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Methicillin-resistant coagulase-negative *Staphylococcus* spp. prevalence in Lithuanian dogs: a cross-sectional study

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ABSTRACT

The aim of this study was to investigate the presence and frequency of methicillin-resistant coagulase negative staphylococci in dogs in Lithuania and to characterize them regarding antimicrobial resistance. In 2012-2013 clinical material was collected from 400 dogs. Three hundred samples from diseased dogs with different clinical conditions (dermatitis, otitis, wound infections, gastrointestinal and respiratory tract infections) as well as 100 samples from pure-breed bitches with reproductive disorders (pyometritis, metritis, partus praematurus), used as breeding animals in kennels, were selected. Twenty MRCNS isolates were obtained from 18 dogs out of 400 tested. All isolates harboured the *mecA* gene while the *mecC* (*mec*LGA251) gene was not found. Ten isolates were detected in vaginal samples of the bitches within 3 large kennels. The prevalence of MRCNS in dogs kept in households was 3.3 % i.e. significantly lower ($P<0.01$) than in dogs kept in large kennels (10 %). Ten different MRCNS species were detected with the highest prevalence for *Staphylococcus haemolyticus*. MRCNS isolates were resistant to macrolides (75 %) due to *erm*(C) and *msr*A genes, and to tetracycline (65 %) due to *tet*(K) and/or *tet*(M) genes. The rate of resistance to gentamicin was 50 % (attributed to *aac(6')-Ie-aph(2")-Ia, aph(3')-IIIa*), and to co-trimoxazole - 40 % *(dfrG* gene). One isolate of *S. lentus* harboured the *dfrK* gene. All isolates were susceptible to linezolid, daptomycin and vancomycin. This study revealed that breeding kennels might be a reservoir of MRCNS strains and may pose a risk for the spread of such strains during mating. The focus on the possible spread of multi-resistant *S. haemolyticus* between companion-animals and humans should be foreseen, as this species plays an important role in human infections as well.

Key words: resistance, genes, pure-breed dogs, kennels, *Staphylococcus haemolyticus*

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Introduction

Although coagulase-positive staphylococci (CPS) are regarded as the most important group in severe infections, coagulase-negative staphylococci (CNS) have emerged as important pathogens as well. Moreover, all species of staphylococci, regardless of their coagulase activity, could be resistant to different classes of antimicrobials used for human and animal treatment. About 80 to 90 % of CNS isolates associated with human hospital infections are methicillin-resistant coagulase-negative staphylococci (MRCNS) (DE MATTOS et al., 2003). Staphylococci resistant to methicillin and other antibiotics have been frequently reported in pets worldwide (COHN and MIDDLETON, 2010; MALIK et al., 2005; MATANOVIĆ et al., 2012). CNS are known as a part of the normal bacterial community of skin and mucosae of pets, but can develop resistance mechanisms to various antibiotics (BAGCIGIL et al., 2007). Nevertheless, their pathogenic potential and the capacity to transfer resistance genes to the CPS species are still under-investigated (MALIK et al., 2005; GANDOLFI-DECRISTOPHORIS et al., 2013). In pets, the pathogenic potential of CNS microorganisms remains to be clearly defined, although there are some reports of infections related to methicillin-resistant CNS in cats and dogs (VAN DUIJKEREN et al., 2004; LITSTER et al., 2007).

Development of new molecular techniques allows accurate identification of CNS (CARBONELLE et al., 2007). This will eventually lead to a better understanding and knowledge of these bacterial species (GANDOLFI-DECRISTOPHORIS et al., 2013).

It is assumed that methicillin-resistance genes evolved in coagulase-negative staphylococci (CNS) and then were horizontally transferred across staphylococci (BARBIER et al., 2010). Particularly *Staphylococcus sciuri* and S. fleurettii are discussed as natural reservoirs of the methicillin-resistance gene mec*A* (HUBER et al., 2011). The *mecA* gene is located on a mobile genetic element called the staphylococcal cassette chromosome (SCC) and confers resistance to methicillin by encoding an altered penicillin-binding protein (PBP2α), which shows limited affinity to beta-lactam antibiotics. There are data about the horizontal gene transfer of SCC*mec* between CPS and CNS species (HANSSEN and ERICSON SOLLID, 2006). The *mecA* gene is widely present among both coagulase-positive and negative staphylococcal species (CATRY et al., 2010; TULINSKI et al., 2012) although knowledge about *mecA* gene distribution in CNS isolated from pets is still sparse. VAN DUIJKEREN et al. (2004) detected methicillin-resistant S. haemolyticus in cats with cystitis and rhinitis, in dogs with bronchitis and pyoderma, and in a mare with vaginitis. Other authors found other MRCNS species in pets. For example, NAKAMURA et al. (2012) isolated methicillin resistant *S. lugdunensis* from a dog with endocarditis, while KERN and PERRETEN (2013) isolated *S. epidermidis*, *S. warneri*, *S. hominis,* as well as some other MRCNS, from dogs and horses.

