other antibiotics to the strains that were resistant to Oxacillin, 39 strains were evaluated, of which

2.6% showed resistance to Ceftazidime, 12.8% to Azithromycin, 15.4% to Cefotaxime, and 20.9% to Gentamicin; on the other hand, they were 100% sensitive to Amikacin, Ciprofloxacin, Ceftriaxone and Cefuroxime; in agreement with Sanabria et al, 2003, who evaluated strains of methicillin-resistant *Staphylococcus aureus* and all showed cross-resistance to at least 4 antibiotics. Although research on bacterial susceptibility and resistance profile for *S. aureus* exists, no further work was found on the analysis of antibiotic cross-reactivity for our microorganism under study.

Regarding the detection of possible clinical factors associated with bacterial colonisation of *Staphylococcus aureus*, the data provided by the survey after evaluation of the positive isolates determined that 9.3% of the participants had recurrent pharyngeal infections and 41.3% had a history of acute respiratory infections, including colds and flu, either due to some type of respiratory virus that favours bacterial colonisation, 42% suffered from allergies, and 46% were in the habit of self-medicating; The background was related to prevalent results, to students with positive and resistant isolates to the antibiotics evaluated. Self-medication was the common clinical factor in all positive *Staphylococcus aureus* isolates. Villafañe et al., 2013 considers that hospitalisation and living with people who work in hospitals are risk factors that allow the acquisition of nosocomial strains that can subsequently be transmitted to communities; López, et al., 2013 found that a considerable percentage of students who lived with family members who worked in a health centre (31.4%), and suffered from asthma (9.3%).

Regarding the detection of possible clinical factors associated with *Staphylococcus aureus* bacterial colonisation, the data provided by the survey after the evaluation of positive isolates determined that 9.3% of the participants had recurrent pharyngeal infections and 41.3% had a history of acute respiratory infections, including colds and flu, either due to some type of respiratory virus that favours bacterial colonisation, 42% suffered from allergies, and 46% were in the habit of self-medicating; The background was related to prevalent results, to students with positive isolates that were resistant to the antibiotics evaluated. Self-medication was the common clinical factor in all positive *Staphylococcus aureus* isolates. Villafañe et al., 2013 considers that hospitalisation and living with people who work in hospitals are risk factors that allow the acquisition of nosocomial strains that can subsequently be transmitted to communities; López, et al., 2012 found that a considerable percentage of students who lived with family members who worked in a health centre (31.4%), and suffered from asthma (9.3%). A total of 150 nasal swab samples were analysed, resulting in 43 positive isolates for *Staphylococcus aureus*, representing 28.6% of the total study population. Of the *Staphylococcus aureus* isolates, 90.6% were resistant to Oxacillin, 19% to Sulfamethoxazole - Trimethoprim, 95.3% to penicillin, 18.6% to Vancomycin and 38% to Cefoxitin, with all isolates being sensitive to Imipenem.

When analysing the cross-reactivity of the 39 strains of *Staphylococcus aureus* resistant to Oxacillin with other antibiotics, especially with cephalosporins, it was determined that 2.6% showed resistance to Ceftazidime, 12.8% to Azithromycin, 15.4% to Cefotaxime and 20.9% to Gentamicin; all the strains tested were sensitive to Amikacin, Ciprofloxacin, Ceftriaxone and Cefuroxime. Of all the students at the Universidad Nacional Pedro Ruiz Gallo who participated in the present investigation, it was determined that the Professional School of Biological Sciences had the highest frequency of *Staphylococcus aureus* isolates.

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Conflict of interest declaration

The authors declare that there is no conflict of interest.

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