doi: 10.17221/96/2015-VETMED

Methicillin-resistant coagulase-negative staphylococci in healthy dogs

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ABSTRACT: The objective of this study was to evaluate the prevalence of coagulase-negative staphylococci in healthy dogs and to determine whether methicillin-resistant staphylococci expressed the m*ecA* gene. Nasal and rectal swab samples were taken from 50 clinically healthy dogs. The prevalence of coagulase-negative staphylococci was evaluated according to phenotypic properties. The agar diffusion method was applied to evaluate antimicrobial resistance and the prevalence of methicillin resistance was determined using PCR analysing the m*ecA* gene. A total of 59 coagulase-negative staphylococcus strains were isolated from the nostrils and rectums of 37 (74%) clinically healthy dogs. The prevalence of coagulase-negative staphylococci in female dogs was significantly higher compared with male dogs ($P < 0.05$). The results of antimicrobial susceptibility testing showed that 6.7% of the strains were resistant to oxacillin, 23.7% were resistant to penicillin, 22% to ampicillin and 16.9% to erythromycin. The m*ecA* PCR revealed one oxacillin-sensitive and four oxacillin-resistant coagulase-negative staphylococci strains to be m*ecA* carriers. *Staphylococcus sciuri* (60%) and *Staphylococcus warneri* (20%) were the most prevalent species among methicillin-resistant coagulase negative staphylococci. High antimicrobial resistance rates for these bacteria were observed against penicillin (100%), ampicillin (100%), oxacillin (80%), erythromycin (80%) and gentamicin (60%). All strains were susceptible to vancomycin and enrofloxacin. It is assumed that methicillin-resistance genes evolved in coagulase-negative staphylococcus and were then horizontally transferred among staphylococci.

Keywords: *Staphylococcus sciuri*; antimicrobial resistant; m*ecA*; age; nasal; rectal

Coagulase-negative staphylococci (CoNS) are a diverse group of commensals inhabiting the skin and mucous membranes of humans and animals; however, some species are known as important opportunistic pathogens (Zell et al. 2008; Karakulska et al. 2012; Kern and Perreten 2013), and are considered to act as human pathogens in hospital environments (Vengust et al. 2006). The role of CoNS as animal pathogens is less understood (Karakulska et al. 2012). Some species of CoNS are involved in disease; in pets they typically cause bacteraemia, pneumonia, rhinitis, furuncles, abscesses, pyoderma, keratitis, conjunctivitis, otitis externa and uterine infections (Kloos and Bannerman 1995; Litster et al. 2007; Hariharan et al. 2009; Suter et al. 2017).

Methicillin resistance is one of the most serious antibiotic resistance mechanisms found in staphylococci, and represents a public health issue that can increase both the rate of failure of antibiotic therapy and mortality rates of human and animal diseases (Chah et al. 2014). Methicillin resistance is associated with the presence of the *mecA* gene, which encodes an additional penicillin-binding protein (PBP2A or PBP2'). This protein has a lower affinity for all beta-lactam antibiotics. The m*ecA* gene is located on a mobile genetic element

Supported by the Project Cost ES 1403 Action – "New and emerging challenges and opportunities in wastewater reuse (Nereus)".

doi: 10.17221/96/2015-VETMED

called staphylococcal cassette chromosome *mec* (SCC*mec*) (Tulinski et al. 2012). Companion animals are frequently implicated as potential reservoirs of methicillin-resistant staphylococci (Chah et al. 2014). This assumption is mainly based on studies reporting antibiotic resistance in clinical coagulase-positive staphylococci isolates from dogs and humans (Gandolfi-Decristophoris et al. 2013; Drougkaa et al. 2016). However, a clear picture of the distribution, diversity and methicillin resistance of CoNS species in pets is lacking. Only a few researchers have studied antimicrobial resistance in CoNS isolates recovered from pets. Van Duijkeren et al. (2004) detected methicillin-resistant *Staphylococcus haemolyticus* in cats and dogs with cystitis, rhinitis, bronchitis and pyoderma. Methicillin resistance was reported in *Staphylococcus epidermidis*, *Staphylococcus hominis*, *Staphylococcus simulans*, *Staphylococcus scriuri* (*S. sciuri*), *Staphylococcus warneri* (*S. warneri*), *Staphylococcus arlettae* and *Staphylococcus haemolyticus* isolated from healthy and diseased dogs and cats by Malik et al. (2006), Garza-Gonzalez et al. (2010) and Aslantas et al. (2013). In recent studies, methicillin-resistant *Staphylococcus cohnii* subsp. *urealyticus* and *Staphylococcus haemolyticus* were isolated from the ear and nasal swabs of a clinically healthy dog by Bean et al. (2017a; Bean et al. 2017b).

