

## Presence and antimicrobial resistance of coagulase-negative staphylococci isolated from animals in a Veterinary Teaching Hospital in Cyprus

HALIT SUKUR\*, OMER MEMDUH ESENDAL

Department of Microbiology, Faculty of Veterinary Medicine, Near East University, Nicosia, North Cyprus

\*Corresponding author: [halit.sukur@neu.edu.tr](mailto:halit.sukur@neu.edu.tr)

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**Abstract:** Coagulase-negative staphylococci (CoNS) are a group of commensal microorganisms residing on the skin and mucous membranes of both humans and animals. Until recently, they have been regarded as non-pathogenic to livestock and companion animals, but since then, their clinical importance in veterinary medicine has increased with the discovery of their potential pathogenic roles in animals causing skin and soft tissue infections together with spontaneous abortions and mastitis. Scientific data concerning the presence of CoNS in North Cyprus are very limited. Therefore, the purpose of the study reported herein was to investigate the presence and antimicrobial resistance patterns of CoNS species isolated from various animals presented at the Veterinary Teaching Hospital in North Cyprus between July 2018 and 2019. Staphylococci were isolated from 37.0% (87/235) of the samples submitted, within which 60.9% (53/87) and 39.1% (34/87) were identified as coagulase-positive staphylococci (CoPS) and coagulase-negative staphylococci (CoNS), respectively. Among the CoNS, *S. chromogenes* was the most predominantly isolated species (14/34, 41.2%), followed by *S. capitis* (5/34, 14.7%) and *S. simulans* (4/34, 11.8%). Of these 34 CoNS, 24 (70.6%) and 10 (29.4%) were identified as MRCoNS and MSCoNS, respectively. The CoNS isolates showed relatively high levels of resistance towards amoxicillin/clavulanic acid (19/34, 55.9%), tetracycline (14/34, 41.2%) and penicillin (13/34, 38.2%). In conclusion, the presence of CoNS, especially MRCoNS, and the detection of multiple drug resistant (MDR) species with a high prevalence were regarded as being important since they might limit and have negative effects on the therapeutic treatment options of staphylococcal infections in animals, and might have both public and veterinary concerns.

**Keywords:** cows; companion animals; CoNS; North Cyprus; prevalence

Until the last two decades, coagulase-negative staphylococci (CoNS) have been regarded as non-pathogenic to livestock as well as companion animals and have generally been discarded in veterinary diagnostic laboratories with the assumption of being unimportant contaminants (Cain et al. 2011). Nevertheless, this concept has changed recently with the discovery of *Staphylococcus lugdunensis* and *Staphylococcus schleiferi* subsp. *schleiferi*, as emerging potential zoonotic pathogens in dogs, cats and other animals causing skin and

soft tissue infections, as well as spontaneous abortions (Cain et al. 2011; Rook et al. 2012; Davis et al. 2013; Ardigo et al. 2014). Studies showing the role of CoNS in the induction of udder infections and inflammations in livestock have simultaneously fortified this aspect (Bochniarz and Wawron 2011).

Although *Staphylococcus aureus*, a CoPS species, has been considered among the major aetiological agents of mastitis in dairy animals, the significance of the CoNS species as the putative causative agents in mastitis has increased considerably in many

countries recently (Vanderhaeghen et al. 2015; Bhat et al. 2017; Srednik et al. 2017; Vakkamaki et al. 2017; Nobrega et al. 2018). At present, more than 15 CoNS species have been described in the aetiology of mastitis in dairy cows, with *S. chromogenes*, *S. simulans*, *S. xylosus*, *S. epidermidis*, *S. hyicus* and *S. haemolyticus* being the most commonly isolated species (Cervinkova et al. 2013; Alekikh 2015; Vanderhaeghen et al. 2015; Zigo et al. 2019). Since commonly isolated CoNS, in particular methicillin-resistant CoNS (MRCoNS) from bovine mastitis, could display resistance to a wide range of antimicrobial agents, they would drastically limit the therapeutic options in staphylococcal infections (Srednik et al. 2017).