The aim of this study was to investigate the presence and frequency of MRCNS in dogs with a variety of clinical conditions in Lithuania, and to characterize them regarding antimicrobial resistance.

Materials and methods

Location and samples. In 2012-2013 clinical material was collected from 400 dogs in Lithuania. Three hundred samples from diseased dogs with different clinical conditions (dermatitis, otitis, wound infections, gastrointestinal and respiratory tract infections), as well as 100 samples from pure-breed bitches with reproductive disorders (pyometritis, metritis, partus praematurus), used as breeding animals in kennels, were selected for this study.

Samples were collected using sterile Amies media swabs (Liofilchem, Italy) or other necessary instruments, under aseptic conditions. Samples were delivered to the laboratory the same day.

Isolation and identification of CNS. Clinical material was inoculated onto 5 % Sheep Blood Agar, Mannitol Salt Agar (Liofilchem, Italy), Mannitol Salt Agar supplemented with 4 mg/L cefoxitin (Sigma-Aldrich), Brilliance MRSA 2 Agar (Oxoid, Thermo Scientific) as well as onto Contrast MRSA Broth (Oxoid, Thermo Scientific).

Staphylococci were identified up to the genus level according to morphology characteristics, catalase production, gram-staining, and susceptibility to furazolidone, as well as other generally accepted methods. Species identification was performed according to pigment and coagulase production, the presence of protein A and clumping factor, as well as biochemical properties using RapID Staph Plus (Thermo Scientific) identification system (QUINN et al., 2013).

DNA extraction. DNA material for molecular testing was obtained after bacterial lysis, according to the extraction protocol prepared by the Community Reference Laboratory for Antimicrobial Resistance (ANONYMOUS, 2009) with slight modifications. Briefly, a loopful of colonies were taken from the surface of the Mueller Hinton Agar and transferred to phosphate buffered saline (pH 7.3). The content was centrifuged for 5 min. Then the supernatant was discarded and the pellet re-suspended in Tris-EDTA (TE) buffer. The suspension was heated using a thermomixer at 100 °C degrees for 10 minutes. The boiled suspension was transferred directly onto ice and diluted to 1:10 in TE.

Taxonomic verification. The genus specific 16S rRNA gene was investigated by PCR (Table 1). 16S rRNA sequencing for species confirmation was performed using an ABI3730XL sequencer. The universal primers 27F and 515R were used as described previously (KIM et al., 2008). Sequences were analysed using Molecular Evolutionary Genetic Analysis software (MEGA, version 6). A basic local alignment search tool

(BLAST) was used for comparison of obtained sequences with sequences presented in the database of the National Centre of Biotechnology Information (NCBI, 2014).

