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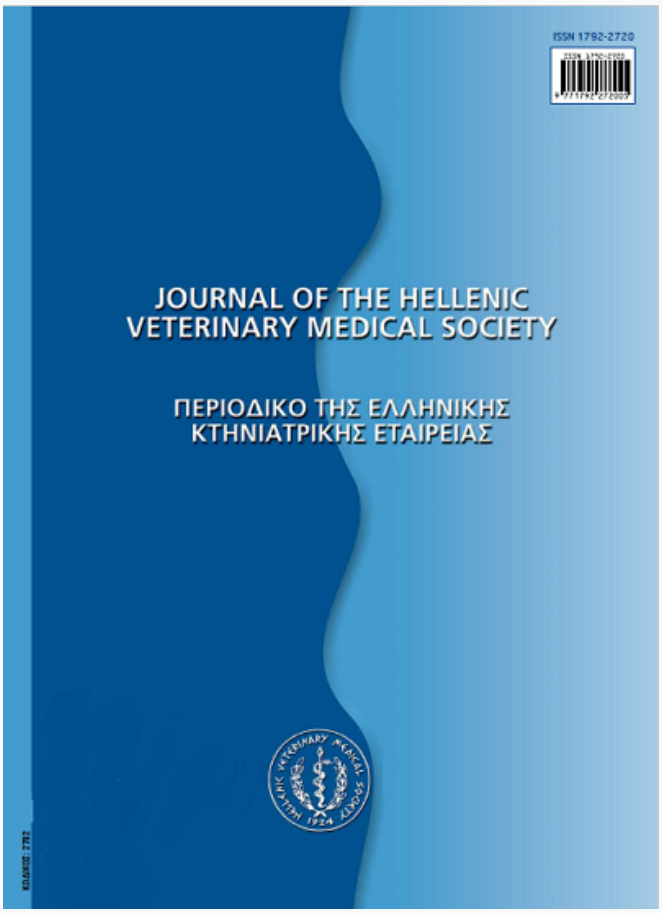

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Research article
Ερευνητικό άρθρο

Nasal carriage and antimicrobial susceptibility of Coagulase - Negative Staphylococci (CoNS) among healthy veterinary students in Greece

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Ρινική φορεία και αντιμικροβιακή ευαισθησία αρνητικών στην πηκτάση σταφυλόκοκκων σε φοιτητές της Κτηνιατρικής στην Ελλάδα

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ABSTRACT. During last decades CoNS and especially the methicillin-resistant (MRCoNS) ones have become important pathogens and their infections are usually associated with healthcare settings. CoNS are considered as source of antimicrobial resistance traits for other bacteria and thus the evaluation of their prevalence in the community contributes significantly to the risk assessment in relation to public health. The aim of the present study was the investigation of the nasal carriage and antimicrobial susceptibly of CoNS among healthy veterinary students. From 81 healthy students of the School of Veterinary Medicine, Aristotle University of Thessaloniki, Greece, 24 strains were isolated from 22 (27.16%) students. Of them 54% were identified as *Staphylococcus haemolyticus,* 45.8% as *Staphylococcus warneri,* 16.6% as *Staphylococcus epidermidis*, 4.2% as *Staphylococcus pasteuri* and 4.2% as *Staphylococcus capitis*. All isolates were resistant to penicillin, 33.3% were resistant to amoxicillin/clavulanic acid, 29.2% to erythromycin, 4.2% to oxacillin, 4.2% to gentamycin and 4.2% to trimethoprim/sulfamethoxazole. The resistant to oxacillin isolate belonged to the *S. epidermidis* species and proved to carry the *mec*A gene.

This study showed that the rate of nasal carriage of CoNS among veterinary students in Greece was low. The analysis of the standardised questionnaire, that was completed for each participating student during sampling, showed that pet owners tested positive for the coagulase-negative staphylococci were at a significantly lower rate (p-value=0.007) compared to non-pet owners. Moreover, among the participants who had visited a hospital over the last six months, the percentage of positive results in coagulase-negative staphylococci was significantly lower compared to that of the other participants (p-value=0.048). Although only one student found to carry methicillin-resistant *S. epidermidis*, its presence is evidence that this pathogen may circulate among veterinarians and the personnel of veterinary health establishments. Surveillance programs should also be performed in veterinary units because the emergence resistant bacteria in this environment may represent a risk to public health.