The impact of CoNS in bovine mastitis has been studied extensively throughout the world. CoNS species have been detected in clinical and/or sub-clinical bovine mastitis cases with a frequency ranging from 13.04% to 95.6%. Many species, such as *S. chromogenes*, *S. haemolyticus*, *S. xylosus*, *S. cohnii*, *S. hominis*, *S. saprophyticus*, *S. lentus*, *S. lugdunensis* and *S. simulans*, have been reported to be the most commonly isolated species in different regions (El-Jakee et al. 2013; Hosseinzadeh and Saei 2014; Bhat et al. 2017; Vakkamaki et al. 2017). Upon extensive isolation of *S. chromogenes* and *S. haemolyticus* from dairy cattle, Hosseinzadeh and Saei (2014) have stated that these species should be considered as emerging pathogens causing mastitis in the North West of Iran.

Srednik et al. (2017) have shown that the CoNS species that is commonly isolated from bovine mastitis could display resistance to a wide range of antimicrobial agents including penicillin, oxacillin and cefoxitin. They concluded that CoNS could pose a potential public health threat with respect to the antimicrobial resistance, and in particular, the emergence of methicillin-resistant (MR) phenotypes would limit the therapeutic options. Nobrega et al. (2018) have reported resistance to quinupristin/dalfopristin, erythromycin,  $\beta$ -lactams and tetracyclines among 25 CoNS isolated from Canadian dairy herds.

Various CoNS species with different antibacterial resistance patterns have been isolated from diseased and healthy companion animals (Karakulska et al. 2012; Mallardo et al. 2013; Ruzauskas et al. 2014; Loncaric et al. 2016). In Poland, Karakulska et al. (2012) evaluated the nasal microbiota of 42 healthy horses with a special emphasis on the presence of CoNS species and their antimicrobial resistance

to 18 antimicrobials, particularly methicillin resistance. In Italy, Mallardo et al. (2013) studied the prevalence of methicillin-resistant staphylococci (MRS) in horses by examining the nasal swab samples of 191 healthy individuals, and reported the isolation of MRS from 68 (35.6%) carrier horses. Loncaric et al. (2016) isolated MRCoNS, including *S. haemolyticus* and *S. fleurettii*, from different animal species in Lower Austria. A total of 13 MRCoNS, comprised of 6 methicillin-resistant *S. haemolyticus* (MRSH) and 7 methicillin-resistant *S. fleurettii*, were isolated from 4 horses, 7 cats and 2 pigs, with antimicrobial resistance to  $\beta$ -lactam antibiotics, erythromycin, fluoroquinolones, and gentamicin residing on the same farm. Among the MRCoNS, antimicrobial resistance was detected to  $\beta$ -lactam antibiotics, erythromycin, fluoroquinolones, and gentamicin. After examining 754 samples collected from companion animals in Lithuania, Ruzauskas et al. (2014) reported the isolation of 12 methicillin-resistant *S. haemolyticus* (MRSH) strains, 11 from dogs and one from a kennel owner, where no MRSH were observed in any of the examined horses or cats. The authors stated that the most frequent resistance of the 11 methicillin-resistant *S. haemolyticus* (MRSH) strains from the dogs was that to benzylpenicillin (91.7%), erythromycin (91.7%), gentamicin (75.0%), tetracycline (66.7%), fluoroquinolones (41.7%) and co-trimoxazole (41.7%), respectively.

Scientific data concerning the presence of CoNS in animals are very limited in Cyprus. The only data were published by Darbaz et al. (2018). While investigating the udder health and milk quality parameters of dairy farms in North Cyprus, Darbaz et al. (2018) have examined bulk tank milk samples from 138 dairy farms between October 2009 and September 2010, and reported that CoNS species were isolated in the first rank with a frequency of 22.7%. Nevertheless, they have neither identified the isolates on the species level, nor determined their antimicrobial susceptibilities. Apart from this, there is no other information about the presence of CoNS in animals, particularly, companion animals in Cyprus.

Therefore, the objective of this preliminary study was to investigate the presence, prevalence, and antimicrobial resistance patterns of coagulase-negative staphylococci in animals presented at the Veterinary Teaching Hospital in Cyprus between July 2018 and 2019.

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## MATERIAL AND METHODS

### Clinical samples

A total of 235 clinical samples including milk samples, skin scrapings and swabs, as well as swab samples from the eyes, ears, and nostrils were collected from patients that were brought to the Near East University Animal Hospital between August 1, 2018 and July 31, 2019. All the patients had manifested different clinical conditions like mastitis, dermatitis, otitis, rhinitis, ophthalmologic disorders and wound infections with a suspected staphylococcal aetiology.

