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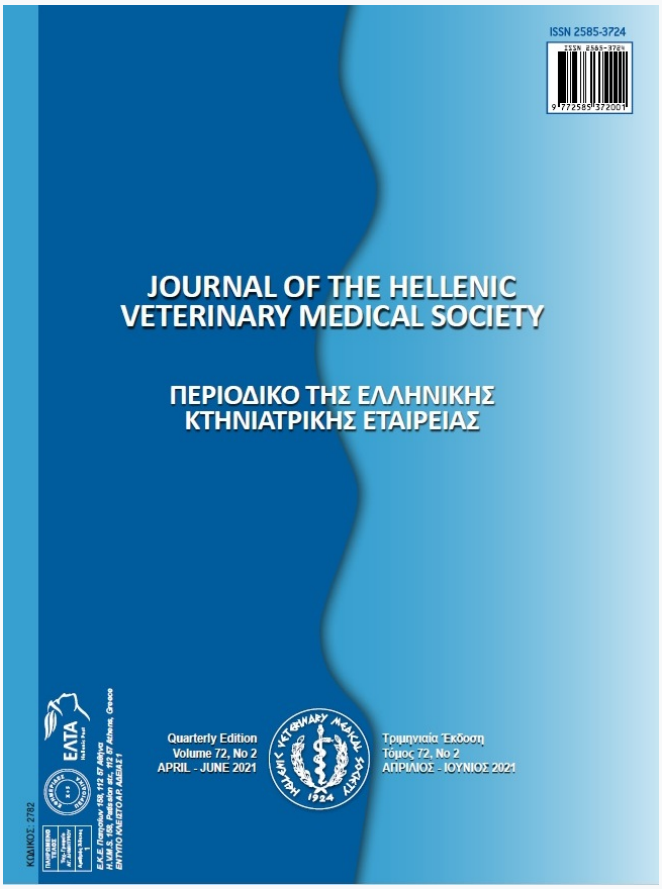

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Research article
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Nasal carriage of *Staphylococcus aureus* among healthy veterinary students in Greece, 2017-2018: A cross-sectional cohort study

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ABSTRACT: The epidemiology of *Staphylococcus* spp., *Staphylococcus aureus* and MRSA among veterinary students in Greece during 2017-2018 is reported. Nasal swabs and a standardized questionnaire from 160 healthy veterinary students were used to identify potential risk factors for colonization. Antimicrobial susceptibility testing, *pvl, mecA, mecC*, staphylococcal enterotoxin genes and PFGE were used to characterize *S. aureus* isolates. Overall, 76% and 19% of the students were colonized by *Staphylococcus* spp. and *S. aureus* but none by MRSA. Students with a prior visit to a hospital were 1.33 and 2.25 times more likely to be colonized by *Staphylococcus* spp. and *S. aureus,* respectively while, 94% of the *S. aureus* isolates were resistant to penicillin, 68% to amoxicillin/clavulanic acid and 12% were multidrug-resistant. Staphylococcal enterotoxin genes were detected in 32% of the *S. aureus* isolates, while PFGE showed heterogeneity. Although MRSA was not detected, the high rate of *Staphylococcus* spp. colonization suggests the need of sustained implementation of strict hygiene practices among students and the staff involved in veterinary training. The results of the present study add useful information for the assessment of the risks associated with staphylococcal infection in veterinary students.

Keywords: *Staphylococcus*, colonization, PFGE, antimicrobials, MRSA

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INTRODUCTION

Staphylococcus aureus (*S. aureus*) is a common bacterium found on the skin and nasal cavities of healthy people. Approximately 25-40% of the population is colonized by *S. aureus*. It is also a common cause of skin and soft tissue infections and sometimes causes severe disease such as pneumonia, bacteraemia, meningitis, sepsis, and pericarditis exhibiting a high burden in terms of morbidity and mortality (Frana et al. 2013; Cassini et al. 2019). *S. aureus* is also responsible for food poisoning due to oral intake of Staphylococcal enteroxins (SEs) present in foods (Zhao et al. 2017; Khemiri et al. 2019) which leads to a significant health and economic in accute gastroenteritis (Papadopoulos et al. 2019a). *S. aureus* bacteria harbouring the *mec*A gene are resistant to methicillin and other β-lactam antimicrobials and are referred to as methicillin-resistant *S. aureus* (MRSA) (Frana et al. 2013). MRSA has been considered a major hospital associated pathogen (HA-MRSA), and has become a serious threat in hospitals worldwide but has been also found associated to community setting (CA-MRSA) and to livestock (LA-MRSA) (Pantosti 2012).In Greece, the prevalence of MRSA in clinical isolates of *S. aureus* has been 36-40% during 2015-2018 and remains one of the highest in Europe; the EU/EEA population-weighted mean percentage of MRSA was 16.4% in 2018 (ECDC 2019).