***Keywords*:** Coagulase negative staphylococci, veterinary students, antimicrobial susceptibility, *mec*A gene, MALDI-TOF MS

ΠΕΡΙΛΗΨΗ. Τις τελευταίες δεκαετίες οι Πηκτάση Αρνητικοί Σταφυλόκοκκοι (Coagulase Negative Staphylococci –CONS) και ειδικά οι ανθεκτικοί στη μεθικιλλίνη (MRCoNS) αποτελούν σημαντικά παθογόνα και οι λοιμώξεις που προκαλούν συνδέονται με χώρους υγειονομικής περίθαλψης. Οι CoNS θεωρούνται πηγή μετάδοσης σε άλλα βακτήρια γενετικού υλικού που διέπει την αντιμικροβιακή αντοχή. Η εκτίμηση του επιπολασμού τους στους ανθρώπους συμβάλλει σημαντικά στην αξιολόγηση του κινδύνου για τη Δημόσια Υγεία. Σκοπός της παρούσας μελέτης ήταν η διερεύνηση της ρινικής φορείας CoNS σε υγιείς φοιτητές Κτηνιατρικής. Κατά την εξέταση ρινικών επιχρισμάτων 81 υγιών φοιτητών του Τμήματος Κτηνιατρικής του ΑΠΘ, απομονώθηκαν 24 στελέχη από 22 (27,16%) φοιτητές εκ των οποίων 54% ήταν *Staphylococcus haemolyticus,* 45,8% *Staphylococcus warneri,* 16,6% *Staphylococcus epidermidis*, 4,2% *Staphylococcus pasteuri* και 4,2% *Staphylococcus capitis*. Όλα τα στελέχη ήταν ανθεκτικά στην πενικιλλίνη, 33,3% ήταν ανθεκτικά στην αμοξυκιλλίνη / κλαβουλανικό οξύ, 29,2% στην ερυθρομυκίνη, 4,2% στην τριμεθοπρίμη / σουλφαμεθοξαζόλη 4,2% στην οξακιλλίνη και 4,2% στη γενταμυκίνη. Το ανθεκτικό στην οξακιλλίνη στέλεχος ανήκε στο είδος *S. epidermidis* και αποδείχθηκε ότι φέρει το γονίδιο *mec*A. Η παρούσα μελέτη κατέδειξε ότι το ποσοστό ρινικής φορείας CoNS σε φοιτητές Κτηνιατρικής στην Ελλάδα ήταν χαμηλό. Οι ιδιοκτήτες ζώων ήταν σε σημαντικά χαμηλότερο βαθμό θετικοί στους αρνητικούς στην πηκτάση σταφυλόκοκκους, σε σχέση με τους μη έχοντες κάποιο ζώο συντροφιάς (p-value=0.007), ενώ οι συμμετέχοντες που είχαν επισκεφθεί νοσοκομείο το τελευταίο εξάμηνο, ήταν σε στατιστικά χαμηλότερο ποσοστό θετικοί στους αρνητικούς στην πηκτάση σταφυλόκοκκους σε σχέση με τους υπολοίπους (p-value=0.048). Παρά το γεγονός ότι μόνο ένας φοιτητής βρέθηκε να μεταφέρει ανθεκτικό στη μεθικιλλίνη *S. epidermidis*, η παρουσία του είναι ένδειξη ότι αυτό το παθογόνο μπορεί να κυκλοφορεί σε κτηνίατρους και προσωπικό κτηνιατρικών μονάδων υγείας. Κατά συνέπεια συστήνονται προγράμματα επιτήρησης στις κτηνιατρικές μονάδες, μιας και τα ανθεκτικά βακτήρια υπάρχουν σε αυτό το περιβάλλον και μπορεί να αποτελέσουν κίνδυνο για τη δημόσια υγεία.

