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#### **Research Article**

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

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- Staphylococcus aureus
- Resistance
- Pathogenicity factors
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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  $\beta$ -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

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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 humanspecific 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) 24-48h.at 37°C. 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 37°C. 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  $37^{\circ}$ C. 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 94°C for 5 minutes and centrifuged at 14000g for 5 minutes. Its conservation was done at -20°C.

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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<sub>2</sub>, 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

PRIMER	SEQUENCE	POSITIVE CONTROL	REFERENCE	
16st	5' YCAGCTCGTGTCGTGAGATGTY '3 3' AATCATTTGTCCCACCTTCG 5'	S. aureus +		
ErmA	5´ YTCTAAAAAGCATGTAAAAGAAY 3´ 3´ YTGATTATAATTATTTGATAGCTTCY 5´	Р8	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.,	

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ISOLATE	SLIME	ANTIBIOTIC RESISTANCE						
		Amoxicillin Clavulanic Acid	Erythromycin	Enrofloxacin	Doxycycline	Gentamicin		
00	+	S	S	S	S	S		
01	-	S	Ι	S	S	S		
04	-	S	Ι	S	S	S		
06	-	S	Ι	S	S	S		
09	+	S	S	S	S	S		
12	-	S	Ι	S	S	S		
16	-	S	S	S	S	S		
21	+	S	Ι	Ι	S	S		
24	+	S	Ι	R	R	S		
27	-	S	Ι	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.									
	PCR								
ISOLATE	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

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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 multiresistant. 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.

#### REFERENCES

- 1. Frana TS, Beahm AR, Hanson BM, Kinyon JM, Layman LL, Karriker LA, et al. Isolation and Characterization of Methicillin-Resistant Staphylococcus aureus from Pork Farms and Visiting Veterinary Students. PLoS ONE. 2013; 8: 53738.
- 2. Varshney AK, Kuzmicheva GA, Lin J, Sunley KM, Bowling RA, Kwan TY, et al. A natural human monoclonal antibody targeting Staphylococcus Protein A protects against Staphylococcus aureus bacteremia. PLoS

ONE. 2018; 13: 0190537.

- Adani S, Bhowmick T, Weinstein MP, Narayanan N. Clinical Outcomes of Patients with Methicillin-Resistant Staphylococcus aureus Bacteremia Treated with Vancomycin at an Institution with Suppressed MIC Reporting: Impact of Vancomycin MIC. Antimicrob Agents Chemother. 2018; 62: 02512-17.
- Peacock SJ, de Silva I, Lowy FD. What determines nasal carriage of Staphylococcus aureus? Trends Microbiol. 2001; 9: 605-610.
- Lindsay JA. Hospital-associated MRSA and antibiotic resistance-what have we learned from genomics? Int J Med Microbiol. 2013; 303: 318-323.
- 6. Johnson AP. Methicillin-resistant Staphylococcus aureus: the European landscape. J Antimicrob Chemother. 2011; 66: 43-48.
- Kock R, Becker K, Cookson B, van Gemert-Pijnen JE, Harbarth S, Kluytmans J, et al. Methicillin-resistant Staphylococcus aureus (MRSA): burden of disease and control challenges in Europe. Euro Surveill. 2010; 15: 19688.
- Garcia-Graells C, Antoine J, Larsen J, Catry B, Skov R, Denis O. Livestock veterinarians at high risk of acquiring methicillin-resistant Staphylococcus aureus ST398. EpidemiolInfect. 2012; 140: 383-389.
- Hau SJ, Kellner S, Eberle KC, Waack U, Brockmeier SL, Haan JS, et al. Methicillin-Resistant Staphylococcus aureus Sequence Type (ST) 5 Isolates from Health Care and Agricultural Sources Adhere Equivalently to Human Keratinocytes. Appl Environ Microbiol. 2018; 84: 02073-17.
- 10. Kinross P, Petersen A, Skov R, Van Hauwermeiren E, Pantosti A, Laurent F, et al. Livestock-associated meticillin-resistant Staphylococcus aureus (MRSA) among human MRSA isolates, European Union/ European Economic Area countries, 2013. Euro Surveill. 2017; 22.
- 11.Bal AM, Coombs GW, Holden MTG, Lindsay JA, Nimmo GR, Tattevin P, et al. Genomic insights into the emergence and spread of international clones of healthcare-, community- and livestock-associated meticillinresistant Staphylococcus aureus: Blurring of the traditional definitions. J Glob Antimicrob Resist. 2016; 6: 95-101.
- 12.Wulf M, van Nes A, Eikelenboom-Boskamp A, de Vries J, Melchers W, Klaassen C, et al. Methicillin-resistant Staphylococcus aureus in veterinary doctors and students, the Netherlands. Emerging Infect Dis. 2006; 12: 1939-1941.
- 13.Aklilu E, Zunita Z, Hassan L, Cheng CH. Molecular epidemiology of methicillin-resistant Staphylococcus aureus (MRSA) among veterinary students and personnel at a veterinary hospital in Malaysia. Vet Microbiol. 2013; 164: 352-358.
- 14.Duquette RA, Nuttall TJ. Methicillin-resistant Staphylococcus aureus in dogs and cats: an emerging problem? J Small Anim Pract. 2004; 45: 591-597.
- 15.Watts JL, Fajt, VR, Schwarz S, Papich MG, Fritsche TR, Silley P, et al. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals: approved standard. Wayne, PA: CLSI. 4<sup>th</sup> Edn. 2013.
- 16. Morosini MI, Cercenado E, Ardanuy C, Torres C. Phenotypic detection of resistance mechanisms in gram-positive bacteria. Enferm Infecc Microbiol Clin. 2012; 30: 325-332.
- 17. Christensen GD, Simpson WA, Bisno AL, Beachey EH. Adherence of slime-producing strains of Staphylococcus epidermidis to smooth surfaces. Infect Immun. 1982; 37: 318-326.
- Mende K, Beckius ML, Zera WC, Yu X, Li P, Tribble DR, et al. Lack of Doxycycline Antimalarial Prophylaxis Impact on Staphylococcus