The *mecA* gene is transmitted from one staphylococcal species to another, and *mecA* genes were carried by a common ancestor of both *S. aureus* and CoNS species. The gene has been inherited by all present-day staphylococcal species. We hypothesised that methicillin-resistant staphylococci would express the *mecA* gene. The aim of our study, therefore, was to evaluate the prevalence of coagulase-negative staphylococci in healthy dogs and to determine whether methicillin-resistant staphylococci expressed the m*ecA* gene.

MATERIAL AND METHODS

The study was carried out in 2013–2014. Nasal and rectal swabs were obtained from 50 clinically healthy dogs by veterinarians in their owner's homes. The owners completed a questionnaire on their companion animals (pets), which provided information on the breed, age, sex, housing conditions and health status of dogs. The work was performed in compliance with Lithuanian animal welfare regulations (No. B1-866, 2012; No. XI-2271, 2012), and was approved by the Lithuanian Committee of the Veterinary Medicine and Zootechnical Sciences (Protocol No. 09/2012). Samples were collected using sterile cotton swabs soaked with saline solution. The swab was inserted approximately 0.5–1 cm into the nasal cavities of dogs and approximately 1 cm into the rectums of pets. All swabs were placed in Amies transport medium (Amies, Liofilchem, Italy) and stored at +4 °C until culture (not longer than 24 h).

Fifty swabs from nasal cavities and 50 swabs from rectums were obtained from 28 female and 22 male dogs in this study. The ages of the dogs under study ranged from nine weeks to 19 years. The most commonly examined dogs were purebreds (32); the remainder were mixed-breeds dogs (18). Twenty-six dogs were medium-sized breeds, 17 of the pets were small-breed and seven were large-breed dogs. Most dogs were short-haired (32), while 18 were long-haired. Forty-six of the investigated dogs were kept both inside and outside the house, and four of the dogs were kept only outside.

Nasal and rectal swabs were placed in Trypticase Soya broth (Difco, USA) and incubated at +37 °C for 24 h. About 100 µl of broth were streaked onto mannitol salt agar (Oxoid, England). Plates were incubated aerobically at +37 °C for 48 h. Colonies that failed to produce any change on the medium were picked and inoculated onto 5% blood agar (BA) at +37 °C for 24 h. Isolates thought to belong to *Staphylococcus* species on the basis of colony morphology (creamy, greyish, white or yellow) and haemolytic strains on the surface of BA were collected. Pure colonies were obtained by sub-culturing presumed staphylococcal colonies onto nutrient agar (Oxoid, United Kingdom). The ability of the investigated staphylococcus strains to produce coagulase was determined using the tube coagulase test (Rabbit plasma, Pro-Lab, Bromborough, UK) according the manufacturer's instructions. The commercial Integral System Staphylococci systems (Liofilchem, Italy) were used for the identification of methicillin resistance CoNS species. Staphylococcal strains were stored at –70 °C in trypticase soy broth (Difco, US) with 10% glycerol before being tested.

The phenotypic antibiotic resistance of coagulase-negative staphylococcus isolates to 12 antimicrobial agents was determined using the Kirby-Bauer disc diffusion method (CLSI 2010). The following

doi: 10.17221/96/2015-VETMED

antimicrobial agents were tested: oxacillin (1 µg), amoxicillin (30 µg), amoxicillin with clavulanic acid (20 µg + 10 µg), penicillin (10 IU), ampicillin (10 µg), vancomycin (30 µg), erythromycin (15 µg), fusidic acid (10 µg), tetracycline (30 µg), gentamicin (10 µg), enrofloxacin (µg) and cefovecin (µg) (Liofilchem, Italy).