Λέξεις κλειδιά: Πηκτάση Αρνητικοί Σταφυλόκοκκοι, φοιτητές κτηνιατρικής, αντιμικροβιακή αντοχή, γονίδιο *mec*A, MALDI-TOF MS

INTRODUCTION

Coagulase-negative staphylococci (CoNS) are ubiquitous in nature, residing on skin and mucous membranes in humans and animals. Unlike their positive counterparts, CoNS were generally considered as harmless commensals producing few virulence patterns. During last decades CoNS and especially the methicillin-resistant ones (MR-CoNS) have become important pathogens and their infections are usually associated with healthcare settings. Professionals from both human and veterinary medicine are at higher contamination risk. *S.epidermidis*, *S. haemolyticus* and *S. saprophyticus* exhibit increasing abilities as opportunistic emerging pathogens (Piette and Verschraegen 2009). MR-CoNS represent a serious concern in hospital acquired infections (Ziebuhr et al. 2006), while *S. epidermidis* is the prominent CONS pathogen causing bacteremia in device-related infections (Wielders et al. 2001).

There are several correlated risk factors to CoNS occurrence such as profession, contact with animals, smoking and use of antibiotics that need to be evaluated when assessing the prevalence of CoNS in a certain population (Gomez-Sanz et al., 2013; Paul et al., 2011; Goslings & Buchli, 1958; Kaygusuz et al., 2004; Marples et al., 1969). CoNS are historically more resistant to antimicrobials than *S. aureus*, including b-lactam antibiotics (Wedley et al., 2014). Furthermore, their increasing virulence and resistance to antimicrobials is associated with their ability to colonize, proliferate and produce biofilms on biomaterials (Holland et al., 2011). Staphylococci become resistant by the acquisition of the *mec*A gene, which encodes a penicillin-binding protein (PBP2a) with a low affinity for b-lactams; strains producing PBP2a are resistant to all b-lactam antibiotics (Chambers 1997).

So far, attention has been paid in hospital infections with MRSA and MR-CoNS, while the exposure of vets in these pathogenic agents has not been investigated widely. Since there is no research in Greece on the exposure of the Veterinary community to CoNS as well as MR-CoNS, this study comes to fill in a part of the gap by assessing the current situation in one of the Veterinary Schools in Greece. In the present study, we investigated a possible occupational health risk, the nasal carriage CoNS in healthy veterinary students and also their antimicrobial susceptibility in order to evaluate if they can be easily controlled or not.

MATERIALS AND METHODS

Sampling

Nasal swabs were collected from 81 healthy students of the School of Veterinary Medicine, Aristotle University of Thessaloniki, Greece during November and December 2015. Sampling of nasal cavities was performed by swabbing both nares with the Sterile Transport Swabs STUART (FL MEDICAL Torreglia (PD) Italy). Swabs were transported immediately for analysis in the Laboratory of Food Hygiene of the Veterinary School, Aristotle University.

Questionnaire

A standardised questionnaire was completed for each participating student, during sampling, in order to collect data with reference to the lifestyle and habits that the students have and which would be correlated as potential risk factors. Questions were categorised in the following sections: personal data, health profile, life style.

Isolation of *Staphylococcus* spp.

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 h incubation at 35 °C, 10 μl of the enrichment was plated on Baird-Parker agar with Egg Yolk Tellurite (Oxoid, Unipath, Basingstoke, UK) and Mannitol Salt agar (biolab, PH EUR – USP, Budapest, Hungary) and incubated for 24-48 h at 35 °C. 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 °C. Colonies were selected according to their appearance, black both with and without opaque haloes from Baird Parker agar and both red and yellow from Mannitol Salt agar. After incubation, all cultures on TSA were transported to the laboratory of Hygiene and Epidemiology, Faculty of Medicine, University of Thessaly, for identification by MALDI-TOF MS.

Identification of *Staphylococcus* spp.

Identification at species level was performed by Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS), using a Microflex LT (Bruker Daltonik GmbH, Germany)

mass spectrometer. For *Staphylococcus species* identification with MALDI-TOF MS, an ethanol/formic acid protein extraction method was used according to the manufacturer's standardized protocols provided by Bruker Daltonics in order to increase spectrum quality. One μl of the extraction was placed on a steel MALDI target plate. Afterwards, it was overlaid with one μl of a saturated solution of a-cyano-4-hydroxycinnamic acid (HCCA) (Bruker Daltonics, Bremen, Germany) and allowed to co-crystalize at room temperature. Spectra were automatically acquired in a linear positive mode, at a laser frequency of 20Hz, within a mass range from 2,000 to 20,000 Da, with AutoExecute acquisition software (Flex control 3.4, Bruker Daltonics, Bremen, Germany). The spectra were externally calibrated using DH5alpha. Raw spectra were processed using MALDI BioTyper v.3.1 software (Bruker Daltonik GmbH, Germany). Results were classified using modified score values proposed by the manufacturer.