The samples were directly collected by the clinicians working in the Hospital under aseptic conditions and delivered for routine bacteriological diagnosis to the Microbiology Laboratory of the same Institute. This study was approved by the Near East University Animal Research Ethics Committee (Decision No. 2018/21).

### Isolation and identification of CoNS

All the clinical samples were inoculated onto 5% sheep Blood Agar (43041; Biomerieux, Marcy-l'Étoile, France), MacConkey Agar (105465; Merck, Darmstadt, Germany) and Eosin Methylene Blue Agar (101347; Merck, Darmstadt, Germany) for routine diagnosis and incubated aerobically at 37 °C for 24–48 hours. The concurrently relevant samples were also inoculated on Sabouraud Dextrose Agar (CM0041; Oxoid, Massachusetts, USA) and incubated accordingly for the presence of any possible dermatophytes.

The isolates grown on the MacConkey Agar and EMB agar were discarded and only the isolates with a staphylococcal morphology were processed for further analyses.

The staphylococcus species were identified at the genus level according to their morphological features, catalase production, and Gram-staining characteristics. They were further identified at the species level according to the coagulase production, the presence of the clumping factor, as well as the biochemical properties as determined by using the Vitek 2 compact automated system (BioMérieux, Marcy l'Étoile, France) according to the manufacturer's guidelines as used elsewhere (Badger-Emeka 2017).

### Antimicrobial susceptibility testing

The antimicrobial resistance profiles of the CoNS isolates were determined by using the Vitek 2 compact automated system (BioMérieux, Marcy l'Étoile, France) according to the manufacturer's guidelines as used elsewhere (Badger-Emeka 2017), for the following 27 antimicrobials: amoxicillin/clavulanic acid, ampicillin/sulbactam, ceftriaxone, cephalexin, chloramphenicol, ciprofloxacin, clindamycin, daptomycin, enrofloxacin, erythromycin, fosfomycin, fusidic acid, gentamicin, imipenem, kanamycin, linezolid, marbofloxacin, mupirocin, nitrofurantoin, penicillin, polymyxin B, rifampin, teicoplanin, tetracycline, tigecycline, trimethoprim/sulfamethoxazole and vancomycin. The methicillin resistance of the isolates was interpreted according to the antimicrobial susceptibility test results of the cefoxitin and/or oxacillin, as the screening antibiotics, as reported earlier (Dupieux et al. 2016).

## RESULTS

### Bacterial isolates

During the study period between August 1, 2018 and July 31, 2019, a total of 235 clinical samples were delivered for routine bacteriological diagnosis. Among these samples, while 27.2% (64/235) did not yield any bacterial growth, Gram-negative bacteria were isolated from 28.5% (67/235) and Gram-positive bacteria were isolated from 44.3% (104/235). Based on the microscopic appearances and other basic features, 83.7% (87/104) of the Gram-positive isolates were defined as Staphylococci. With the aid of a coagulase test, the Staphylococcal isolates were identified as 60.9% CoPS (53/87) and 39.1% CoNS (34/87). A total of 34 CoNS strains isolated from 28 animals (cows,  $n = 4$ , cats,  $n = 7$ , horses,  $n = 8$ , dogs,  $n = 6$ , sheep,  $n = 1$ , chicken,  $n = 1$  and monkey,  $n = 1$ ) were selected for further identification at the species level. Considering the methicillin resistance of these 34 CoNS, 24 (70.6%) and 10 (29.4%) were identified as methicillin resistant CoNS (MRCoNS) and methicillin sensitive CoNS (MSCoNS), respectively. The results are summarised in Table 1. According to these results, the prevalence of CoNS was estimated at 39.1% among the staphylococcal isolates and 14.5% among all the examined clinical samples. Similarly, the prevalence of MRCoNS

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Table 1. The bacteriological results of the clinical samples presented at the Veterinary Teaching Hospital between August 1, 2018 and July 31, 2019