Table 1. Oligonucleotide primers used in this study

Primer name	Sequence (5' - 3')	Size, bp and T(ºC)	Target gene	Source
mecA1	GGGATCATAGCGTCATTATTC	527 (61)	*mecA*	Anonymous, 2009
mecA2	AACGATTGTGACACGATAGCC			
mecC1	GCTCCTAATGCTAATGCA	204 (50)	*mec*LGA251	Cuny et al., 2011
mecC2	TAAGCAATAATGACTACC			
blaZ1	CAGTTCACATGCCAAAGAG	772 (50)	*blaZ*	Schnellmann et al., 2006
blaZ2	TACACTCTTGGCGGTTTC			
tetM1	GTTAAATAGTGTTCTTGGAG	656 (45)	*tet*(M)	Aarestrup et al., 2000
tetM2	CTAAGATATGGCTCTAACAA			
tetK1	TTAGGTGAAGGGTTAGGTCC	718 (55)	*tet*(K)	Aarestrup et al., 2000
tetK2	GCAAACTCATTCCAGAAGCA			
aac6-aph2F	CAGAGCCTTGGGAAGATGAAG	348 (61)	*aac(6')-Ie-aph(2")-Ia*	Perreten et al., 2005
aac6-aph2R	CCTCGTGTAATTCATGTTCTGGC			
aph3-IIF	CCGCTGCGTAAAAGATAC	609 (57)	*aph(3')-IIIa*	Perreten et al., 2005
aph3-IIR	GTCATACCACTTGTCCGC			
dftrG1	TTTCTTTGATTGCTGCGATG	501 (51)	*dfrG*	Couto et al., 2014
dfrG2	AACGCACCCGTTAACTCAAT			
dfrK1	GCTGCGATGGATAAGAACAG	214 (50)	*dfrK*	Kadlec et al., 2010b
dfrK2	GGACGATTTCACAACCATTAAAGC			
ermA1	AAGCGGTAAAACCCCTCTGAG	442 (53)	*erm*(A)	Jensen et al., 2002
ermA2	TCAAAGCCTGTCGGAATTGG			
ermC1	ATCTTTGAAATCGGCTCAGG	295 (48)	*erm*(C)	Jensen et al., 2002
ermC2	CAAACCCGTATTCCACGATT			
msrA1	GCTTAACATGGATGTGG	1230 (55)	*msrA*	Perreten et al., 2005
msrA2	GATTGTCCTGTTAATTCCC			
16S1	GTGCCAGCAGCCGCGGTAA	886 (61)	*16S* staph	Anonymous, 2009
16S2	AGACCCGGGAACGTATTCAC			

Antimicrobial susceptibility testing. Antimicrobial susceptibility testing was performed using the broth microdilution method. Sensititre® plates and the ARIS 2X automated system (Thermo Scientific) were used with the following antimicrobials: daptomycin, ciprofloxacin, clindamycin, erythromycin, gentamicin, levofloxacin, linezolid, oxacillin, penicillin, tetracycline, quinupristin/dalfopristin, vancomycin, co-trimoxazole and rifampin. Interpretation of results was carried out using the manufacturer's software

(SWIN®) adapted to the clinical breakpoints of the European Committee on antimicrobial susceptibility testing (EUCAST). The quality control strain *S. aureus* ATCC 29213 was included in each assay for validation purposes.

PCR assay for antimicrobial genes. Detection of genes encoding antimicrobial resistance *(mecA, mecC, blaZ, tet*(K), *tet*(M), *erm*(A), *erm*(C), *msrA*, *aac(6')-Ie-aph(2")-Ia, aph(3')-IIIa*, *dfrG* and *dfrK*) was performed by PCR. Annealing temperatures and oligonucleotides used are presented in Table 1.

Statistical analysis. Statistical analysis was performed using the "R 1.8.1" package (http://www.r-project.org/). The comparison between categorical variables was calculated by chi-square and Fisher's exact test. Results were considered statistically significant if $P<0.05$.

Results

The rate of *Staphylococcus* spp. isolation from the clinical material from the dogs was 86.5 %. Twenty MRCNS isolates were obtained from 18 dogs out of the 400 tested, i.e. the prevalence rate of dogs carrying MRCNS isolates was 4.5 %. All isolates harboured the *mecA* gene, while the *mecC* (*mec*LGA251) gene was not found. Ten isolates were detected in vaginal samples of bitches from 3 large kennels. The prevalence of MRCNS in dogs kept in households was 3.3 % i.e. significantly lower ($P<0.01$) than in dogs kept in large kennels (10 %). Biochemical testing correctly identified only 12 of the 20 CNS to the species level, compared to 16S RNA gene sequencing. Species distribution, antimicrobial susceptibility phenotypes and genes encoding resistance in MRCNS isolates are presented in Table 2.

Ten different MRCNS species were detected, with the highest prevalence was of *S. haemolyticus* (35 %). Different resistance profiles were determined in the isolates of this species (Table 2). Six isolates of *S. haemolyticus* demonstrated resistance to at least three different classes of antimicrobials, and only a single isolate showed resistance to beta-lactams and macrolides alone.