Many countries have developed and implemented national recommendations for preventing the spread of MRSA, including screening, isolation and decolonization of the MRSA carriers and prudent antimicrobial use (Köck et al. 2014). In order to slow the spread of MRSA in Europe, comprehensive MRSA strategies targeting all healthcare sectors remain essential. On the other hand, the monitoring of MRSA in animals and food is currently voluntary and only performed in a limited number of countries. Recently transmission of LA-MRSA to humans by food-producing animals has been described, especially in persons with close contact to animals and mostly in farmers (Graveland et al. 2011), slaughter and abattoir workers (Papadopoulos et al. 2018; Drougka et al. 2019; Papadopoulos et al. 2019c; Papadopoulos et al. 2019d), companion animals' owners (Ferreira et al. 2011) and veterinarians (Hanselman et al. 2006), demonstrating a severe occupational risk for veterinary professionals.Studies have been also conducted among exclusively veterinary students or personnel in veterinary hospitals; the occupational risk associated to veterinary students has also been addressed and reported by works describing infections by MRSA (Wulf et al. 2006; Aklilu et al. 2013; Frana et al. 2013; Huang and Chou 2019). However, in Greece, the occupational risk for colonization among healthy veterinary students that are in contact with animals is still unknown.

Therefore, the aim of this study was to estimate the prevalence of *Staphylococcus* spp, *S. aureus* and MRSA nasal carriage among healthy veterinary students in Greece, to describe the isolated strains of *S. aureus* and to identify possible risk factors of colonization.

MATERIALS AND METHODS

Sampling frame and sample collection

All the students studying in 5th semester of two consecutive years (2017-2018) in the School of Veterinary Medicine, Aristotle University of Thessaloniki in Greece were asked to participate in the study. All individuals that accepted to participate (174 out of 208; rejection rate of 16%) were previously informed of the screening procedure (Figure1). A standardized questionnaire was completed for each participating student, during sampling, in order to collect data with reference to the lifestyle and habits that the students had, and which would be correlated as potential risk factors. Students that reported symptoms of infectious disease during the last two weeks of the interview/sampling day were excluded from the study. Sampling of nasal cavities was performed by swabbing both nostrils with Sterile Transport Swabs STUART (FL MEDICAL, Torreglia, Italy). Swabs were transported immediately for analysis in the Laboratory of Hygiene of Foods of Animal Origin-Veterinary Public Health, of the School of Veterinary Medicine, Aristotle University of Thessaloniki. An informed consent signed by all the participants was obtained prior to enrollment.

Isolation and identification of *Staphylococcus* spp and *S. aureus*

Swabs were placed for enrichment in tubes containing 5 ml Tryptone Soy broth (TSB, LAMB M, Lancashire, United Kingdom) with 6.5% NaCl and 0.3% yeast extract. After 18-24 h incubation at 35 ^{o}C, 10 μl of the enrichment was plated on Baird-Parker agar with Egg Yolk Tellurite (Oxoid, Unipath, Basingstoke, UK) and incubated at 35 ^{o}C for 24-48 h. After incubation, 3-4 colonies from each plate were transferred on Tryptone Soy agar (LAB M Limited, Lancashire, United Kingdom) and incubated for 24 h at 35 ^{o}C. Colonies were selected according to their appearance, black both with and without opaque ha-

loes from Baird Parker. Identification as *Staphylococcus* spp and presumptive *S. aureus* was based upon Gram-staining, catalase reaction, mannitol fermentation and coagulase test, morphological and cultural characteristics. One *Staphylococcus* spp. (suspected *S. aureus*) per student was stored at -80 ºC in TSB containing 20% glycerol.