Total number of clinical samples tested	235
Number of culture negative samples	64/235 (27.2%)
Number of samples with Gram-negative bacteria	67/235 (28.5%)
Number of samples with Gram-positive bacteria	104/235 (44.3%)
Number of samples with Staphylococci	87/104 (83.7%)
Number of samples with other Gram-positive agents	17/104 (16.3%)
Number of CoPS	53/87 (60.9%)
Number of CoNS	34/87 (39.1%)
Number of MRCoNS	24/34 (70.6%)
Number of MSCoNS	10/34 (29.4%)

was estimated at 70.6% (24/34) among the CoNS and 10.2% (24/235) among all the examined clinical samples. The distribution of the CoNS and MRCoNS species isolated from the 28 clinical samples is shown in Table 2. Among the 34 CoNS isolates, *S. chromogenes* (41.2%) was determined to be the most prevalent, followed by *S. capitis* (14.7%) and *S. simulans* (11.8%). On the other hand, among the 24 MRCoNS isolates, *S. chromogenes* (41.7%) was again found to be the most widespread, followed *S. capitis* (16.7%) and *S. epidermidis* (8.3%).

Table 2. The CoNS (MRCoNS) isolated from the clinical samples presented at the Veterinary Teaching Hospital between August 1, 2018 and July 31, 2019

Bacterial species	Number of isolates	Frequency
<i>S. chromogenes</i>	14 (10)*	41.2% (41.7%)*
<i>S. capitis</i>	5 (4)	14.7% (16.7%)
<i>S. simulans</i>	4 (2)	11.8% (8.3%)
<i>S. epidermidis</i>	3 (2)	8.9% (8.3%)
<i>S. haemolyticus</i>	3 (2)	8.9% (8.3%)
<i>S. caprae</i>	1 (–)	2.9% (–)
<i>S. gallinarum</i>	1 (1)	2.9% (4.2%)
<i>S. hominis</i>	1 (1)	2.9% (4.2%)
<i>S. kloosi</i>	1 (1)	2.9% (4.2%)
<i>S. xyloso</i>	1 (1)	2.9% (4.2%)
Total	34 (24)	100.0% (100.0%)

\*Data within brackets represent the MRCoNS

### Antimicrobial resistance

When the antimicrobial susceptibility testing of 34 CoNS and 24 MRCoNS isolates against a panel of 27 antimicrobials was evaluated, they were observed to show resistance to 15 antimicrobials. The results are shown in Table 3. The CoNS isolates showed relatively high levels of resistance towards amoxicillin/clavulanic acid (55.9%), tetracycline (41.2%) and penicillin (38.2%), followed by other antimicrobials tested.

Table 3. The antimicrobial resistance results of the CoNS and MRCoNS isolates

Antimicrobial*	CoNS		MRCoNS	
	frequency	percent (n/N)**	frequency	percent (n/N)**
Amoxicillin/clavulanic acid	19	55.9 (19/34)	18	75.0 (18/24)
Tetracycline	14	41.2 (14/34)	9	37.5 (9/24)
Penicillin	13	38.2 (13/34)	9	37.5 (9/24)
Trimethoprim/sulfamethoxazole	8	23.5 (8/34)	7	29.2 (7/24)
Erythromycin	6	17.6 (6/34)	4	16.7 (4/24)
Ampicillin/sulbactam	5	14.7 (5/34)	5	20.8 (5/24)
Cephalexin	4	11.8 (4/34)	4	16.7 (4/24)
Enrofloxacin	3	8.8 (3/34)	2	8.3 (2/24)
Clindamycin	3	8.8 (3/34)	1	4.2 (1/24)
Polymyxin B	3	8.8 (3/34)	3	12.5 (3/24)
Ceftriaxone	2	5.9 (2/34)	2	8.3 (2/24)
Gentamicin	2	5.9 (2/34)	2	8.3 (2/24)
Fosfomycin	2	5.9 (2/34)	–	–
Fusidic Acid	1	2.9 (1/34)	1	4.2 (1/24)
Mupirocin	1	2.9 (1/34)	–	–

\*Cefoxitin and/or Oxacillin excluded; \*\*n is the number of resistant, N is the number tested



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Similarly, the MRCoNS isolates showed high levels of resistance towards amoxicillin/clavulanic acid (75.0%), tetracycline (37.5%) and penicillin (37.5%), followed by others. The overall isolation results of CoNS from the animals and their antimicrobial resistance patterns are shown in Table 4.