The resistance to non-beta-lactamic antimicrobials of isolated MRCNS depended on the class of antimicrobials. The highest resistance prevalence was demonstrated to macrolides (75 %) due to the *ermC* and *msrA* genes, and to tetracycline (65 %) due to *tet*(K) and/or *tet*(M) genes. The rate of resistance to gentamicin was 50 % (attributed to *aac(6')-Ie-aph(2")-Ia, aph(3')-IIIa*), and to co-trimoxazole - 40 % *(dfrG* gene). One isolate of *S. lentus* harboured the novel *dfrK* gene encoding resistance to trimethoprim. Half of the isolates were resistant to fluoroquinolones. All isolates were susceptible to linezolid, daptomycin and vancomycin. Two isolates were resistant to rifampin and two isolates were intermediately susceptible to quinupristin/dalfopristin. The minimum inhibitory concentration (MIC) distributions of antimicrobials tested are presented in Table 3.

Table 2. Resistance profiles and source of the *Staphylococcus* spp. canine isolates

Species	Phenotype[1]	Genotype	Source
S. equorum	OX	*mecA*	nares
S. capitis	OX,CIP, LE, CLI, ERY, GEN, TE, SXT	*mecA*, *dfr*G, *aac(6')-Ie-aph(2")-Ia*	vagina
S. capitis	OX	*mecA*,	nares
S. schleiferi	OX, CLI, ERY, GEN, TE	*mecA*, *aac(6')-Ie-aph(2")-Ia*, *tet*M	vagina
S. lentus	OX, CLI, ERY, TE, SXT	*mecA*, *dfr*G, *erm*C, *tet*K, *tet*M,	vagina
S. lentus	OX, CIP, LE, CLI, ERY, GEN, TE, SXT	*mecA*, *aac(6')-Ie-aph(2")-Ia, aph(3')-IIIa, dfr*K	vagina
S. epidermidis	OX,CIP, LE, GEN, TE	*mecA*, *blaZ*, *aac(6')-Ie-aph(2")-Ia*	skin
S. epidermidis	OX, CLI, ERY	*mecA*,	vagina
S. sciuri	OX, CLI, ERY, TE	*mecA*, *blaZ*	skin
S. sciuri	OX, CIP, LE, CLI, ERY, GEN, RIF, TE	*mecA*, *erm*A, *aac(6')-Ie-aph(2")-Ia*, *tet*M	vagina
S. xylosus	OX, CIP, LE, CLI, ERY, GEN, TE, SXT	*mecA*, *dfr*G, *erm*C, *tet*K	vagina
S. felis	OX	*mecA*	vagina
S. chromogenes	OX, GEN. TE, SXT	*mecA*, *dfrG, blaZ, ermC, msrA*, *aac(6')-Ie-aph(2")-Ia, aph(3')-IIIa*	vagina
S. haemolyticus	OX, CLI, ERY, TE	*mecA*, *tetK*, *blaZ*	nares
S. haemolyticus	CIP, LE, CLI, ERY, RIF, TE, SXT	*mecA*, *dfrG, blaZ, msrA, tetK, tetM*, *aac(6')-Ie-aph(2")-Ia, aph(3')-IIIa*	mouth
S. haemolyticus	OX, CIP, CLI, ERY, GEN, TE, SXT	*mecA, dfrG, blaZ, ermC, msrA, aac(6')-Ie-aph(2")-Ia, aph(3')-IIIa*	vagina
S. haemolyticus	OX, CLI, ERY	*mecA*, *msrA*	skin
S. haemolyticus	OX, CIP, LE, CLI, ERY, GEN, SXT	*mecA*, *dfr*G, *blaZ*, *msr*A, *aac(6')-Ie-aph(2")-Ia, aph(3')-IIIa*	skin
S. haemolyticus	OX, CIP, LE, CLI, ERY, GEN, TE	*mecA, blaZ, tetM, aph(3')-IIIa*	skin
|S. haemolyticus	OX, CIP, LE, CLI, ERY, GEN	*mecA*, *aac(6')-Ie-aph(2")-Ia*	skin

[1] OX - oxacillin; CIP - ciprofloxacin; LE - levofloxacin; CLI - clindamycin; ERY - erythromycin; GEN - gentamicin; TE - tetracycline; SXT - co-trimoxazole; RIF - rifampin

Table 3. Minimum inhibitory concentration distributions of the methicillin-resistant CNS isolates