Antimicrobial Susceptibility

Antimicrobial susceptibility testing to 11 antimicrobials was performed by agar dilution method according to CLSI (2008) guidelines and EUCAST (2015) guidelines for MIC. These antibiotics were b-lactam [penicillin (P), amoxicillin/clavulanic acid (AMC) and oxacillin (OX)], glycopeptides [vancomycin (VA)], macrolids [erythromycin (E)], fluoroquinolones [ciprofloxacin (CP)], chloramhenicol (C), rifamycins [rifampicin (RA)], tetracycline (TET), trimethoprim (TR) gentamicin (G) and trimethoprim/sulfamethoxazole (SXT) (BioChemica, Applichem, Illinois, US).

Identification of the *mec*A gene

DNA extraction from staphylococcal cells was performed according to Casey et al. (2006), and the detection of the *mec*A gene was achieved by PCR according to Murakami et al. (1991) using the primers (50 AAAATC GATGGTAAAGGTTGGC) corresponded to nucleotides 1282 to 1303, and (50 AGTTCTGCAGTACCGGATTTGC) complementary to nucleotides 1793–1814.

Statistical Analysis

A data base for the information collected from the questionnaires was created in Microsoft Excel (v.2007). Data were analyzed using the statistical package SPSS v.19. Quantitative variables are presented as mean values with standard deviation or as median with Interquartile range (IQR). Qualitative variables are given as absolute frequencies with percentages. Chi-square test or Fischer's exact test was used to investigate associations between variables. A p-value less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Table 1 presents in detail the descriptive data of the students participated in this study. To the best of our knowledge this is the first time in Greece and among very few worldwide (Rall et al., 2010; Paul et al., 2011; Huber et al., 2011; Yilmaz et al., 2015) that nasal carriage of CoNS is being assessed in Veterinary students, even though this issue is considered rather crucial on public health grounds as veterinary professionals are considered among others (Rall et al., 2010; Paul et al., 2011; Huber et al., 2011; Yilmaz et al., 2015) as high risk group for increase staphylococcal carriage.

Out of 81 samples taken from veterinary students, 59 staphylococci were isolated (72.8%), that is considered as an expected value, due to the fact that staphylococci consist natural micro flora of humans (Gordts et al., 2000). Out of 59 staphylococci, 24 were identified by MALDI-TOF as CoNS. Table 2 presents in detail the CoNS identified per sample, alongside with the MALDI-TOF reliability score. In total 27.16% of the students sampled were found to carry CoNS. It is notable that in two cases of veterinary students, dual carriage of CoNS was found (Table 3). From the students found positive in CoNS, 54% had *S. haemolyticus*, 45.8% had *S. warneri*, 16.6% had S. *epidermidis*, 4.2% had *S. pasteuri* and 4.2% had *S. capitis* (Table 3). Thus, it was expected to find more species of CoNS, such as *S. hominis* and *S. lugdunensis*, as they are considered as very common species isolated from nasal cavity (Ohara-Nemoto et al., 2008; Rasmussen et al., 2000). Other studies declare similar CoNS diversity ; Ohara-Nemoto et al. (2008) declares *S. epidermidis* (41.1 %) and *S. hominis*, *S. warneri, S. intermedius, S. capitis, S. haemolyticus, S. lugdunensis* and *S. gallinarum* ranging in order from 12.5 to 1.8 %, while Rall et al. (2010) declares 45.1% *S. warneri*, 28% *S. epidermidis,*1.2% *S. capitis* and 1,2% *S. xylosus*.

All isolates were resistant to penicillin, 33.3% were

Table 1. Sample population descriptive characteristics

		N	%
	Men	43	53,1
Gender	Women	38	46,9
	18-20	3	3,7
	20-22	51	63,0
	22-24	20	24,7
Age	>24	7	8,6
	2008	1	1,2
	2009	2	2,5
	2010	1	1,2
	2011	2	2,5
Enrty Year in Vet School	2012	74	91,4
	2013	1	1,2
Clinical modules attendance	No	0	0,0
	Yes	81	100,0
	1 year	31	38,3
	1,5 year	15	18,5
	2 years	22	27,2
	3 years	1	1,2
	3 months	2	2,5
If Yes, for how long?	4years	1	1,2
	on going	2	2,5
	No	42	51,9
Pet owner	Yes	39	48,1
	Cat	10	12,3
	Rabbit	1	1,2
	Hamster	1	1,2
	Squirel	1	1,2
	Dog	17	21,0
Kind of pet	Dog and Cat	5	6,2