Table 4. Coagulase-negative Staphylococci isolates from animals and their antimicrobial resistance patterns

Animal species	Sample	Isolate	Resistance pattern
Cow No. 1	milk	<i>S. epidermidis</i>	ENR
Cow No. 2	milk	<i>S. chromogenes</i>	ERY, CLI, PEN, TET
		<i>S. simulans</i>	ERY, CLI, PEN, TET, SXT
Cow No. 3	milk	<i>S. chromogenes</i>	MRCoNS, AMC, LEX, PEN
Cow No. 4	milk	<i>S. capitis</i>	MRCoNS, AMC, PEN, PMB, TET
		<i>S. epidermidis</i>	MRCoNS, AMC, PEN, TET
Cat No. 1	eye	<i>S. chromogenes</i>	MUP
Cat No. 2	wound*	<i>S. caprae</i>	FOF
Cat No. 3	skin	<i>S. chromogenes</i>	–
Cat No. 4	ear	<i>S. chromogenes</i>	–
		<i>S. simulans</i>	–
Cat No. 5	ear	<i>S. simulans</i>	MRCoNS, CRO
Cat No. 6	nose	<i>S. simulans</i>	MRCoNS, AMC, SXT
Cat No. 7	ear*	<i>S. chromogenes</i>	MRCoNS, AMC, PEN
Horse No. 1	skin	<i>S. capitis</i>	FOF, TET
Horse No. 2	skin	<i>S. capitis</i>	MRCoNS, AMC, LEX, PEN
Horse No. 3	skin	<i>S. haemolyticus</i>	MRCoNS, SAM, LEX, PEN, SXT
Horse No. 4	skin	<i>S. chromogenes</i>	MRCoNS, LEX, PEN, SXT
Horse No. 5	skin	<i>S. chromogenes</i>	MRCoNS, AMC
Horse No. 6	skin	<i>S. chromogenes</i>	MRCoNS, AMC
Horse No. 7	skin*	<i>S. xylosus</i>	MRCoNS, AMC, PMB, TET
		<i>S. capitis</i>	MRCoNS, AMC, PMB, TET
Horse No. 8	skin**	<i>S. chromogenes</i>	MRCoNS, AMC, PEN, SXT
Dog No. 1	skin	<i>S. epidermidis</i>	MRCoNS, ERY, FA, TET
Dog No. 2	skin	<i>S. chromogenes</i>	MRCoNS, AMC, ERY, TET
Dog No. 3	ear	<i>S. haemolyticus</i>	–
Dog No. 4	ear*	<i>S. capitis</i>	MRCoNS
Dog No. 5	skin	<i>S. chromogenes</i>	MRCoNS, AMC, SXT
Dog No. 6	skin	<i>S. chromogenes</i>	MRCoNS, AMC
Sheep	nose	<i>S. chromogenes</i>	MRCoNS, TET, SXT
Monkey	skin	<i>S. haemolyticus</i>	MRCoNS, AMC, SAM, CRO, ENR, ERY, GEN, CLI, PEN, TET, SXT
		<i>S. kloosi</i>	MRCoNS, AMC, SAM
Chicken	nec	<i>S. gallinarum</i>	MRCoNS, AMC, SAM, ENR
		<i>S. hominis</i>	MRCoNS, AMC, SAM, ERY, GEN, TET

\*Mix infection with *S. aureus*; \*\*Mix infection with *S. aureus* and *S. hyicus*

AMC = amoxicillin/clavulanic acid; CLI = clindamycin; CRO = ceftriaxone; ENR = enrofloxacin; ERY = erythromycin; FA = fusidic acid; FOF = Fosfomycin; GEN = gentamicin; LEX = cephalexin; MUP = mupirocin; Nec = necropsy material; PEN = penicillin; PMB = polymyxin B; SAM = ampicillin/sulbactam; SXT = trimethoprim/sulfamethoxazole; TET = tetracycline

## DISCUSSION

The presence and aetiological role of CoNS in clinical and subclinical bovine mastitis have been studied by many researchers around the world. Various CoNS species including *S. sciuri* (Cervinkova et al. 2013), *S. chromogenes*, *S. epidermidis* and *S. haemolyticus* (Alekhish 2015; Srednik et al. 2017; Zigo et al. 2019) have been described as the most prevalent isolates obtained from either the clinical or subclinical bovine mastitis. After detecting *S. chromogenes* and *S. epidermidis* as the most frequently isolated species with frequencies of 19.4% and 16.7%, respectively, Alekhish (2015) concluded that the subclinical mastitis caused by CoNS should be considered as a significant health issue for dairy heifers in Jordan.

