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Staphylococcus aureus in Veterinary Students of Different Levels: Prevalence, Risk Factors and Antimicrobial Resistance

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Abstract

Staphylococcus aureus is a coccus housed in healthy people but also implicated in fatal infections. The emergence of multi-resistant strains, like MRSA, lead to a highly specific antibiotic treatment and produce prominent mortality rates, in animals and mankind. Veterinarians, health workers, and people who have continued contact with animals suffer greater risks because of the interspecies transmission of the bacteria.

In this study, the significance of veterinary students as *S. aureus* carriers was evaluated, along with its prevalence, the Erythromycin, Enrofloxacin, Doxycycline, Gentamicin and Amoxicillin-Clavulanic Acid resistance featured, and its molecular basis. Additionally, some pathogenicity factors were evaluated.

A 44% of *S. aureus* prevalence was found. None of the factors collected showed a statistical correlation with the presence or non-presence of *S. aureus*. Slime production was detected in 45.45% of isolates. Among the 22 *S. aureus* isolates, 10 (45.45%) showed resistance or an intermedius result to one (36.36%), two (4.54%) or three (4.54%) antibiotics. Erythromycin was, by difference, the antibiotic with the highest percentage of resistant or intermedius isolates (10/22, 45.45%), followed by Enrofloxacin (2/22, 9.09%) and Doxycycline (1/22, 4.54%). All the isolates were susceptible to Amoxicillin-Clavulanic Acid and Gentamicin.

All the isolates harboured the 16st genes. Three isolates harboured Erythromycin resistance genes (13.63%), two of them *ErmC*, and one *ErmB* and *ErmC*. Three of the isolates harboured Tetracycline resistance genes, all of them *TetK* (13.63%). The pathogenicity factor PVL gene was detected in only one isolate (4.54%). The pathogenicity factor ACME gene was detected in four isolates (18.18%).

ABBREVIATIONS

***S. aureus*:** *Staphylococcus aureus*; MRSA: Methicillin Resistant *Staphylococcus aureus*; PVL: Panton Valentine Leucocidin; ACME: Arginine Catabolic Mobile Element; LA-MRSA: Livestock-associated MRSA; HA-MRSA: Healthcare-associated MRSA; CA-MRSA: Community-associated MRSA; PCR: Polymerase Chain Reaction.

INTRODUCTION

Staphylococcus aureus is a Gram-positive coccus naturally housed in healthy people's nasal cavity and skin, colonizing around 25-40% of the population [1]. In people with a compromised immune system, *S. aureus* infections become severe or fatal if it achieves to break the exterior body barrier. Generally, bacteraemia is implicated in fatal infections, which had a 65-70% mortality before the spread of the antibiotics use. Nowadays, despite the latest techniques and the availability of some new antibiotics, due to the development of antimicrobial resistance, there is 20-40% mortality within 30 days of bacteraemia [1].

There has been an increase in the level of drug resistance since 1960, especially methicillin-resistance, correlated with high morbidity, mortality and health-care costs due to factors like an increased virulence and a lesser effective treatment [3]. Methicillin-Resistant *S. aureus* (MRSA) appears as a result of the acquisition of the capacity of encoding methicillin resistance, through a relatively stable *staphylococcal* cassette chromosome [4]. This feature guaranteed resistance to β-lactam antibiotics, such as cephalosporins [5].

There has been a growing attention on MRSA hosted in animals, especially in pigs, noted in countries like Spain [6]. It is estimated that, annually, more than 150000 patients are affected with MRSA infections, and those represent an extra cost of EUR 380 million for EU healthcare system [7]. Reasonably higher rates

of nasal carriage of MRSA by humans in contact with pigs (for example, veterinarians) have been shown in epidemiological studies [6]. After 3-4 hours of exposure to a MRSA-positive pig farm, the bacteria could be found 22% of the time in veterinary students' samples, but it didn't become established [1].

Livestock-associated MRSA (LA-MRSA), compared to healthcare (HA-MRSA) and community-associated MRSA (CA-MRSA), show less transmissibility and virulence [8]. Each one of these has obtained characteristics that help them to survive in a specific environment. HA-MRSA has developed numerous antibiotic resistance genes that adapt itself to the hospital settings. On the other hand, CA-MRSA possesses the arginine catabolic mobile element (ACME), which through the modulation of the skin pH and the degradation of polyamines enhances its survival in the human skin. At last, LA-MRSA has lost human-specific virulence factors to gain others specific ones for the livestock species they live in [9].