## **⊘**SciMedCentral

aureus Tetracycline Resistance. Diagn Microbiol Infect Dis. 2016; 86: 211-220.

- 19. Vento TJ, Calvano TP, Cole DW, Mende K, Rini EA, Tully CC, et al. Staphylococcus aureus colonization of healthy military service members in the United States and Afghanistan. BMC Infect Dis. 2013; 13: 325.
- 20.Aarestrup FM, Agerso Y, Gerner-Smidt P, Madsen M, Jensen LB. Comparison of antimicrobial resistance phenotypes and resistance genes in Enterococcus faecalis and Enterococcus faecium from humans in the community, broilers, and pigs in Denmark. Diagn Microbiol Infect Dis. 2000; 37: 127-137.
- 21. Strommenger B, Kettlitz C, Werner G, Witte W. Multiplex PCR assay for simultaneous detection of nine clinically relevant antibiotic resistance genes in Staphylococcus aureus. J Clin Microbiol. 2003; 41: 4089-4094.
- 22.Sutcliffe J, Grebe T, Tait-Kamradt A, Wondrack L. Detection of erythromycin-resistant determinants by PCR. Antimicrob Agents Chemother. 1996; 40: 2562-2566.
- 23.Gomes IM, Marlow MA, Pinheiro MG, Freitas M de FN de, Fonseca FF, Cardoso CAA, et al. Risk factors for Staphylococcus aureus and methicillin-resistant S aureuscolonization among health care workers in pediatrics departments. Am J Infect Control. 2014; 42: 918-920.
- 24. Bocker S, Gervelmeyer A, Monnet DL, Molbak K, Skov RL. Methicillinresistant Staphylococcus aureus: risk factors associated with community-onset infections in Denmark. Clinical MicrobiolInfect. 2008; 14: 942-948.
- 25.Nah SY, Chung MH, Park JE, Durey A, Kim M, Lee JS. Infective endocarditis caused by methicillin-resistant Staphylococcus aureus in a young woman after ear piercing: a case report. J Med Case Rep. 2011; 5: 336.
- 26.Kulkarni R, Antala S, Wang A, Amaral FE, Rampersaud R, LaRussa SJ, et al. Cigarette Smoke Increases Staphylococcus aureus Biofilm Formation via Oxidative Stress. Infect Immun. 2012; 80: 3804-3811.
- 27. McEachern EK, Hwang JH, Sladewski KM, Nicatia S, Dewitz C, Mathew DP, et al. Analysis of the Effects of Cigarette Smoke on Staphylococcal Virulence Phenotypes. InfectImmun. 2015; 83: 2443-2452.
- 28. Sánchez A, Prats-van der Ham M, Tatay-Dualde J, Paterna A, de la Fe C, Gómez-Martín Á, et al. Zoonoses in Veterinary Students: A Systematic Review of the Literature. PLoS One. 2017; 12: 0169534.
- 29. Sanchez A, Ham MP der, Tatay-Dualde J, Paterna A, Fe C de la, Gomez-Martin A, et al. Zoonoses in Veterinary Students: A Systematic Review of the Literature. PLoS ONE. 2017; 12: 0169534.
- 30. Mendes A, Costa PM da, Rego D, BeCa N, Alves C, Moreira T, et al. Contamination of public transports by Staphylococcus aureus and its carriage by biomedical students: point-prevalence, related risk factors and molecular characterization of methicillin-resistant strains. Public Health. 2015; 8: 1125-1131.
- 31.Sader HS, Farrell DJ, Jones RN. Antimicrobial susceptibility of Grampositive cocci isolated from skin and skin-structure infections in European medical centres. Int J Antimicrob Agents. 2010; 36: 28-32.
- 32. Lewis HC, Molbak K, Reese C, Aarestrup FM, Selchau M, Sorum M, et al. Pigs as Source of Methicillin-Resistant Staphylococcus aureus CC398 Infections in Humans, Denmark. Emerg Infect Dis. 2008; 14: 1383-1389.