		N	%
Antibiotic use	No	66	82,5
(<3 months)	Yes	14	17,5
	amoxicillin/clavulanic	2	2,5
	ceclor	1	1,2
	ciproxin, Procef, mefoxil,	1	1,2
	Ponstan	1	1,2
	Vibramycin	1	1,2
	cefuroxime	1	1,2
	Amoxycillin, clavulonic	1	1,2
Antibiotic type	Mouth wash antiseptic	1	1,2
Antibiotic use	No	65	82,3
(<6 months)	Yes	14	17,7
	cefaclor, clindamycin, ciprofloxacin, nitrofurantoin, amoxicillin, cefuroxime	1	1,2
		1	1,2
	Ponstan	1	1,2
	cefuroxime	1	1,2
Antibiotic type	amoxicillin	1	1,2
	No	45	55,6
Smoking	Yes	36	44,4
	Frequently	11	40,7
Smoking Frequency	Occasionally	16	59,3
	No	72	88,9
Suregery (<1 year)	Yes	9	11,1
	Skin	1	1,2
	Dental	2	2,5
	Respiratory	1	1,2
Type of surgery	Digestive	2	2,5
Known respiratory	No	68	85,0
Disease	Yes	12	15,0

resistant to amoxicillin/clavulanic acid, 29.2% to erythromycin, 4.2% to oxacillin, 4.2% to gentamicin and 4.2% to trimethoprim/sulfomethoxazone (Table 4). The resistant to oxacillin isolate belonged to the *S. epidermidis* species proved to carry the *mec*A gene. *S. epidermidis* as well as other commensal staphylococcal species have been recognized as important sources of genes facilitating MRSA infection (Otto, 2013). Yilmaz et al, (2015) reported that from 43.8% of the veterinarians and 44.6% of the examined veterinary students in Turkey were found positive to MR-CoNS and their isolates were identified as *S. epidermidis* (n=33), *S. hominis subsp. hominis* (n=2) and *S. lentus* (n=1) from veterinarians and *S. epidemidis* (n=26), *S. haemolyticus* (n=5), *S. cohnii* (n=4) and *S. hominis subsp. hominis* (n=2) from students.

Table 2. CoNs occurrence per sampled Veterinary Student

	Sample ID	CoNS species	Reliability Score
1	A.13.1	*Staphylococcus epidermidis*	2,048
2	A.14.2	*Staphylococcus haemolyticus*	2,159
3	A.19.1	*Staphylococcus haemolyticus*	2,115
4	A.21.1	*Staphylococcus haemolyticus*	2,162
5	A.23.2	*Staphylococcus haemolyticus*	2,009
6	B.1.1	*Staphylococcus warneri*	1,920
7	B.5.2	*Staphylococcus warneri*	1,945
8	B.6.1	*Staphylococcus warneri*	1,926
9	B.6.3	*Staphylococcus epidermidis*	2,010
10	B.7.2	*Staphylococcus capitis*	2,134
11	B.8.1	*Staphylococcus epidermidis*	1,906
12	B.10.1	*Staphylococcus warneri*	1,753
13	B.10.2	*Staphylococcus haemolyticus*	1,902
14	B.11.1	*Staphylococcus haemolyticus*	2,159
15	B.12.2	*Staphylococcus warneri*	2,206
16	B.13.2	*Staphylococcus haemolyticus*	2,028
17	C.10.2	*Staphylococcus warneri*	1,944
18	C.11.1	*Staphylococcus warneri*	1,931
19	C.12.2	*Staphylococcus pasteuri*	1,862
20	C.18.2	*Staphylococcus warneri*	2,010
21	C.21.3	*Staphylococcus warneri*	1,961
22	C.23.1	*Staphylococcus haemolyticus*	2,111
23	D.9.1	*Staphylococcus haemolyticus*	2,240
24	D.16	*Staphylococcus haemolyticus*	1,921