LA-MRSA situation in Europe is directly correlated with the pig and calf farms density present in the territory [8]. The death of four individuals in Denmark, 2014, infected with the CC398 strain of LA-MRSA attract the attention from the European media and political people, who start to consider the importance of those cases. Due to this event, MRSA is included under the denomination of 'special health issue' of 'Antimicrobial Resistance' [10]. Besides pigs and cows, the CC398 strain has been found in poultry, horses, dogs, cats and rodents; with a swiftly increasing prevalence worldwide. In Southeast Asia, CC9 is the primary strain and, in addition, other strains have been reported, including ST425, CC121, CC5, among others [11].

It has been established by some studies that people involved in the health science field are more likely to carry *S. aureus* and, especially, MRSA. A short-term exposure to LA-MRSA-positive pork farms makes possible the detection of the bacteria in veterinary students 22% of the time, despite the negative result obtained later because of the lack of establishment of the strain [1]. In Veterinary students and doctors in contact with farms, the MRSA prevalence was 160 times higher than in patients at hospital admissions [12]. The spread of MRSA is promoted by the lack of awareness about the bacteria among veterinary students and veterinarians [13] that may be the primary source of infection for animals at veterinary hospitals [14].

The main objective of this study was to evaluate the significance of veterinary students as *S. aureus* carriers. Other objectives were to assess the prevalence of *S. aureus* in veterinary students of the University of Las Palmas de Gran Canaria (Canary Island, Spain), to look for correlation between the presence of the bacteria and some related risk factors, to assess the presence of antibiotic resistance, studying its potential evolution through the years of the veterinary medicine degree and to establish its molecular basis.

MATERIALS AND METHODS

Study subjects and sampling

Samples were collected from 50 students, 25 from the first and second years of the Veterinary Medicine Degree, and another 25 from the fourth and fifth years. Samples were taken from the right and left nostrils with sterile cotton swabs. Each pair of samples came along with a survey that helps with the future evaluation of the results. This survey asks about the contact of the test subjects with animals or health centres, besides about the contact of those who live with them and recent treatments or health issues that can affect the results.

Isolation and identification of S. aureus

All samples were cultured on Mannitol Salt Agar (Difco, Mo, USA) 24-48 h. at 37ºC. Due to the fermentation of mannitol, colonies suspicious to be *S. aureus* appear as yellow with yellow zones on the media. The selected ones were isolated on Mannitol Salt Agar and were checked out through a Gram stain and microscopy observation. If the bacteria were Gram-positive cocci arranged in clusters, the production of catalase enzyme was evaluated doing the reaction with hydrogen peroxide. Lastly, if catalase production was detected, two agglutinationtests were done: Pastorex Staph-Plus (BioRad) for Clumping Factor, Protein A and capsular polysaccharides 5 and 8; and Staph-Plus (BioMerieux) for Clumping Factor, Protein A and Glicopolysaccharide Antigen 18. If agglutination occurred in one of these tests, the bacteria were considered *S. aureus*.

Antimicrobial susceptibility assays

The antimicrobial susceptibility was evaluated with an Agar Diffusion test (Kirby-Bauer test) on Mueller-Hinton Agar (Difco, Mo, USA) [15]. Antibiotics tested were Amoxicillin-Clavulanic Acid, Erythromycin, Enrofloxacin, Doxycycline and Gentamicin. A D-test for Erythromycin and Clindamycin resistance was also done to the isolates that appear resistant to Erythromycin in the Kirby-Bauer test. Because of the relationship between Macrolides resistance and Clindamycin in staphylococci, the D-test clarifies the association of these two. The test was done putting, separated by 15-25mm, a 15µg Erythromycin disc and a 2µg Clindamycin disc in a Mueller-Hinton Agar (Difco, Mo, USA) previously cultured, resembling the Kirby-Bauer test. If the Clindamycin halo is detected as sensitive, but there is a flattening, it reflects an inducible expression of Clindamycin resistance. If this happens without flattening, Erythromycin resistance came up from an active expulsion pump. This test can reflect a common resistance to both antibiotics too [16].

Slime production

A variation of the Christensen method was used for a quantitative evaluation of the slime production [17]. Bacteria were cultured on 2ml of Brain Heart Infusion (BHI) 24h at 37ºC. The content was drained, and the tube was washed with a Methyl Violet solution. When a bacterial growth halo was observed stained in the tube, it was considered that the bacteria produced slime.