- 33.Brook I, Hausfeld JN. Microbiology of acute and chronic maxillary sinusitis in smokers and nonsmokers. Ann Otol Rhinol Laryngol. 2011; 120: 707-712.
- 34. Andersen PS, Larsen LA, Fowler VG, Stegger M, Skov RL, Christensen K. Risk factors for Staphylococcus aureus nasal colonization in Danish middle-aged and elderly twins. Eur J Clin Microbiol Infect Dis. 2013; 32: 1321-1326.
- 35. Melek IM, Duran N, Guuml, Duran lay G, Duman T, Okuyucu E. The frequency of slime, adhesin and methicillin resistance genes among staphylococci isolated from nasal samples of multiple sclerosis patients. AJMR. 2011; 5: 5453-5460.
- 36. Rezaei M, Moniri R, Mousavi SGA, Shiade MJ. Prevalence of Biofilm Formation Among Methicillin Resistance Staphylococcus aureus Isolated From Nasal Carriers. Jundishapur J Microbiol. 2013; 6: 1-5.
- 37. Gowrishankar S, Kamaladevi A, Balamurugan K, Pandian SK. In Vitro and In Vivo Biofilm Characterization of Methicillin-Resistant Staphylococcus aureus from Patients Associated with Pharyngitis Infection. Biomed Res Int. 2016; 2016: 1289157.
- 38. Krismer B, Peschel A. Does Staphylococcus aureus nasal colonization involve biofilm formation? Future Microbiol. 2011; 6: 489-493.
- 39. Parisi A, Caruso M, Normanno G, Latorre L, Sottili R, Miccolupo A, et al. Prevalence, antimicrobial susceptibility and molecular typing of Methicillin-Resistant Staphylococcus aureus (MRSA) in bulk tank milk from southern Italy. Food Microbiol. 2016; 58: 36-42.
- 40. Locatelli C, Cremonesi P, Caprioli A, Carfora V, Ianzano A, Barberio A, et al. Occurrence of methicillin-resistant Staphylococcus aureus in dairy cattle herds, related swine farms, and humans in contact with herds. J Dairy Sci. 2017; 100: 608-619.
- 41.Wan TW, Hung WC, Tsai JC, Lin YT, Lee H, Hsueh PR, et al. Novel structure of Enterococcus faecium-originated ermB-positive Tn1546-like element in Staphylococcus aureus. Antimic Agents Chemother. 2016; 60: 6108-6114.
- 42.Ong MHL, Ho WY, Ng WW, Chew CH. High Prevalence of tetMas Compared to tetK Amongst Methicillin-Resistant Staphylococcus aureus (MRSA) Isolates from Hospitals in Perak, Malaysia. Jundishapur J Microbiol. 2017; 10: 1-6.
- 43. Montagnani C, Cocchi P, Bianchi L, Resti M, de Martino M, Galli L. Severe infections caused by Panton-Valentine leukocidin-positive Staphylococcus aureus in infants: report of three cases and review of literature. Acta Paediatr. 2013; 102: 284-287.
- 44. Thurlow LR, Joshi GS, Clark J R, Spontak JS, Neely CJ, Maile R, et al. Functional Modularity of the Arginine Catabolic Mobile Element Contributes to the Success of USA300 Methicillin-Resistant Staphylococcus aureus. Cell Host Microbe. 2013; 13: 100-107.
- 45. Ellington MJ, Yearwood L, Ganner M, East C, Kearns AM. Distribution of the ACME-arcA gene among methicillin-resistant Staphylococcus aureus from England and Wales. J Antimicrob Chemother. 2008; 61: 73-77.
- 46. Hedlund L, Hellmark B, Soderquist B. Presence of arginine catabolic mobile element among community-acquired meticillin-resistant Staphylococcus aureus is linked to a specific genetic background. APMIS. 2013; 121: 221-225.

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