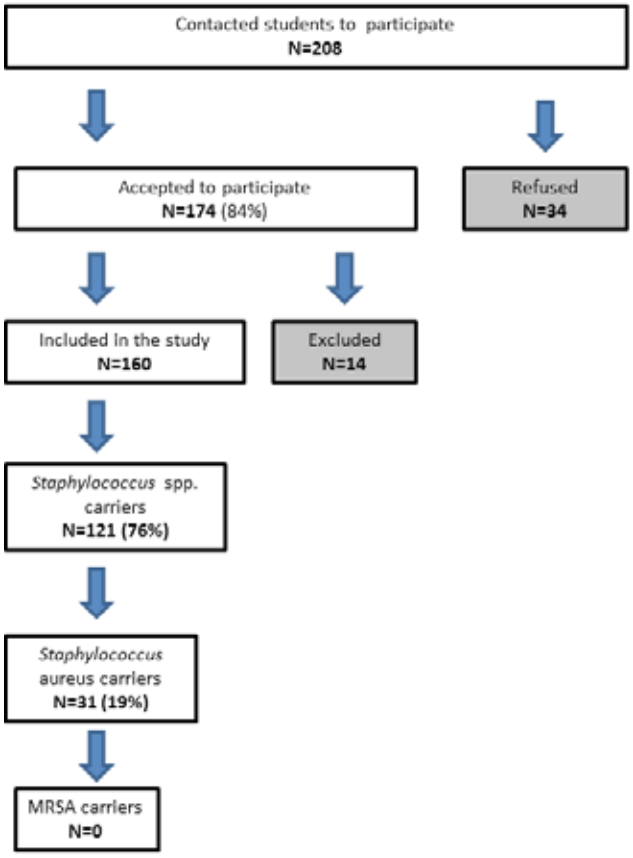

Figure 1: Flow chart describing the populations of students for each particular step of the study, Greece, 2017-18, N=208

One presumptive *S. aureus* isolate from each sample was confirmed by PCR targeting the *S. aureus* species-specific determinants *coa* (coagulase) and *nuc* (nuclease) genes. Extraction of genomic DNA from bacterial cultures was conducted according to the protocol of DNA purification from Gram-positive bacteria by the Pure Link Genomic DNA kit (Invitrogen, Carlsbad CA). A 500- to 650-bp fragment of the *coa* gene and a 416-bp fragment of the *nuc* gene were amplified using previously described primer sets (Hookey et al. 1998; Sudagidan and Aydin 2008) and PCR conditions (Zdragas et al. 2015). The PCR amplicons were separated in 1.5% agarose gels stained with ethidium bromide and visualized under UV illumination (TEX-20 M, Life Technologies, GibcoBRL System). A sample from a healthy student was defined as positive for colonization if it contained at least one *Staphylococcus* spp or *S. aureus* isolate, respectively.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility of the *S. aureus* isolates was determined by the agar-dilution method in Mueller-Hinton agar (MHA, Merck) according to the Clinical and Laboratory Standard Institute guidelines (CLSI, 2009). Briefly, plates were prepared by incorporating the appropriate amount of antimicrobial agent into MHA. For each bacterial isolate the inoculum was prepared by adjusting the turbidity to 0.5 McFarland and was applied rapidly to the agar surfaces using a multi-channel pipet (Eppendorf, Merck) capable of transferring multiple inocula to each plate. The results were evaluated after incubation at 35 °C for 24 h. Susceptibility towards the following 15 antimicrobials was evaluated with the final concentration in μg/mlin brackets: penicillin, P (0.25); oxacillin, Ox (0.25 and 2); amoxicillin/clavulanic acid, Amc (1/0.125); tetracycline, T (1); erythromycin, E (1); vancomycin, V (2 and 4); chloramphenicol, C (8); ciprofloxacin, Cp (1); trimethoprim/sulfamethoxazole, Sxt (2/38); trimethoprim, Tm (2); gentamicin, G (1); amikacin, Ak (8); kanamycin, K (8); rifampicin (0.5); clindamycin Cl (0.25). Multidrug-resistance (MDR) was defined as previously proposed (Magiorakos et al. 2012). *S. aureus* ATTC 25923, *Escherichia coli* ATCC 25922 and *Enterococcus faecalis* ATCC 29212 were used as control strains.