DNA extraction and PCR

Bacteria were cultured on 2ml of Brain Heart Infusion (BHI) 24h at 37ºC. The sediment extracted from 1.5ml of culture, centrifuged twice at 14000g for 5 minutes, was resuspended in 0.5ml of distilled water, and it was heated at 94ºC for 5 minutes and centrifuged at 14000g for 5 minutes. Its conservation was done at -20ºC.

Nine Polymerase Chain Reactions (PCR) were done to detect nine different genes: 16S rDNA as a *S. aureus* amplification control; *ErmA*, *ErmB*, and *ErmC*as Erythromycin specific resistance genes; *TetM*, *TetL*, and *TetK* as Doxycycline specific resistance genes; and *LukPV* and *ArcA* as PVL and ACME genes (pathogenicity factors). These PCR were done as previously described [18-22]. The final mixes used in the reaction had a 25µl volume, with 1 or 2 µl of DNA, Tris-HCl NaCl, $MgCL_2$, 3-phosphate deoxyribonucleic, primers and Taq Polymerase (Bioline, UK). The primers are described in Table 1. A Bio-Rad Thermo-Cycler was used.

The amplification products were analysed by electrophoresis in 2% Agarose gels and stained with DNA-Dye Non Tox (PanReac AppliChem). A negative control composed of sterile water and a positive control for each primer (Table 1) were used.

Statistical analysis

Through a linear regression analysis, the correlation between the presence of the bacteria and the potential influential data retrieved through the surveys was analysed. If a p value is equal or lower than 0.05, it can be said that a correlation exists between the data and the presence of *S. aureus* in our samples. These analyses were made through the American Centre of Disease Control and Prevention tool "EpiInfo".

RESULTS AND DISCUSSION

Samples

Among the 50 collected samples, 22 yielded *S. aureus* (44%), 13 from the first and second year (52% of the students of these years), and 9 from the fourth and fifth year (36% of this group of students). A study done in a veterinary hospital in Malaysia reported a prevalence of MRSA of 23.3% in veterinary students and personnel [13]. Among 152 students and doctors in contact with livestock from the Netherlands, another study found a MRSA carriage of 4.6% [12]. In a study made in Denmark and Belgium, a 9-5% and a 1-4% of MRSA prevalence was reported in livestock and veterinarians, respectively [8]. A 33.1% prevalence of *S. aureus* and a 5.1% of MRSA among healthcare workers in paediatrics departments were reported by a study in Brazil [23]. The highest prevalence found in our study could be explained by the lower number of samples.

Despite all the probably related factors extracted from the literature, asked in the survey and collected, none of them showed a statistical correlation with the presence or non-presence of *S. aureus* in our study. Each fact is explained individually below.

The mean student age was 21.36 with a range from 18 to 41 years. This is not considered a risk factor among the studies consulted, but there is a reported difference between children and the elderly for MRSA infections [24], and it is a confounding factor in another study [23]. There were 32 female and 18 male students (64% and 36%, respectively). There are no significant differences registered in the male/female ratio. Only 9 of the students (18%) wore a nasal piercing. These are related to MRSA bloodstream infections, like endocarditis, when are performed under suboptimal hygienic conditions in places where *S. aureus* normally colonized the body [21,25]. Four of the students (8%) were tobacco smokers, 2 of them sporadic smokers, and the other 2 regular smokers (4%). However, 16 of the students (32%) were smokers of unspecified substances, 15 of them sporadic smokers (30%), and one regular smoker (2%). It is known that the exposure to cigarette smoke increases bio film formation and host cell adherence [26], increase its resistance to macrophage killing, cell lysis and antimicrobial peptide [27]; so, it may be an additional factor that contributes to the susceptibility to *S. aureus* infections in smokers.

Among the survey respondents, 43 had animals (86%), mainly dogs (69.7%) and cats (48.8%). Dogs and cats have been reported as carriers of LA-MRSA, specifically the strain CC398 [11], and may serve as reservoirs. The contact with animals is a daily routine for a veterinary student, but the addition of owning a pet in home increase this association. Eighteen of the survey respondents had done external practices (36%), 17 had done the small animal's clinical service (34%), and 10 the large animal's

Table 1: Primers used in PCR.