Table 3. CoNs occurrence among Veterinary students population

CoNS species	No of positive students	%
S. warneri, S. epidermidis	2	8.3
S. warneri, S. haemolyticus	2	8.3
S. warneri	7	29.2
S. haemolyticus	9	37.5
S. epidermidis	2	8.3
S. capitis	1	4.2
S. pasteuri	1	4.2

Table 4. Antimicrobial resistance profiles of CoNS isolated from nasal cavities of Vet Students

Species	Resistance Profile *	No of Strains	*mec*A gene
Staphylococcus warneri	P,A/C	2	
	P, E	1	
	P	5	
	P, E, T/S	1	
Staphylococcus haemolyticus	P	4	
	P,A/C	2	
	P,E	3	
	P,E,,T/S	1	
Staphylococcus epidermidis	P	2	
	P,G,O-0.5,O-4,A/C	1	+
Staphylococcus pasteuri	P, A/C	1	
Staphylococcus capitis	P,E,A/C	1	

** penicillin 0.25 (P), erythromycin 2(E), gentamicin 2 (G), oxacillin 0.5 (O-0.5), oxacillin 4 (O-4), trimethoprim/sulfamethoxazole 4/76 (T/S), amoxicillin/clavulanic acid 8/2 (A/C)*

Abadi et al., (2015) found 71.7% of school students in India to carry CoNS and among them 16.7% were MR-CoNS. The isolated CoNS species were *S. lugdunensis* (26.1%), *S. epidermidis* (24.9%), *S. haemolyticus* (24.4%), *S. saprophyticus* (13.7%), *S. schleiferi* (10%) and *S. simulans* (0.9%). Huber et al., (2011) reported that 49.3 % of samples from professionals involved in the production of foods of animal origin (farmers, veterinarians and slaughterhouse employees) were positive to MR-CoNS with predominant species *S. haemolyticus* (47.5%) and *S. epidermidis* (40.3%). High nasal carriage of 67.6% and 60.2% of MR-CoNS was observed among pig farmers and veterinarians respectively. The prevalence for slaughterhouse employees was by far lower set at the percent of 26.3%. The population participated may not be considered as representative for the veterinary professional population in Greece, but nevertheless it gives an initial idea of the current situation with regards to CoNS occurrence, that is considered important on public health grounds. The detailed demographic data collected alongside with data on lifestyle, habits and health status, as presented in Table 1, are considered crucial for evaluating possible associations to the occurrence of staphylococci (Gordts et al., 2000; Kock et al., 2016; Gomez-Sanz et al., 2013) and therefore have been assessed in detail.

Table 5 presents in detail the examined associated factors with CoNS occurrence in the Veterinary students' population sampled. It is notable that vet students that didn't own a pet presented a statistically significant difference (p-value=0.007) in CoNS occurrence, that is contradictory to the current literature (Gomez-Sanz et al., 2013; Paul et al., 2011). Additionally, hospital visits of students in the last 6 months has been also found to be negative associated with CoNS occurrence (p-value=0.048), that is also in contradiction with the literature, where it is stated that hospital visits are correlated with increased staphylococci occurrence for at least 21 weeks (Goslings & Buchli, 1958). Both these results might be due to the relatively small sample tested in this study and are considered as random findings that cannot be further assessed. Possibly, longer monitoring period with increased sample size may have shown different

Table 5. Questionnaire data correlation with CoNS occurrence in the Veterinary student's population sampled

		CoNS				
		NEGATIVE		POSITIVE		
		N	%	N	%	p-value
Gender	Men	27	62,8	16	37,2	0,112*
	Women	30	78,9	8	21,1	
Age	18-22	38	70,4	16	29,6	1,000*
	>22	19	70,4	8	29,6	
Pet owner	No	24	57,1	18	42,9	**0,007***
	Yes	33	84,6	6	15,4	
Hospital Visit (<6months)	No	34	63,0	20	37,0	**0,048***
	Yes	22	84,6	4	15,4	
Intensive Care Unit visit (<6 months)	No	54	69,2	24	30,8	0,551**
	Yes	3	100,0	0	0,0	
Hospitalization (<6 months)	No	52	69,3	23	30,7	1,000**
	Yes	4	80,0	1	20,0	
Hospitalization (<1 year)	No	56	70,0	24	30,0	1,000**
	Yes	1	100,0	0	0,0	
Medical staff in the family	No	33	63,5	19	36,5	0,068*
	Yes	24	82,8	5	17,2	
Surgery (<1year)	No	50	69,4	22	30,6	0,718**
	Yes	7	77,8	2	22,2	
Antibiotics use (<3 months)	No	47	71,2	19	28,8	0,749**
	Yes	9	64,3	5	35,7	
Antibiotics use (<6 months)	No	47	72,3	18	27,7	0,338**
	Yes	8	57,1	6	42,9	
Smoking	No	34	75,6	11	24,4	0,253*
	Yes	23	63,9	13	36,1	
Smoking Frequency	Frequently	9	81,8	2	18,2	0,405**
	Occasionally	10	62,5	6	37,5	
Known respiratory disease	No	45	66,2	23	33,8	0,096**
	Yes	11	91,7	1	8,3	
Known other disease	No	51	68,9	23	31,1	0,668**
	Yes	6	85,7	1	14,3	
Known skin disease (<6 months)	No	53	69,7	23	30,3	1,000**
	Yes	4	80,0	1	20,0	