Identification of the *mec*A and *mec*C genes

The detection of the *mec*A gene in the phenotypically resistant (OX 0.25) *S. aureus* isolates was achieved by PCR according to Murakami et al. (1991) using the primers (5' AAAATC GATGGTAAAGGTTGGC) corresponded to nucleotides 1282 to 1303 and (5' AGTTCTGCAGTACCGGATTTGC) complementary to nucleotides 1793-1814. The PCR was also performed for the detection of the *mec*C according the method described by Stegger et al. (2012) using the primers 5'-GAAAAAAAGGCTTAGAACGCCTC-3' and 5'-GAAGATCTTTTCCGTTTTCAGC-3'.

Detection of staphylococcal enterotoxin(SEs) genes

Five specific primer sets, previously described by Jarraud et al. 2002), were used for the detection of genes encoding for the five classic SEs (*sea*, *seb*, *sec*, *sed*, *see*). Amplifications of SE-coding genes were performed as single PCR assays.

Table 1: Risk factors for nasal carriage by *Staphylococcus* spp among healthy veterinary students, N=160, Greece 2017-2018.

Characteristics	Colonized by *Staphylococcus* spp(N)	Total	Colonized by *Staphylococcus* spp %	Prevalence ratio (PR)	95% C.I	p value	Adjusted Prevalence ratio (PR)	95% C.I	p value
Sex									
male	61	78	74.39	1.03	0.87-1.23	0.71			
female	60	82	76.92	ref	ref				
Age (years)									
20	39	53	73.58	ref	ref				
21	43	57	75.44	1.03	0.82-1.28	0.82			
22	24	30	80.00	1.09	0.85-1.38	0.49			
23	9	13	69.23	0.94	0.63-1.40	0.76			
24	6	7	85.71	1.16	0.83-1.64	0.38			
Year enter									
2013	10	13	76.92	ref	ref				
2014	24	32	75.00	0.97	0.68-1.40	0.89			
2015	48	62	77.42	1.00	0.73-1.40	0.97			
2016	39	53	73.58	0.96	0.68-1.34	0.80			
Years in clinic									
1	39	53	73.58	ref	ref				
2	76	100	76.00	1.03	0.85-1.26	0.75			
3	6	7	85.71	1.16	0.83-1.64	0.38			
Pet									
yes	92	72	78.26	1.08	0.90-1.30	0.38			
no	68	49	72.06	ref	ref				
Smoking									
yes	44	58	75.86	1.00	0.84-1.21	0.96			
no	77	102	75.49	ref	ref				
Skin infection during last 3 months									
yes	12	14	85.71	1.15	0.91-1.45	0.25			
no	109	146	74.66	ref	ref				
Surgery during last 3 months									
yes	12	13	92.31	1.24	1.04-1.50	0.019			
no	109	147	74.15	ref	ref				
Respiratory disease during last 3 months									
yes	15	17	88.24	1.19	0.98-1.45	0.09			
no	106	143	74.13	ref	ref				
Visited hospital during last 3 months									
yes	43	47	91.49	1.33	1.14-1.54	0.00	1.33	1.14-1.54	0.00
no	78	113	69.03	ref	ref		ref	ref	
Use of antibiotics during last 3 months									
yes	17	24	70.83	0.93	0.70-1.22	0.58			
no	104	136	76.47	ref	ref				
Use of antibiotics during last 6 months									
yes	22	29	75.86	1.00	0.80-1.26	0.97			
no	99	131	75.57	ref	ref				

Table 2: Risk factors for nasal carriage by *S. aureus* among healthy veterinary students, N=160, Greece 2017-2018.