In the present study, among the subclinical mastitic milk samples obtained from 4 cows, 6 CoNS strains were isolated (Table 4). The isolates were identified as *S. chromogenes* (33.3%), *S. epidermidis* (33.3%), *S. simulans* (16.7%), and *S. capitis* (16.7%). of the 6 CoNS strains cultured, 3 (50%) were determined to be MR. The three MRCoNS strains were identified as *S. chromogenes*, *S. epidermidis*, and *S. capitis* (16.7% each). In two samples, the simultaneous isolation of MSCoNS and MRCoNS were observed. No other bacterial or fungal agents were isolated from the examined milk samples. As has been stated earlier (Hosseinzadeh and Saei 2014; Vakkamaki et al. 2017), the isolation of a CoNS species in a pure culture was regarded as remarkable, and they were ascribed as the causative agents of the subclinical mastitis cases examined herein. The results presented in the current study were in full agreement with the results of Alekhish (2015), Srednik et al. (2017) and Zigo et al. (2019) who reported *S. chromogenes* as the most prevalent species, whereas, there were slight differences with the findings of Cervinkova et al. (2013), El-Jakee et al. (2013) and Hosseinzadeh and Saei (2014) who described other species as the most widespread. Such differences were not considered as unexpected because of the possible differences of the CoNS strains circulating among the cattle populations in a given geographic location.

The presence and antibiotic resistance profiles of CoNS in diseased and/or healthy companion animals have also been studied by many researchers (Karakulska et al. 2012; Kern and Perreten 2013; Mallardo et al. 2013; Ruzauskas et al. 2014; Metiner et al. 2015; Qekwana et al. 2017; Siugzdaite

and Gabinaitiene 2017). From 216 feline samples, the isolation of *S. felis* and *S. simulans* (0.9% each) with relatively high levels of resistance towards ampicillin (32.4%), penicillin-G (29.0%), clindamycin (34.2%) and lincospectin (31.6%) were reported by Qekwana et al. (2017). A number of CoNS species has been described as members of nasal microbiota in healthy horses. From 42 healthy horses, Karakulska et al. (2012) have isolated 87 CoNS isolates with *S. equorum* (14.9%) being the most prevalent species. They reported the highest resistance to tetracycline (17.2%) followed by penicillin, erythromycin, amoxicillin/clavulanic acid, and gentamicin. Mallardo et al. (2013) identified *S. sciuri* and *S. lentus* as the most predominant MRCoNS species among 191 healthy horses with 100% resistance to amoxicillin/clavulanic acid, ampicillin and lincomycin. Ruzauskas et al. (2014) have reported the isolation of 12 methicillin-resistant *S. haemolyticus* (MRSH) strains from dogs with varying resistance patterns to benzylpenicillin, erythromycin, gentamicin, tetracycline, fluoroquinolones and co-trimoxazole. Metiner et al. (2015) have stated the isolation of *S. chromogenes* as the most prevalent CoNS from dogs with otitis externa, with a resistance profile of 60% of the isolates to sulfamethoxazole/trimethoprim, erythromycin, and clindamycin. Siugzdaite and Gabinaitiene (2017) have reported the presence *S. sciuri* and *S. warneri* as the most prevalent MRCoNS species in clinically healthy dogs with high antimicrobial resistance rates against penicillin, ampicillin, oxacillin, erythromycin and gentamicin. Kern and Perreten (2013) have identified 43 MRCoNS as *S. epidermidis* ( $n = 20$ ), *S. haemolyticus* ( $n = 17$ ), *S. hominis* ( $n = 3$ ), *S. capitis* ( $n = 1$ ), *S. cohnii* ( $n = 1$ ) and *S. warneri* ( $n = 1$ ) from the clinical samples of 16 cats, 20 dogs and 7 horses and noted that, apart from the  $\beta$ -lactam antibiotics, the most frequent resistance was observed to gentamicin/kanamycin (79.1%), macrolides/lincosamides (72.1%), tetracycline (51.2%), streptomycin (46.5%), trimethoprim, (39.5%), sulfamethoxazole (79.1%) and fluoroquinolones (69.8%). The authors concluded that MRCoNS from the animal infection sites were multiple drug-resistant (MDR) strains that represented a new challenge in the prevention and therapy of infections in veterinary clinics.