** Pearson chisquare test*

*** Fishers exact test*

trends in CoNS occurrence in relation to certain risk factors. As far as other risk factors are considered, frequent smoking and antibiotics use present a trend in higher occurrence of CoNS, as reported by others (Kaygusuz et al., 2004; Marples et al., 1969) without though presenting statistically significant difference. Future research could possibly focus in correlating risk factors related to demographic data (gender, age), lifestyle, habits and health status to the occurrence of CoNS and MR-CoNS as well.

CONCLUSIONS

Although only one student was found to carry methicillin-resistant *S. epidermidis*, its presence is evidence that this pathogen may circulate among veterinarians and the personnel of veterinary health establishments. Generally poor information is available about the prevalence of MR-CoNS in veterinary health units and the community and thus more studies are needed for the evaluation of the prevalence and antimicrobial resistance of CoNS. Surveillance programs should also be performed in veterinary units because the emergence of resistant bacteria in this environment may represent a risk to public health.

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REFERENCES

Abadi, M.I.M., Moniri, R., Khorshidi, A., Piroozmand, A., Mousavi, S.G.A., Dastehgoli, K. and Ghazikalayeh, H.M. (2015) Molecular characteristics of nasal carriage Methicillin-Resistant Coagulase Negative Staphylococci in school students. *Jundisapur J microbiol 8*(6):e18591, DOI:10.5812/jjm.18591v2.

Aires-de-Sousa, M., Boye, K., de Lencastre, H., Deplano, A., Enright, M.C., Etienne, J., Friedrich, A., Harmsen, D. et al. (2006) High interlaboratory reproducibility of DNA sequence-based typing of bacteria in a multicenter study. *J Clin Microbiol* 44:619–621.

Casey, A.L., Worthington, T., Caddick, J.M., Hilton, A.C., Lambert, P.A. and Elliott, T.S.J. (2006) RAPD for the typing of coagulase-negative staphylococci implicated in catheter-related bloodstream infection. J Infect 52: 282–289.

Chambers, H.F. (1997) Methicillin resistance in staphylococci: molecular and biochemical basis and clinical implications. Clin Microbiol Rev 10: 781–791.

CLSI (2008) Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals Approved Standard CLSI Document M31-A3. Wayne, PA, USA: Clinical and Laboratory Standards Institute.

EUCAST (2015) Breakpoint table for bacteria, http://www.eucast.org/ clinical _ breakpoints/

Gomez-Sanz, E., Torres, C., Lozano, C., & Zarazaga, M. (2013). High diversity of *Staphylococcus aureus* and *Staphylococcus pseudintermedius* lineages and toxigenic traits in healthy pet-owninh household members. Underestimating normal household contact? *Comp. Immunol. Microbiol. Infect. Dis., 36*(1): 83-94.

Gordts, F., Halewyck, S., Pierard, D., Kaufman, L., & Clement, P. A. (2000) Microbiology of the middle meatus: a comparison between normal adults and children. *Journal of Laryngology & Otology, 114*(3):184-188.

Goslings, W. R., & Buchli, K. (1958) Nasal carrier rate of antibiotic-resistant staphylococci; influence of hospitalization on carrier rate in patients, and their household contacts. *AMA Arch Intern Med, 102*(5):691-715.

Harmsen, D., Claus, H., Witte, W., Rothg€anger, J., Claus, H., Turnwald, D. and Vogel, U. (2003) Typing of methicillinresistant *Staphylococcus aureus* in a university hospital setting by using novel software for spa repeat determination and database management. *J Clin Microbiol 41*:5442–5448.