Characteristics	Colonized by *S. aureus* (N)	Total	Colonized by *S. aureus* %	Prevalence ratio (PR)	95% C.I	p value	Adjusted Prevalence ratio (PR)	95% C.I	p value
Sex									
male	13	78	21.95	0.76	0.40-1.44	0.4			
female	18	82	16.67	ref	ref				
Age (years)									
20	11	53	20.75	ref	ref				
21	10	57	17.54	0.85	0.39-1.83	0.67			
22	4	30	13.33	0.64	0.22-1.84	0.41			
23	4	13	30.77	1.48	0.56-3.91	0.43			
24	2	7	28.57	1.38	0.38-4.97	0.63			
Year enter									
2013	3	13	23.08	ref	ref				
2014	6	32	18.75	0.81	0.24-2.77	0.74			
2015	11	62	17.74	0.77	0.25-2.38	0.65			
2016	11	53	20.75	0.90	0.29-2.77	0.85			
Years in clinic									
1	11	53	20.75	ref	ref				
2	18	100	18	0.87	0.44-1.70	0.68			
3	2	7	28.57	1.38	0.38-4.97	0.63			
Pet									
yes	19	92	20.65	1.17	0.61-2.24	0.64			
no	12	68	17.65	ref	ref				
Smoking									
yes	10	58	17.24	0.84	0.42-1.65	0.61			
no	21	102	20.59	ref	ref				
Skin infection during last 3 months									
yes	6	9	42.86	2.5	1.24-5.05	**0.01**			
no	24	151	17.12	ref	ref				
Surgery during last 3 months									
yes	4	13	30.77	1.68	0.69-4.05	0.25			
no	27	147	18.37	ref	ref				
Respiratory disease during last 3 months									
yes	6	16	35.29	2.02	0.97-4.21	0.06			
no	25	144	17.48	ref	ref				
Visited hospital during last 3 months									
yes	15	47	31.91	2.25	1.21-4.18	0.01	2.25	1.21-4.18	0.01
no	16	113	14.16	ref	ref	ref	ref	ref	ref
Use of antibiotics during last 3 months									
yes	7	24	29.17	1.65	0.80-3.40	0.17			
no	24	136	17.65	ref	ref				
Use of antibiotics during last 6 months									
yes	9	29	31.03	1.85	0.95-3.59	0.07			
no	22	131	16.79	ref	ref				

Pulsed Field Gel Electrophoresis (PFGE)

PFGE analysis was conducted following the Pulse-Net protocol (McDougal et al. 2003) using the size standard, electrophoretic conditions, dendrogram construction and comparison criteria previously described (Papadopoulos et al. 2018). Clusters were selected using a cut-off at the 80% level of genetic similarity. The diversity of PFGE type distribution was calculated using the Simpson's diversity Index (D), which ranges from 0 (no diversity) to 1 (extreme diversity) as a measure for PFGE type diversity.

Statistical analysis

Frequencies were obtained and proportions were calculated for categorical variables, age was also treated as categorical variable for comparisons as age ranked from 21 to 28 years old. Categorical variables were compared using the Chi square test or the Fisher exact test. Prevalence ratios (PR), 95% confidence intervals (CI), and P values were calculated. The association between influencing factors and *Staphylococcus* spp. or *S. aureus* nasal colonization was examined using multivariable logistic regression models. Multivariable logistic regression analysis of all variables with a P-value of <0.2 indication significance was also performed. A P value of ≤0.05 was considered statistically significant. All statistical measures were estimated using survey data analysis methods (SVY commands) from STATA package. All analyses were performed using STATA 14 (STATA CORP LP, College Station, Texas, USA).

RESULTS

Prevalence of *Staphylococcus* spp, *S. aureus* and-MRSA in veterinary students

A total of 174 students voluntarily accepted to participate in the study. A total of 14 students were excluded from the study, due to symptoms of illness during the last 15 days before the interview/screening. A total of 160 healthy students were included and sampled with a median age of 21years (range 20-24 years) and 51% (78/160) were females (Figure 1). Table 2 presents in detail the descriptive and the corresponding demographic and lifestyle data of the students participated in this study.

One hundred twenty-one out of 160 (76%) found to be colonized by *Staphylococcus* spp, and 31 (19%) by *S. aureus* (Figure 1). All the 31 confirmed as *S. aureus* isolates carried the *coa* (coagulase) and *nuc* (nuclease) genes. Distributions of *Staphylococcus* spp and *S. aureus* carriers and non-carriers stratified by population characteristics and variables associated with carriage in the univariate analysis are shown in Tables 1 and 2 respectively.

The univariate analysis revealed that students that had visited a hospital (ambulatory consultation and/or visit to an impatient and/or hospital admission and/or surgery) during the last three months were 1.33 times more likely to be colonized by *Staphylococcus* spp (OR 1.33 CI 1.14-1.54). However, students that had a skin infection during the last three months were 2.5 times more likely to be colonized by *S. aureus* (OR 2.5 CI 1.24-5.05) and those who had visited a hospital during the last three months were 2.25 times more likely to be colonized by *S. aureus* (OR 2.25 CI 1.14-1.54). In order to assess the relationship between the potential predictors, taking under consideration potential confounders among the influencing factors, a multivariate logistic regression model was used. This model demonstrated that when controlling for the effects of the other influencing factors, the relationships found in the univariate analyses changed. Only a visit to the hospital during the last three months was found significantly associated with any of the abovementioned characteristics. More specific details are presented in Tables 1 and 2. The adjusted prevalence ratio for colonization by *Staphylococcus* spp was 1.33 (CI 1.14-1.54 95%) and for *S. aureus* 2.25 (CI 1.21-4.18 95%) according to the final regression model. None of the variables was identified as a protective factor for nasal colonization. Correlation of carriage with a specific veterinary-related factor, like having a pet or attending more years in the clinic, could not be established.