PRIMER	SEQUENCE	POSITIVE CONTROL	REFERENCE
16st	5´ YCAGCTCGTGTCGTGAGATGTY ´3 3´ AATCATTTGTCCCACCTTCG 5´	*S. aureus* +	--
ErmA	5´ YTCTAAAAAGCATGTAAAAGAAY 3´ 3´ YTGATTATAATTATTTGATAGCTTCY 5´	P8	Sutcliffe et al.,
ErmB	5´ YGAAAAGGTACTCAACCAAATAY 3´ 3´ YCATTTGTTAAATTCATGGCAATGAY 5´	G5-11	Sutcliffe et al.,
ErmC	5´ YTCAAAACATAATATAGATAAAY 3´ 3´ YTAACTGCTAAATTTGTTATAATCGY 5´	L9	Sutcliffe et al.,
TetL	5´ YCATTTGGTCTTATTGGATCGY! 3´ 3´ YCAATATCACCAGAGCAGGCTY 5´	16A	Aarestrup et al.,
TetL	5´ YGTTAAATAGTGTTCTTGGAGY 3´ 3´ YCTAAGATATGGCTCTAACAAY 5´	16A	Aarestrup et al.,
TetK	5´ YTAGGGGGAATAATAGCACATTY 3´ 3´ YAATCCGCCCATAACAAATAY 5´	1A	Aarestrup et al.,
LukPV	5´ YATCATTAGGTAAATGTCTGGACATGATCCAY 3´ 5´ YGCATCAACTGTATTGGATAGCAAAAGCY 3´	4A	Vento et al.,
arcA	5´ YGAGCCAGAAGTACGCGAGY 3´ 5´ YCACGTAACTTGCTAGAACGAGY 3´	*S. aureus* +	Vento et al.,

Table 2: Slime production and antimicrobial resistance.

ISOLATE	SLIME	ANTIBIOTIC RESISTANCE				
		Amoxicillin Clavulanic Acid	Erythromycin	Enrofloxacin	Doxycycline	Gentamicin
00	+	S	S	S	S	S
01	-	S	I	S	S	S
04	-	S	I	S	S	S
06	-	S	I	S	S	S
09	+	S	S	S	S	S
12	-	S	I	S	S	S
16	-	S	S	S	S	S
21	+	S	I	I	S	S
24	+	S	I	R	R	S
27	-	S	I	S	S	S
29	-	S	S	S	S	S
30	+	S	S	S	S	S
31	+	S	R	S	S	S
34	+	S	R	S	S	S
36	-	S	S	S	S	S
40	-	S	S	S	S	S
41	+	S	S	S	S	S
42	-	S	S	S	S	S
43	-	S	S	S	S	S
44	-	S	R	S	S	S
45	+	S	S	S	S	S
46	+	S	S	S	S	S

S: Susceptible; I: Intermediate; R: Resistant

Table 3: Resistance and pathogenicity factors genes detected by PCR.

ISOLATE	PCR								
	AMPLIFICATION CONTROL	ERYTHROMYCIN			DOXYCYCLINE			PATHOGENICITY FACTORS	
	16st	ErmA	ErmB	ErmC	TetL	TetM	TetK	lukPV	arcA
00	+	-	-	-	-	-	-	-	-
01	+	-	-	-	-	-	-	-	-
04	+	-	-	-	-	-	-	-	-
06	+	-	-	-	-	-	-	-	-
09	+	-	-	-	-	-	-	-	+
12	+	-	-	-	-	-	-	-	-
16	+	-	-	-	-	-	-	-	+
21	+	-	-	+	-	-	+	-	-
24	+	-	-	-	-	-	+	-	-
27	+	-	-	-	-	-	-	-	+
29	+	-	-	-	-	-	-	-	-
30	+	-	-	-	-	-	-	-	-
31	+	-	+	+	-	-	-	-	-
34	+	-	-	+	-	-	+	-	-
36	+	-	-	-	-	-	-	-	-
40	+	-	-	-	-	-	-	+	-
41	+	-	-	-	-	-	-	-	-
42	+	-	-	-	-	-	-	-	-
43	+	-	-	-	-	-	-	-	+
44	+	-	-	-	-	-	-	-	-
45	+	-	-	-	-	-	-	-	-
46	+	-	-	-	-	-	-	-	-

clinical service (20%). Veterinary students normally have contact with sources of zoonotic pathogens since the first years of their studies [28], but the clinical services done in their final year, and the 100 hours of compulsory external practices increase the time a student approach to the field work, and, in turn, raise the risk of a colonization.