In this study, a total of 16 MRCoNS species were isolated from the cats, horses and dogs (Table 4). The feline isolates were identified as *S. simulans* and *S. chromogenes*, the equine isolates were identified

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as *S. chromogenes*, *S. capitis*, *S. haemolyticus* and *S. xylosus*, and the canine isolates were identified as *S. chromogenes*, *S. capitis* and *S. epidermidis*. All the MRCoNS isolates showed relatively high levels of resistance towards amoxicillin/clavulanic acid (75.0%), tetracycline (37.5%) and penicillin (37.5%), followed by the other tested antimicrobials. The results of the feline strains were in concordance, to some extent, with previous studies (Qekwana et al. 2017). In contrast to the findings of Qekwana et al. (2017), neither the *S. intermedius* group representing CoPS nor the *S. felis* one representing CoNS were isolated from the diseased cats. On the other hand, in concordance with the same study, the *S. aureus* strains representing CoPS and the *S. simulans* strains representing CoNS were isolated with higher rates. Regarding the equine strains (Table 4), the isolation of the *S. chromogenes*, *S. capitis*, *S. haemolyticus*, and *S. xylosus* species representing CoNS was in full agreement with the results of Karakulska et al. (2012). On the other hand, except *S. pseudintermedius*, the isolation of the *S. chromogenes*, *S. capitis*, *S. haemolyticus*, and *S. xylosus* species representing MRCoNS together with *S. aureus* was similarly in full agreement with the results of Mallardo et al. (2013). In respect to the canine strains (Table 4), the isolation of *S. chromogenes*, *S. epidermidis* and *S. haemolyticus* was regarded to be in concordance with the results of Kern and Perreten (2013) and Metiner et al. (2015), where, in contrast to the findings of Ruzauskas et al. (2014), no MRSH was isolated from the dogs. The differences observed in the species distribution and antimicrobial resistance patterns of MRCoNS were mainly attributed to the ecological diversity of the microbiota of the local animal populations and the environment, the discrepancies in the geographical sampling area, and to some extent, disparities in the used bacteriological methods.

To the best of the authors' knowledge, both the distribution and antimicrobial resistance patterns of the CoNS species were reported in dairy cattle and companion animals for the first time in Cyprus. A total of 34 CoNS strains were isolated from different animal species (Table 1). The most predominantly isolated strains were identified as *S. chromogenes* (41.2%), *S. capitis* (14.7%) and *S. simulans* (11.8%) (Table 2). Among these strains, 70.6% of them were identified as MRCoNS. The CoNS species were found to be resistant to amoxicillin/clavulanic acid (55.9%), tetracycline (41.2%)

and penicillin (38.2%). An increase in the resistance to amoxicillin/clavulanic acid (75.0%) was observed among the MRCoNS species (Table 3). Additionally, 79.1% of the MRCoNS species were determined to be MDR. As has been reported earlier by Qekwana et al. (2017) and Srednik et al. (2017), the presence of antimicrobial resistant CoNS, especially MRCoNS, and MDR species was regarded as an important issue since these might limit and have negative effects on the therapeutic treatment options of staphylococcal infections in animals, and might have both public and veterinary concerns.

In conclusion, the results obtained in this study showed that CoNS species were present in dairy cattle and the companion animals in Cyprus. The isolation of the antimicrobial resistant CoNS, especially MRCoNS and the detection of MDR staphylococci in a high percentage suggested that these agents should be investigated more carefully in diagnostic laboratories for a definitive diagnosis. Considering the present work as a preliminary study to determine the current status of CoNS in the region, more detailed studies, primarily molecular ones, should be carried out in order to fully characterise the strains circulating in the dairy and companion animals in the country. The authors believe that the results of this preliminary study will pioneer the set-up of such research.

## Conflict of interest

The authors declare no conflict of interest.

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