Antimicrobial susceptibility testing

The antibiotic resistance patterns of the 31 *S. aureus* isolates are presented in Figure 2. Drug-resistance to at least one antimicrobial agent was observed in all 31 isolates. Resistance to penicillin allocated the highest rate of resistance (94%), following by amoxicillin/clavulanic acid (68%) and erythromycin (23%). Lower resistance rates were observed in ciprofloxacin, clindamycin and tetracycline (6%). Eight isolates showing resistance to low concentration of oxacillin (0.25μg/ml) were identified. However, none of the 31 isolated carried the *mec*A or the *mec*C genes or exhibited resistance to >2 μg/mlof oxacillin thus MRSA strain was not isolated. The *pvl* gene was not detected among all the *S. aureus* isolates in this study; all of them were susceptible to amikacin, kanamycin, gen-

tamicin, rifampicin, vancomycin and trimethoprim with or without sulphamethoxazole (Table 3). Three out of the 31 (10%) isolates showed resistance against antibiotics belonging to three or more antibiotic classes and consequently were characterized as multi-drug resistant. The most common profile was Pe-AC (39%) followed by Pe and Pe-pOx-AC (13%) (Figure 2).

Detection of SEsgenes and *pvl* genes

Detection of SEs genes is shown in Figure 2. Among all the 31 *S. aureus* isolates, at least one of the SEs genes was detected in 10 (32%) of them. The *sec* was the most common detected (19%) followed by *seb* (13%) and *sea* (10%). In two isolates (6.5%), both *seb* and *sec* were detected, while *sea* and *sec*, *sea* and *seb* were both detected in 3.2%. No significant difference (P >0.05) was identified among the risk factors and the detection of any of the tested SEs genes. However, *pvl* gene positive isolate was not identified.

Pulsed Field Gel Electrophoresis

Twenty-eight distinct PFGE types were identified among *S. aureus* isolates with overall similarity 52.6%; 22 of them assigned to five main clusters (80% similarity cut off) consisting from two to seven isolates. The Simpson's index of diversity was calculated as D = 0.974 (Figure 2). Only two cases of students sharing the same PFGE type were identified; the first one with two students no 020 and 069 (same age and same AMR profile). The second case included three students no 101, 107 and 111. This case had different AMR profiles and different enterotoxin profiles. Overall, endemic clones circulating among the students were not identified.

Table 3: Antimicrobial susceptibility testing of *S. aureus* isolates among healthy veterinary students, N=31, Greece 2017-2018.

Antimicrobial	Concentration (mg/l)	N	%
Penicillin	0.25	29	94
Oxacillin	0.25	10	32
Oxacillin	2	0	0
Trimethoprim/ Sulphamethoxazole	2/38	0	0
Gentamycin	1	0	0
Erythromycin	1	7	23
Amikacin	8	0	0
Kanamycin	8	0	0
Tetracycline	1	2	6
Trimethoprim	2	0	0
Amoxicillin/ clavulanic acid	1/0.125	21	68
Ciprofloxacin	1	2	6
Rifampicin	0.5	0	0
Vancomycin	2	0	0
Vancomycin	4	0	0
Clindamycin	0.25	2	6
Chloramphenicol	8	0	0