Only 7 (14%) of the students lived with someone who works with animals, and 8 (16%) lived with someone who work in a health institution. Eight of the students had worked in a health centre (16%), 4 of them on the last 3 months (8%). Working in a healthcare institution is recognized as an important risk factor for infection [23], and animals, mostly livestock, have been pointed out as great reservoirs of MRSA [6-8,10]. Antibiotic treatment was given lately to 22 of the students (44%), in the last 3 months for 9 of them (18%); one had received immunotherapy treatment recently, and 11 had received cortico therapy (22%), 2 of them nowadays, 3 recently and 6 in the past. Despite the exclusion of recent antibiotic users in some studies [19], it seems like there is no significant association between this and the *S. aureus* nasal carriage. This idea is supported by other studies [29].

Only one of the survey respondents was hospitalized in the last 6 months. Six of the students were suffering a skin or soft issues infection (12%), 5 were suffering or had suffered sinusitis (10%), 11 were suffering or had suffered asthma (22%), and 17 rhinitis (34%). Skin and soft tissue infections (SSTIs) were the most common *S. aureus* infection reported in Europe, being the pathogen in 71% of the cases, with 22.5% of the isolates being MRSA [30]. Sinusitis have been reported in some case-control studies [24,31], and smokers who suffer chronic or acute sinusitis have a higher incidence of *S. aureus* as the pathogen [32]. Asthma has a relatively weak association with *S. aureus* nasal colonization [33].

It should be noted that *S. aureus* was isolated from the only person that was hospitalized among the students. Being hospitalized seems to be a risk factor for MRSA infection. More precisely, a study reported that being hospitalized generate a high rate of MRSA infection, especially a period of hospitalization longer than 7 days within the last 6 months [24]. Other study reported only one MRSA carrier, a female veterinary student who had been hospitalized six months prior to the screening and had been subjected to intensive antimicrobial therapy [29].

None of the data collected shown any statistical influence on the presence or non-presence of *S. aureus*. Through a linear regression analysis, we studied the correlation between the presence of the bacteria and the potential influential data. All the p values appear higher than the limit ($p > 0.05$). The closest one to that threshold was the reception of antibiotic treatment in the last year, with a p value equal to 0.07. Correlations were not found probably because the small number of samples included in our study.

Slime production

Slime production was detected in 10 isolates (45.45%). The results are shown in Table (2). In other studies, slime production was found in a 77.6% in nasal samples of multiple sclerosis patients [34], and in a 36.5% in emergency department patients [35]. This ability to generate bio film is demonstrated through the presence of *Ica* and adhesion genes, and let the bacteria become multidrug resistant in some cases, thanks to the alleviate of the immune system and the resistance of the recalcitrant bio films [36]. However, a dispersed mode of growth is favoured rather than a bio film-related mode during *S. aureus* nasal colonization [37].

Antibiotic resistance

Among the 22 *S. aureus* isolated, 10 (45.45%) showed resistance or an intermedius result to one (36.36%), two (4.54%) or three (4.54%) antibiotics. Antimicrobial multi-resistance is defined when resistance to three or more different classes of antimicrobial drugs is found [24]. In a study conducted with MRSA strains from pigs and veterinary students, 95.55% of the isolates were resistant to 3 or more antibiotics, and one was resistant to 10 antibiotics [1]. In another study, all MRSA isolates were resistant from 6 to 11 antibiotics, with a variable rate of resistance to Ampicillin, Amoxicillin/clavulanic Acid, and Enrofloxacin [38].

The highest percentage of resistant or intermedius isolates was found against Erythromycin (10/22, 45.45%), followed by Enrofloxacin (2/22, 9.09%) and Doxycycline (1/22, 4.54%). All the isolates were susceptible to Amoxicillin-Clavulanic Acid and Gentamicin. The results are shown in Table (2). In a study where *S. aureus* was evaluated in dairy cattle herds, related swine farms and humans in contact with herds, 57.8% of the isolates were resistant to Gentamicin, 65.6% to Erythromycin, and 70.3% to Enrofloxacin [39]. In another study, with samples from pigs and veterinary students in contact with them, a significant difference in resistance levels was seen with Enrofloxacin, being the students' samples more resistant [1]. Regarding MRSA in bulk tank milk, 82% of the strains showed resistance to Amoxicillin/Clavulanic Acid, and 9% to Enrofloxacin [38]. *S. aureus* colonies evaluated in healthy military service members presented high susceptibility to Doxycycline, except 3 of the strains [19].