The isolates were sub-cultured from frozen onto trypticase soy agar (Difco, USA) and incubated aerobically for 24–48 h at a temperature of +37 °C. At least three colonies of each isolate were selected and re-suspended in sterile, de-ionized water until the 0.5 standard was reached (DEN-1 McFarland Densitometer, Biosan, Latvia). Suspensions were spread onto Mueller-Hinton agar (Oxoid, England) and disks of each antimicrobial agent were applied. The plate was assessed after 24 hours of incubation at +35 ± 2 °C, and the diameter of inhibition zones was then measured to the nearest millimetre. Results were interpreted according to the Clinical and Laboratory Standards Institute guidelines (CLSI 2010).

The presence of the *mecA* gene, which demonstrates methicillin resistance, was tested using the polymerase chain reaction for all coagulase-negative staphylococcus isolates. Genomic DNA from isolated microorganisms was extracted with Chelex 100 (Sigma, USA). A colony of each staphylococcus strain from trypticase soy agar (Difco, USA) was chosen and placed into a separate sterile Eppendorf tube containing 200 µl of 5% Chelex solution. The suspension was heated at +80 °C for 25 min and boiled at +95 °C for 10 min. The heated solution was then centrifuged for 3 min at 10 000 rpm. The supernatants were transferred to new sterile Eppendorf tubes and used as template DNA in PCR.

The determination of the methicillin resistance genotypes of coagulase-negative staphylococci was carried out using the forward oligonucleotide primer *mec*A-F-1 (5'-TCCAGATTACAACTTCACCAGG-3') and reverse primer *mecA-F-2* (5'-CCACTTCATATCTTGTA-ACG-3') (Grida Lab, Lithuania). Excepted amplicon size was 162 bp (Oliveira and Lencastre 2002). The PCR amplifications were performed in a final volume of 25 µl containing 10 × PCR buffer (100 mM Tris-HCH, pH 8.8, 500 mM KCl, 0.8% Nonidet P-40; MBI, Ferment), 25 mM $MgCl_2$ (MBI, Ferment), 2 mM dNTP mix (MBI, Ferment), 500 IU Taq DNA polymerase (MBI, Ferment), 0.25 µl of each of the oligonucleotides and 3 µl of template DNA. The amplifications were performed on a PTC-100 programmable thermal controller (MJ Research Inc., USA) under the following conditions: initial denaturation at +95 °C for 5 min followed by 30 cycles of +95 °C for 1 min, +54 °C for 1 min, +72 °C for 1 min and a final extension step of +72 °C for 7 min. Electrophoresis of PCR products was performed in TAE buffer (40 mM Tris, 20 mM acetic acid, 1 mM EDTA), at 100 V for 60 min. PCR products were analysed in 1% Top Vision LE GQ Agarose gels (MBI, Fermentas) with 1.3% ethidium bromide under a UV lamp. The GeneRuler TM 100 bp DNA Ladder (MBI, Fermentas) was used to evaluate the sizes of PCR products.

Descriptive statistical analyses were calculated using the SPSS 13.0 statistical package for Windows (2004). The χ^2-test was used to examine the association of coagulase-negative staphylococci and methicillin-resistant coagulase-negative staphylococci with pet characteristics (breed, age, sex, size and health status) and housing conditions. The Kruskal-Wallis test was used to examine the distribution of antibiotic resistance among CoNS strains. A value of $P < 0.05$ was considered to be statistically significant.

RESULTS

The prevalence of coagulase-negative staphylococci

A total of 59 coagulase-negative staphylococcus strains were isolated from the nostrils and/or rectums in 37 out of 50 (74%) clinically healthy dogs. Thirty-two (54.2%) isolates of CoNS were detected in the nostrils and 27 (45.8%) in the rectums of the dogs. The prevalence of coagulase-negative staphylococci at different anatomical sites according to age, sex, breed and housing conditions is shown in Table 1.