	MIC values (mg/L), percentage of isolates, (n = 20)									
Antimicrobial	0.06	0.12	0.25	0.5	1	2	4	8	16	32
CIP				30	20	0	50			
CLI		15	10	0	0	25	35	15		
DAP		5	90	5	0	0				
ERY				25	0	0	70	5		
GEN					50	10	20	10	10	0
LEV			5	30	15	0	0	50		
LZD					10	90	0	0	0	
OXA			0	60	25	15	0	0	0	
PEN	0	0	50	15	15	15	5	0	0	
SYN		25	25	30	10	10	0	0		
RIF	90	0	0	0	0	10	0			
TET					35	0	0	0	50	15
SXT				10	30	20	0	40		
VAN					60	40	0	0	0	0

grey cells - susceptible; white cells - intermediate susceptible; dark cells - resistant the marginal numbers on the right side mean MIC value "≥"; CIP - ciprofloxacin; CLI - clindamycin; DAP - daptomycin; ERY - erythromycin; GEN - gentamicin; LEV - levofloxacin; LZD - linezolid; OXA - oxacillin; PEN - penicillin; SYN - quinupristin/dalfopristin; RIF - rifampin; TET - tetracycline; SXT - co-trimoxazole; VAN - vancomycin

Discussion

Bacteria from the genus *Staphylococcus* are highly prevalent in clinical samples of small animals. Here we found a prevalence of 86.5 %, similar to data obtained by other authors (PENNA et al., 2010). We focused on CNS -species that are often resistant to beta-lactams (DETWILER et al., 2013). Moreover, CNS are often reported as methicillin-resistant with co-resistance to different classes of antimicrobials other than beta-lactams (HUBER et al., 2011; VAN DUIJKEREN et al., 2004). The number of MRCNS isolates revealed that at least 4.5 % of the diseased dogs carried staphylococci resistant to all beta-lactams. Species diversity was high: 10 different species of MRCNS were detected, including species previously rarely isolated from dogs. Classical biochemical tests for species identification are not always capable of identifying staphylococci to the species level (VAN DUIJKEREN et al., 2011) and this was proven in this study as well. Certain substrates (carbohydrates and amino acids) are weakly fermented, thus interpretation of these according to the colour index is subjective. Additionally we tried different commercially available biochemical systems for identification of *Staphylococcus* (data

not presented). However, all of them were unable to identify all CNS species as reliably as PCR or sequence analysis of 16S rRNA subunit.

It is interesting that the most prevalent CNMRS species in dogs was *S. haemolyticus*, the species that is identified as the second most prevalent species of CNS in human-blood cultures (TAKEUCHI et al., 2005) and the one that shows the highest level of antimicrobial resistance (BARROS et al., 2012). Thus, the findings of this study are important for a better understanding of antimicrobial resistance spread between companion-animals and their owners.

In our study CNS were isolated from dogs with different clinical manifestations (3.3 %), although the highest number of MRCNS carriers was detected in kennels holding pure-breed dogs (10 %).

Data on antimicrobial susceptibility revealed that MRCNS most frequently demonstrated resistance against antimicrobials that are used for treatment of dogs, including penicillins (resistance attributed to *bla*Z gene), macrolides (*erm*A and *erm*C genes) and tetracyclines (*tetK* and *tetM* genes). A high rate of resistance to fluoroquinolones (50 %) was also recorded. The MICs of ciprofloxacin and levofloxacin were ≥4 mg/L and ≥8 mg/L respectively. Resistance mechanisms to fluoroquinolones of staphylococci have been well described (DESCLOUX et al., 2008). Resistance occurs as a result of mutational amino acid substitutions in the subunits of the most sensitive (or primary-target) enzyme within the cell (HOOPER, 2000). The high frequency of phenotypical resistance to fluoroquinolones found in our study could be explained by at least two reasons: firstly, fluoroquinolones are frequently used to treat dog infections especially in cases with unsatisfactory clinical practice where broad-spectrum antimicrobials are selected for treatment without sending clinical material to a laboratory for diagnosis and antibiogram; secondly, according to previous data, fluoroquinolones have been extensively used in domestic animals in Lithuania (SEPUTIENE at al., 2006; RUZAUSKAS et al., 2010a). Moreover, poultry products intended for human consumption (that are sometimes used for feeding dogs) are highly contaminated with fluoroquinolone-resistant bacteria (RUZAUSKAS et al., 2010b). A high rate (50 %) of resistance to gentamicin by MRCNS isolates was also found . The genes encoding resistance to aminoglycosides *aac(6')-Ie-aph(2")-Ia* and *aph(3')-IIIa* were detected in this study. The same genes were recently found in most isolates of enterococci isolated from diseased cows, pigs and poultry in Lithuania (SEPUTIENE et al., 2012). Those genes encoding resistance to aminoglycosides were found in *S. pseudintermedius* - a species that is also highly prevalent in companion animals (KADLEC et al., 2010b; VAN DUIJKEREN et al., 2011). This study is the first study in Lithuania where MRCNS were detected in dogs using both phenotypical and genotypical methods. Recently we have detected MRSA ST398 strains in pigs (RUZAUSKAS et al., 2013). In our opinion, the most important reason for the prevalence of methicillin resistant staphylococci in