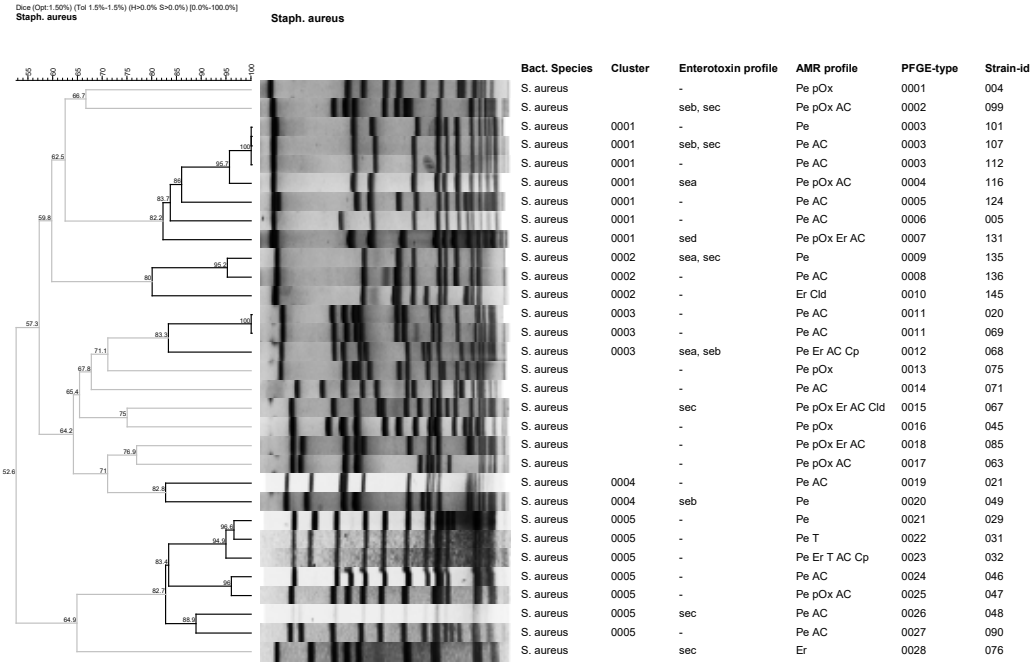

Figure 2: Dendrogram of the 31 *S. aureus* isolates with corresponding antimicrobial resistance profiles, enterotoxin gene profiles and PFGE-types.

DISCUSSION

In this study, *Staphylococcus* spp, *S. aureus* and MRSA nasal carriage among healthy veterinary students was evaluated and the molecular characteristics of the isolated *S. aureus* strains were described. In total, 76% and 19% of the students were colonized by *Staphylococcus* spp and *S. aureus* respectively, while none by MRSA. The present study revealed that only a previous visit to the hospital was independently associated with the carriage of *Staphylococcus* spp and *S. aureus*.

Several studies have been conducted regarding the prevalence of *S. aureus* and MRSA in healthy individuals. Studies in Greece and globally show that about 20% of individuals are persistent *S. aureus* nasal carriers, approximately 30% are intermittent carriers (range 16-70%), and about 50% (range 16-69%) non-carriers (Wertheim et al. 2005; Papadopoulos et al. 2018; Papadopoulos et al. 2019c; Moschou et al. 2020). Results of other studies, targeting medical or university students, are comparable with results from the present study however, most of the studies present a low prevalence of MRSA colonization (<5%) but higher regarding *S. aureus* (29.2-40%) (Rohde et al. 2009; Prates et al. 2010; Roberts et al. 2011; Treesirichod et al. 2013; Petti et al. 2015; Price et al. 2017). Only a few studies have been conducted among exclusively veterinary students or personnel in veterinary hospitals targeting MRSA. The occupational risk associated to veterinary students has also been addressed and reported by others describing infections by MRSA reporting prevalence from 0-23.3% (Wulf et al. 2006; Aklilu et al. 2013; Frana et al. 2013; Youn et al. 2014). Interestingly, studies underscore the importance of occupational exposure mainly to pigs as a risk factor for colonization by MRSA (Frana et al. 2013; Narvaez-Bravo et al. 2016); however, in this study it was not possible to identify students that had contact exclusively with pigs. Overall, the prevalence of *S. aureus* in this study was not high. Nevertheless, there were positive prevalence relationship of *Staphylococcus* spp and *S. aureus* isolates among students visiting hospital.

This study showed that the levels of antimicrobial resistant *S. aureus* were in general low although some strains found to be multidrug resistant. The isolates showed resistance to penicillin and/or amoxicillin/clavulanic acid which are among the most used antibiotics in Greece, country that had the highest rate of non-prescription use of antibiotics during 2016 with nearly 20% or 74.6% of the people using un-prescribed antibiotics (Anonymous 2016). Moreover, these two antimicrobials are also the most over the counter bought antimicrobials by the general population (Anonymous 2017). However, a study in Greece showed that antibiotics are very easy to be asked for and purchased without any justification (Plachouras et al. 2010). Self-medication by 'over the counter' antibiotics appears to be an extensive problem in Greece and this practice may contribute significantly to excess antibiotic use and increased antibiotic resistance.