Age did not influence the colonisation of the nasal cavity and rectum by CoNS in dogs ($P > 0.05$). However, dogs in the 6 to 10-year-old age range showed a comparatively high occurrence of CoNS as compared to the age groups of less than one and greater than 10. The sex of dogs had a significant influence ($P < 0.05$) on the occurrence of CoNS in pets. Higher numbers of these bacteria were found in female dogs than in male dogs. Breed and housing conditions did not influence staphylococcal colo-

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Table 1. The main characteristics of coagulase-negative staphylococcus strains isolated from dogs

Characteristic	Nostrils (n = 32)		Rectum (n = 27)		P-value
	n	%	n	%	
Sex of dogs					
Female	19	59.4	15	55.5	0.0525
Male	13	40.6	12	44.4	
Age of dogs					
< 1	4	12.5	3	11.1	0.590
1–5	15	46.9	11	40.7	
6–10	10	31.3	10	37.0	
> 10	3	9.4	3	11.1	
Daytime location					
Inside and outside	29	90.6	25	92.6	0.0612
Outside only	3	9.4	2	7.4	
Breed of dog					
Mixed	14	43.8	10	37.0	0.601
Pure	18	56.3	17	62.9	
Long-haired	12	37.5	9	33.3	0.425
Short-haired	20	62.5	23	85.2	
Size of dogs					
Small	12	37.5	8	29.6	0.261
Medium	14	43.8	17	62.9	
Large	6	18.8	2	7.4	

P-value considered statistically significant at ≤ 0.05

nisation of the nasal cavity and rectum ($P > 0.05$). However, CoNS were more common in purebred dogs (see Table 1).

Resistance to antimicrobial drugs

The results of the phenotypic susceptibility testing of CoNS strains are shown in Table 2. For the beta-lactam group of antimicrobial agents, 22% of CoNS were resistant to ampicillin ($P < 0.05$), while 6.7, 1.7, 3.4 and 1.7% were resistant to oxacillin, amoxicillin with clavulanic acid, amoxicillin and cefovecin, respectively. The least effective among all beta-lactam antibiotics was penicillin; 23.7% of isolates were resistant ($P < 0.05$).

Additionally, 16.9% of all strains isolated from dogs were resistant to erythromycin and 5.1% to tetracycline. Five per cent of isolates were resistant to gentamicin and 1.7% to fusidic acid. All isolates were sensitive to vancomycin and enrofloxacin.

Table 2. Resistance of coagulase-negative staphylococci to 12 antimicrobial drugs

Antimicrobial agents	Nostrils (n = 32)		Rectum (n = 27)		P-value
	n	%	n	%	
Penicillin	9	28.1	5	18.5	0.041
Oxacillin	3	9.4	1	3.7	0.242
Ampicillin	10	31.3	3	11.1	0.001
Amoxicillin	1	3.1	1	3.7	1
Amoxicillin and clav. acid	0	0	1	3.7	1
Cefovecin	0	0	1	3.7	1
Vancomycin	0	0	0	0	1
Erythromycin	7	21.9	3	11.1	0.159
Tetracycline	2	6.3	1	3.7	1
Enrofloxacin	0	0	0	0	1
Gentamicin	1	3.1	2	7.4	1
Fusidic acid	1	3.1	0	0	1

P-value considered statistically significant at ≤ 0.05

Sixteen per cent (8/50) of pets carried at least one multi-drug resistant staphylococcus isolate. Multi-drug resistance was defined as resistance to at least three drugs belonging to three different classes of antimicrobial agents (Moon et al. 2007).

We examined the presence of the *mecA* gene in 59 strains of coagulase-negative staphylococci using PCR. PCR resulted in the amplification of the *mecA* gene in 8.5% of tested strains. The size of the fragment containing the *mecA* gene was estimated to be 162 bp on the basis of the corresponding regions of the *mecA* genes of human MR *Staphylococcus aureus* (Oliveira and Lencastre 2002).

MecA-positive CoNS were isolated from 10% of investigated dogs. Higher numbers (60%) of *mecA*-positive staphylococci were found in the nostrils and rectums of female dogs compared to male dogs (40%). Sixty per cent of *mecA* carriers were puppies (less than one year old), and 40% were older dogs (greater than six years old). The methicillin-resistant CoNS isolates belonged to two different species, namely, *S. sciuri* and *S. warneri*, with *S. sciuri* (60%) being the predominant species detected. Twenty per cent of *mecA*-positive CoNS were not identified down to the species level.