dogs is associated with the inappropriate usage of fluoroquinolones and cephalosporins in breeding kennels. The anamnesis of the diseases demonstrated periodical usage of those classes of antimicrobials in kennels where reproductive disorders have been prevalent. It is proved that usage of fluoroquinolones as well as cephalosporins might lead to for antimicrobial resistant bacteria (GRECO et al., 2009; VAN DUIJKEREN et al., 2011). Our study revealed that breeding kennels might be a reservoir of MRCNS strains and may pose a risk for spreading such strains during mating. There is no requirement to report methicillin-resistant strain prevalence in kennel, thus other breeders have no information about the microbiological hazards associated with resistant bacteria. Attention should be paid to this problem as methicillin-resistant staphylococci pose a risk not only for animals but also for humans (CATRY et al., 2010; STEGMANN et al., 2010).

Conclusions

Coagulase-negative staphylococci are highly prevalent in dogs with various clinical conditions. Methicillin-resistant staphylococci are mostly distributed in breeding kennels and pose a risk for spreading resistant strains to other pure-breed dogs, their offspring, owners and other animals in close contact. Attention should be paid to the possible spread of resistant *Staphylococcus haemolyticus* between companion-animals and humans.

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SAŽETAK

Cilj ovog istraživanja bio je ustanoviti prisutnost i učestalost koagulaza negativnih stafilokoka otpornih na meticilin (MRKNS) izdvojenih iz pasa u Litvi te odrediti njihovu otpornost na antimikrobne tvari. Klinički materijal bio je prikupljen iz 400 pasa 2012. i 2013. godine. Tri stotine uzoraka bilo je uzeto iz bolesnih pasa s različitim kliničkim znakovima (dermatitis, otitis, infekcije rana, infekcije probavnog i dišnog sustava) te 100 uzoraka iz kuja čistih pasmina s reprodukcijskim poremećajima (pyometritis, metritis, partus praematurus) upotrebljavanih za rasplod u štenarama. Od 400 pretraženih, 20 koagulaza negativnih izolata stafilokoka otpornih na meticilin bilo je izdvojeno iz 18 pasa. Svi izolati imali su gen *mecA*, dok gen *mecC* (*mecL*GA251) nije bio dokazan. Deset izolata bilo je izdvojeno iz uzoraka rodnice kuja iz triju velikih uzgoja. Prevalencija MRKNS u pasa držanih u domaćinstvima iznosila je 3,3 %, tj. bila je značajno manja (P<0,01) nego u pasa držanih u velikim uzgajivačnicama (10 %). Dokazano je 10 različitih vrsta koagulaza negativnih stafilokoka otpornih na meticilin s najvećom prevalencijom za vrstu *Staphylococcus haemolyticus*. MRKNS izolati bili su otporni na makrolide (75 %) zbog *erm*(C) i *msrA* gena i tetraciklin (65 %) zbog posjedovanja *tet*(K) i/ili *tet*(M) gena. Stopa otpornosti na gentamicin bila je 50 % (što se pripisuje genima *aac(6')-Ie-aph(2'')-Ia*, *aph(3')-IIIa)* i na ko-trimoksal – 40 % (gen *dfrG*). Jedan izolat vrste *S. lentus* imao je gen *dfrK*. Svi izolati bili su osjetljivi na linezolid, daptomicin i vankomicin. Ovo istraživanje pokazuje da uzgojne štenare mogu biti rezervoar sojeva MRKNS i mogu predstavljati rizik za širenje takvih sojeva za vrijeme parenja. Treba se usredotočiti na mogući prijenos višestruko otpornih sojeva vrste *S. haemolyticus* s kućnih ljubimaca na čovjeka s obzirom na to da ta vrsta ima važnu ulogu kao uzročnik infekcija u ljudi.

Ključne riječi: otpornost, geni, psi čistog uzgoja, štenare, *Staphylococcus haemolyticus*