D-test was done to Erythromycin resistant isolates. One reflected an inducible expression of Clindamycin resistance; another appeared resistant to both antibiotics, and about the last one, its Erythromycin resistance came up from an active expulsion pump.

Molecular testing

Results are shown in Table (3). All the isolates presented the 16st genes (amplification control). Three isolates harboured Erythromycin resistance genes (13.63%), two of them *ErmC* (21 and 34), and one for *ErmB* and *ErmC* (31). There was one more isolate that presented resistance to Erythromycin but did not carry the gene. The isolate 21 had an intermedius result in the Kirby-Bauer test. A transposon Tn554 (*ErmA*) or a small plasmid (*ErmC*) normally encode Erythromycin resistance [5]. In a study of *S. aureus* colonies of healthy military service members, the *ErmA* gene was found in 17% and 11% of the US and Afghanistan personnel, respectively; and the *ErmC* gene was found in 25% of the MRSA isolated [19]. In another study, where livestock veterinarians were sampled, 62.5% of the bacteria isolated had the *ErmC* gene, but one of the resistant strains did not have the gene [8]. In *S. aureus* strains isolated from blood cultures from a Taiwan Bacteriology Institute with a 12.2% of Erythromycin resistance, the *ErmB* gene was more frequent (35%) that *ErmC* (27% or *ErmA* (21%) [40].

Three of the isolates harboured Tetracycline resistance

genes, all of them *TetK* genes (13.63%). One of these appears as Doxycycline resistant in the antibiogram, but the other two appear as susceptible. In the study implying military service members, in 87% of the *S. aureus* isolates, *TetK* and *TetM* were identified, in a 98% and 94% of Doxycycline resistant strains [19]. In another study, with MRSA isolated from hospitals in Malaysia, *TetM* was more prevalent that *TetK* (97.8% versus 42.7%, respectively) [41].

Only one of the *S. aureus* isolated harboured the pathogenicity factor PVL gene, *Luk-PV* (4.54%). The Panton-Valentine leucocidin is an exotoxin that causes leucocytosis by forming pores in their membrane and tissue necrosis [42]. PVL is not normally found in *S. aureus* or MRSA isolated from animals, or in LA-MRSA [8,10,38]. In a study with *S. Aureus* isolated from healthy military service members, 25% of the MRSA obtained possessed PVL genes [19].

Four of the isolates harboured the pathogenicity factor ACME gene, ArcA (18.18%).The Arginine Catabolic Mobile Element is a feature characteristic of the CA-MRSA, which enable the degradation of polyamines and the pH modulation at the skin surface [43]. In a study with MRSA isolated in England and Wales *Staphylococcus* reference laboratories, the ArcA gen was detected in 17 of 203 samples (8.37%) [44]. In another study, with CA-MRSA obtained from clinical infections and screening procedures in Sweden, ACME genes were detected in 8.8% of the strains [45].

CONCLUSION

The prevalence of *Staphylococcus aureus* in our study was higher than the one found in the literature. Despite all the probably related factors extracted from the literature, asked in the survey and collected, none of them showed statistical correlation with the presence or non-presence of *S. aureus*.

None of our *S. aureus* isolates can be defined as multi-resistant. The highest rate of resistance was detected against Erythromycin, followed by Enrofloxacin and Doxycycline. Two of three Erythromycin resistant isolates detected harboured the *ErmB* and the *ErmC* genes. One isolate harboured the *ErmC* gene and appear as an intermedius result, probably because a weak gene expression. Another mechanism may be involved in the resistant isolate that not presented any of the appointed genes above. The Doxycycline resistant isolate detected harboured the *TetK* gene. Another two isolates harboured the *TetK* gene, despite the lack of Doxycycline resistance, probably due to an absence of gene expression.

Regarding pathogenicity factors, only one isolate harboured PVL gene. This is a lower prevalence than the found in the literature. On the other hand, four isolates harboured the ACME gene, a higher prevalence than the found by other authors.

Relationship among different risk factors or academic course and prevalence of *Staphylococcus aureus* was not found.

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