The phenotypic resistance of *mecA*-positive staphylococcus strains is shown in Table 3. Eighty per cent of methicillin-resistant CoNS strains were resistant to oxacillin, and 40% of isolates were re-

doi: 10.17221/96/2015-VETMED

Table 3. Origin and resistance profile of methicillin-resistant *Staphylococcus* spp. isolated from dogs

mecA-positive staphylococcal species	Origin	Resistance phenotype
S. scriuri	nostrils	PEN-OXA-AML-AMP-ERY-TET-GEN
S. warneri	nostrils	PEN-AMP
Staphylococcus spp.	rectum	PEN-OXA-CEF-AML-AMP-AMC-ERY-TET
S. scriuri	rectum	PEN-OXA-AMP-ERY-GEN
S. scriuri	rectum	PEN-OXA-AMP-ERY-GEN

AMC = amoxicillin with clavulanic acid, AML = amoxicillin, AMP = ampicillin, CEF = cefovecin, ERY = erythromycin, GEN = gentamicin, OXA = oxacillin, PEN = penicillin, TET = tetracycline

sistant to amoxicillin. All (100%) isolates were resistant to penicillin and ampicillin. Twenty per cent of methicillin-resistant CoNS showed resistance to cefovecin and amoxicillin with clavulanic acid, respectively. Tetracycline, gentamicin and erythromycin resistance was detected in 40%, 60% and 80% of isolates, respectively. None of the methicillin-resistant CoNS isolates were resistant to vancomycin, enrofloxacin or fusidic acid. Multidrug resistance was detected in four (80%) methicillin-resistant staphylococcus strains (Table 3).

DISCUSSION

To provide more detailed information on healthy CoNS-carrier dogs and the resistance of the isolated bacteria to antibiotic drugs from different classes, we have studied their occurrence on the nasal and rectal mucosa of fifty dogs. CoNS were isolated from 74% of clinically healthy dogs. The established prevalence of staphylococci is higher than in other published studies where staphylococci were isolated from 60% (Gandolfi-Decristophoris et al. 2013) and 38% of investigated dogs (Wedley et al. 2014). While the rate of isolation of CoNS in dogs was highest from the nasal cavity (54.2%), the perianal region also exhibited a relatively high recovery rate for *Staphylococcus* spp. (45.8%). Our data are in agreement with other studies, which have reported that while CoNS are usually found on the skin, and in the oral and nasal cavities of healthy dogs, they may also colonise regions of the axillae, the pharynx and perineum, gastrointestinal tract and the vagina. Age, bread, size and the daytime location of dogs did not have a significant influence ($P > 0.05$) on the prevalence of CoNS. Only the sex of pets had an influence on the presence of these bacteria. A higher number of CoNS was found in female dogs compared to male dogs. This finding is similar to the conclusion of Gandolfi-Decristophoris et al. (2013), who found that the individual characteristics of pets were not the main risk factor for the carriage of CoNS.

Resistance of staphylococci to methicillin and other antimicrobials is a global problem in the chemotherapy of staphylococcal infections (Chah et al. 2014). Subsequent investigations revealed that 10% of clinically healthy dogs are carriers of methicillin-resistant CoNS. The results obtained in our study show a low rate of methicillin-resistant CoNS carriage in dogs. Most other comparable studies have reported prevalence rates of between 2% and 42% (Kern and Perreten 2013; Chah et al. 2014; Schmidt et al. 2014). Since these studies vary in sampling design, culture methods and conditions, a direct comparison of the results is difficult. However, our data are similar to the findings of Chah et al. (2014) and Aslantas et al. (2013), who found methicillin-resistant CoNS in 12.8% and 15.4% of healthy dogs in Nigeria and a community in Turkey, respectively. Low methicillin-resistant CoNS carriage rates in healthy dogs have been reported by other authors: Gandolfi-Decristophoris et al. (2013) found a methicillin-resistant CoNS prevalence of 4.3% in healthy dogs (swab samples from nostrils and ears of pets were analysed). Malik et al. (2006) isolated methicillin-resistant CoNS from 2.6% of dogs (swab samples were taken from skin lesions of animals). Wedley et al. (2014) reported that methicillin-resistant staphylococci were isolated from the noses of 5.5% of clinically healthy dogs.