Holland, L. M., Conlon, B., & O'Gara, J. P. (2011). Mutation of tagO reveals an essential role for wall teichoic acids in *Staphylococcus epidermidis* biofilm development. *Microbiology, 157*(Pt 2):408-418.

Huber, H., Ziegler, D., Pfluger, V., Vogel, G., Zweifel, C., & Stephan, R. (2011). Prevalence and characteristics of methicillin-resistant coagulase-negative staphylococci from livestock, chicken carcasses, bulk tank milk, minced meat, and contact persons. *BMC Vet Res, 7*, 6. doi: 10.1186/1746-6148-7-6

Kaygusuz, I., Kizirgil, A., Karlidag, T., Keles, E., Yalcin, S., Alpay, H. C., & Yildiz, M. (2004) The effect of smoking on nasal microbial flora. *Saglik Bilimler Tip Dergisi, 18*(3):187-190.

Kock, R., Werner, P., Friedrich, A. W., Fegeler, C., Becker, K., Prevalence of Multiresistant Microorganisms Study, G., & Prevalence of Multiresistant Microorganisms, P. M. M. S. G. (2016) Persistence of nasal colonization with human pathogenic bacteria and associated antimicrobial resistance in the German general population. *New Microbes New Infect, 9*:24-34.

Marples, R. R., Fulton, J. E., Leyden, J., & McGinley, K. J. (1969) Effect of antibiotics on the nasal flora in acne patients. *Arch Dermatol, 99*(6):647-651.

Murakami, K., Minamide, W., Wada, K., Nakamura, E., Teraoka, H. and Watanabe, S. (1991) Identification of methicillin-resistant strains of staphylococci by polymerase chain reaction. *J Clin Microbiol* 29:2240-2244.

Ohara-Nemoto, Y., Haraga, H., Kimura, S., & Nemoto, T. K. (2008) Occurrence of staphylococci in the oral cavities of healthy adults and nasal oral trafficking of the bacteria. *J Med Microbiol, 57*(Pt 1):95-99.

Otto, M. (2013). Coagulase-negative staphylococci as reservoirs of genes facilitating MRSA infection. *Bioassays,35*(1) 4-11.

Piette, A. and Verschraegen, G. (2009) Role of coagulasenegative staphylococci in human disease. *Vet Microbiol 134*:45–54.

Paul, N. C., Moodley, A., Ghibaudo, G., & Guardabassi, L. (2011) Carriage of methicillin-resistant *Staphylococcus pseudintermedius* in small animal veterinarians: indirect evidence of zoonotic transmission. *Zoonoses Public Health, 58*(8):533-539.

Rall, V. L., Sforcin, J. M., Augustini, V. C., Watanabe, M. T., Fernandes, A., Jr., Rall, R., Araujo, J. P. Jr. (2010) Detection of enterotoxin genes of *Staphylococcus* sp isolated from nasal cavities and hands of food handlers. *Braz J Microbiol, 41*(1):59-65.

Rasmussen, T. T., Kirkeby, L. P., Poulsen, K., Reinholdt, J., & Kilian, M. (2000) Resident aerobic microbiota of the adult human nasal cavity. *APMIS, 108*(10):663-675.

Wielders, C.L.C., Vriens, M.R., Brisse, S., de Graaf-Miltenburg, L.A.M., Troelstra, A., Fleer, A., Schmitz, F.J., Verhoef, J. et al. (2001) Evidence for in-vivo transfer of mecA DNA between strains of *Staphylococcus aureus*. *The Lancet 357*:1674–1675.

Yilmaz, M. A., Aslantas, Ö., Özer, B., & Yilmaz, E. Ş. (2015) Nasal carriage of Methicillin- Resistant Coagulase Negative Staphylococci (MR-CoNS) Among Veterinarians and Veterinary Students. *. J. Fac. Vet. Med. Istanbul Univ, 41*(1):69-78.

Ziebuhr, W., Hennig, S., Eckart, M., Kranzler, H., Batzilla, C. and Kozitskaya, S. (2006) Nosocomial infections by *Staphylococcus epidermidis*: how a commensal bacterium turns into a pathogen. *Int J Antimicrob Agents 28*:14–20.