MRSA has been recognized as a major causative agent of healthcare-associated infections (HA-MRSA) in humans for decades (Sergelidis and Angelidis 2017). In recent years, the isolation of MRSA from livestock (LA-MRSA) and companion animals has also been reported (Nemati et al. 2008); HA-MRSA and CA-MRSA are believed to predominantly affect humans and, in general, are not involved in livestock infections. Surprisingly, the present study demonstrated zero prevalence of MRSA among the Greek Veterinary students despite the fact that a study contacted in Greece during 2014 showed a 5.3% of MRSA prevalence among patients visiting hospitals in Central Greece (Tsiodras et al. 2014). Moreover, according to EARS net the EU/EEA population-weighted mean percentage of MRSA was 16.4% in 2018 but in Greece 36.8%; this practically means that more than one out of three invasive staphylococcal infections in Greece are caused by MRSA (Anonymous 2019).

PFGE has been widely used for the characterization of human or animal isolates, sometimes coupled with other molecular (MLST, *spa* typing or WGS) or phenotypic methods (Güven Gökmen et al. 2018; Lakhundi and Zhang 2018). We found a significant genomic variability among the 31 *S. aureus* isolates; this was demonstrated by the large number -28- of distinct PFGE patterns, as well as from the high values of Simpson's index of diversity. Endemic clones circulating among the students were not identified; an explanation could be that colonized students are "persistent" carriers colonized by a single strain for a long time (Wertheim et al. 2005).

Panton-Valentine leucocidin encoded by *pvl* gene is a toxin that lyses leukocytes and strongly associated with skin infections (Lina et al. 1999; Vandenesch et al. 2003). Our results are in line with the study by Roberts et al. (2011) who collected 24 MRSA strains from a university campus in USA and did not identify any positive for *pvl* gene. Similarly, Heller et al. (2009) did not isolate *pvl* positive MRSA strain

among 64 workers in a veterinary clinic in the UK. In Greece, other studies in healthy workers in dairy industry, dairy animals did not confirm the presence of the *pvl* gene (Papadopoulos et al. 2018; Papadopoulos et al. 2019c; Papadopoulos et al. 2019d).

S. aureus enterotoxins (SEs) are major causes of staphylococcal food poisoning (Argudin et al. 2010). Staphylococcal enterotoxin A, B, C and enterotoxin D genes, were confirmed in *S. aureus* isolates from cattle, workers and environmental samples in the dairy industry in Greece (Papadopoulos et al. 2019b; Papadopoulos et al. 2019c) in studies that screened only MRSA isolates. This study shows that 32% *S. aureus* isolates carried at least one SE gene. Several studies worldwide have demonstrated comparable results with ours identifying SEs genes from foods and animals which underscores the significance of these toxins in food poisoning (Vitale et al. 2015; Cheng et al. 2016; Bastos et al. 2017; Zhang et al. 2018).

To our knowledge this is the first study in Greece targeting risk factors of colonization exclusively among veterinary students. Considering the fact that in Greece there are only two Veterinary schools with approximately 200 students per year, we think that sampling 160 students from the one of the schools give our study enough strength to supports the findings. Nevertheless, a possible limitation of the study is that all the students by the time of sampling had already contacts with animals (companion or large animals) during their clinical years. In this aspect it was not possible to gather information and perform comparisons regarding differences between students in preclinical and clinical semesters. Moreover, it was not possible to collect information regarding the contact with different animal species which is of particular interest. The results have not been compared with a control population of non-veterinary students in Greece (suitable age- and gender-matched control group) in order to determine if there is anything unusual about staphylococcal nasal carriage among veterinary students. However, to our knowledge this is the first study in Greece targeting exclusively veterinary students as potential carriers of *Staphylococcus* spp. and *S. aureus.*

CONCLUSIONS

Albeit, studies worldwide have demonstrated veterinarians' occupational risk of *S. aureus* and MRSA colonization, this study showed that possibly colonization did not take place during veterinary studies. Although MRSA was not detected, the high rate of *Staphylococcus* spp. colonization suggests the need of sustained implementation of strict hygiene practices among students and the staff involved in veterinary training.

CONFLICT OF INTEREST

None of the authors declare a conflict of interest.

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