Of the five methicillin-resistant CoNS isolated, *S. sciuri* was the most prevalent species. Previous studies have considered *S. sciuri* as a non-pathogenic commensal bacterium, but it has also been associated with animal diseases such as dermatitis

doi: 10.17221/96/2015-VETMED

in dogs (Hauschild and Wojcik 2007), mastitis in dairy cattle (Rahman et al. 2005) and exudative epidermitis in piglets. A case of human wound infection by a multi-drug resistant strain of *S. sciuri* has also been reported (Nemeghaire et al. 2014). The bacterium has recently become the subject of increased interest after it was discovered that *S. sciuri* strains ubiquitously carry a genetic element (*S. sciuri mecA*) that is closely related to the *mecA* gene found in methicillin-resistant *Staphylococcus aureus* (MRSA) strains. This finding led to the proposal that *S. sciuri mecA* might be the evolutionary origin of the *mecA* element carried by methicillin-resistant staphylococcus (Severin et al. 2010). In our study, the colonisation of the tested dog population by methicillin-resistant *S. scriuri* is similar to the findings of Chah et al. (2014), who reported *S. sciuri* species (62.5%) to be the most prevalent species in dogs in Nigeria. Bagcigil et al. (2007) isolated *mecA*-positive CoNS from dog nasal swabs, and *S. sciuri* with *Staphylococcus haemolyticus* and *Staphylococcus vitulinus* were most prevalent.

S. warneri was the second most prevalent methicillin-resistant CoNS isolated in this study. Like other coagulase-negative staphylococci, it is a common commensal organism found as part of the skin flora in most mammals. The pathogenicity of *S. warneri* for human and animals has been documented; in the recent literature, the agent has been associated with severe bacteraemia and endocarditis in immunocompromised patients (Barigye et al. 2007). The prevalence of methicillin-resistant *S. warneri* in the nasal cavity of companion animals was reported in other studies. Malik et al. (2006) isolated methicillin-resistant staphylococci from ten swab samples from skin and lesions of investigated dogs. *S. haemolyticus* and *S. warneri* (20%) were the predominant species. Aslantas et al. (2013) detected methicillin-resistant *S. warneri* harbouring the *mecA* gene in the nasal cavities of studied dogs. Han et al. (2016) investigated colonisation and the association between the presence of staphylococci in healthy dogs and in their owners. *Staphylococcus* spp. were isolated from 44 (37%) dogs. *S. epidermidis*, *S. pseudintermedius*, *S. aureus*, *S. scheiferi* subsp. *coagulans*, *S. haemolyticus*, *S. sciuri*, *S. saprophyticus* and *S. warneri* were the predominant isolates. Among these, 71.6% were methicillin-resistant and 95.4% of the isolates demonstrated multi-drug resistance irrespective of their origin.

Methicillin resistance is of particular interest, because it confers resistance to all beta-lactams and is also often linked to resistance to other antibiotic classes. In this study, methicillin-resistant CoNS isolates were resistant to all beta-lactams (penicillin (100%), ampicillin (100%), oxacillin (80%), amoxicillin (40%), amoxicillin with clavulanic acid (20%), cefovecin (20%)) and to erythromycin (80%), gentamicin (60%) and tetracycline (40%), respectively. Certain levels of resistance to antimicrobial drugs (penicillin (23.7%), ampicillin (22%), and erythromycin (16.9%)) were even found in staphylococcus strains lacking *mecA* genes. The antimicrobial drugs used in our investigation are approved in Lithuania for the treatment of infectious diseases of small animals and are the most frequently used agents in the veterinary field. Our findings confirm that the frequent use of antimicrobial agents may promote the emergence of resistant strains. The antimicrobial drug resistance of opportunistic pathogens is a critical problem for clinicians because it limits the choice of antibiotic treatment (Decristophoris 2012). Methicillin-resistant CoNS may also be an important reservoir for transmission of bacteria to other animals or humans and/or resistant determinants to other pathogenic bacteria (Wedley et al. 2014). Comparable studies carried out by other researchers have shown that the resistance of methicillin-resistant CoNS to different antimicrobial agents varies. Aslantas et al. (2013) found that methicillin-resistant CoNS isolated from dog nasal cavities were most resistant to oxacillin (100%), erythromycin (56%), tetracycline (52%) and clindamycin (32%); on rare occasions, resistance was found to ciprofloxacin (20%), fusidic acid (4%) and amoxicillin-clavulanic acid (4%). In contrast to the results of Aslantas et al. (2013), all methicillin-resistant CoNS isolates in our study were susceptible to amoxicillin with clavulanic acid, to vancomycin, entofloxacin and fusidic acid. In the study of Chah et al. (2014), no fusidic acid and vancomycin resistance was found among methicillin-resistant CoNS isolated from groin swabs of clinically healthy dogs in Nigeria. Methicillin-resistant CoNS strains were most commonly resistant to beta-lactams (100%), tetracycline (81.3%) and kanamycin (75%). The studies of Decristophoris (2012) and Bean et al. (2017a) on methicillin-resistant CoNS isolated from the nostrils and ears of dogs, reported resistance to many antimicrobial agents frequently used to treat

doi: 10.17221/96/2015-VETMED

staphylococcal infections like beta-lactams, mupirocin, aminoglycoside, macrolide, sulphonamides and tetracycline. Decristophoris (2012) found that 1–21% of methicillin-resistant CoNS were multidrug resistant strains. Relatively low rates (14%) of multi-drug resistant CoNS were found among the studied pets in our research. However, 80% of strains with the *mecA* gene were resistant to at least three drugs belonging to three different classes of antimicrobial agents. Researchers have established that cross-resistance to other antimicrobials is more common in methicillin-resistant than in methicillin-sensitive staphylococcal isolates and may be associated with the carriage of multiple antimicrobial resistance genes on SCCmec cassettes (Smyth et al. 2011; Chah et al. 2014; Couto et al. 2016).

Of the five *mecA*-positive isolates of CoNS, four were oxacillin-resistant, and one was oxacillin-susceptible. Recent studies suggested that amino acid mutations in the *Fem* proteins (involved in cell wall synthesis) might lead to the oxacillin-sensitive methicillin-resistant staphylococcus phenotype, but the association of mutations with the phenotype has not been formally proven (Giannouli et al. 2010; Pu et al. 2014). Furthermore, *Fem* genes that encode proteins which considerably affect the level of methicillin resistance were suggested to be specific only for *Stahylococcus aureus* (Kobayashi et al. 1994). We could not find any reference to the detection of *Fem* genes in CoNS in the literature. Archer and Climo (1994) hypothesised that many strains of staphylococci express the *mecA* gene heterogeneously, and that only a few cells in a population of bacteria may be PBP2A-positive. Methicillin resistance in staphylococci is due to the production of an additional non-native penicillin-binding protein, PBP2A, which is encoded by the *mecA* gene and has low affinity for beta-lactam antibiotics. In our opinion, heterogeneity is more common in CoNS than in *Staphylococcus aureus*. This makes the phenotypic detection of methicillin resistance problematic, especially in CoNS. In the present study, this isolate could have been misclassified as methicillin-susceptible CoNS if genetic detection of *mecA* had not been performed.

The prevalence of methicillin-resistant CoNS in healthy pets is determined to be low (10%) in Lithuania. Resistance against beta-lactam antimicrobial agents, erythromycin, gentamicin and tetracycline was most frequently observed among CoNS strains with the *mecA* gene. The most effective antimicrobial agents against methicillin-resistant CoNS were vancomycin, enrofloxacin and fusidic acid. It is assumed that methicillin-resistance genes evolved in coagulase-negative staphylococci and were then horizontally transferred among staphylococci.

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doi: 10.17221/96/2015-VETMED

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Received: April 2, 2015
Accepted after corrections: August 12, 2017