Acta Microbiologica et Immunologica Hungarica 64 (3), pp. 301–312 (2017)
DOI: 10.1556/030.63.2016.021
First published online December 13, 2016

ANTIMICROBIAL RESISTANCE IN *ENTEROCOCCUS* STRAINS ISOLATED FROM HEALTHY DOMESTIC DOGS

FABRIZIO BERTELLONI, CLAUDIA SALVADORI, GIULIA LOTTI, DOMENICO CERRI and VALENTINA VIRGINIA EBANI*

Department of Veterinary Science, University of Pisa, Pisa, Italy

(Received: 12 May 2016; accepted: 21 October 2016)

Enterococci are opportunistic bacteria that cause severe infections in animals and humans, capable to acquire, express, and transfer antimicrobial resistance. Susceptibility to 21 antimicrobial agents was tested by the disk diffusion method in 222 *Enterococcus* spp. strains isolated from the fecal samples of 287 healthy domestic dogs. Vancomycin and ampicillin minimum inhibitory concentrations (MICs) and high-level aminoglycoside resistance (HLAR) tests were also performed. Isolates showed resistance mainly to streptomycin (88.7%), neomycin (80.6%), and tetracycline (69.4%). Forty-two (18.9%) isolates showed an HLAR to streptomycin and 15 (6.7%) to gentamicin. Vancomycin and ampicillin MIC values showed 1 and 18 resistant strains, respectively. One hundred and thirty-six (61.2%) strains were classified as multidrug resistant and six (2.7%) strains as possibly extensively drug-resistant bacteria. *Enterococcus faecium* and *Enterococcus faecalis* were the most prevalent antimicrobial resistant species. Companion animals, which often live in close contact with their owners and share the same environment, represent a serious source of enterococci resistant to several antibiotics; for this reason, they may be a hazard for public health by providing a conduit for the entrance of resistance genes into the community.

Keywords: *Enterococcus* spp., dogs, feces, antimicrobial resistance, high-level aminoglycoside resistance (HLAR)

Introduction

Enterococcus species are Gram-positive bacteria belonging to the gastrointestinal microbiota of humans and animals, mammals and birds, and are widely distributed in the environment such as terrestrial and water habitats [1]. Some

*Corresponding author; E-mail: valentina.virginia.ebani@unipi.it

Enterococcus species are used as probiotics to treat diarrhea and improve host immunity [2].

Enterococci are opportunistic pathogens for humans and animals. *Enterococcus faecalis* and *Enterococcus faecium* have become particularly important etiological agents of human nosocomial infections, including urinary tract infections, endocarditis, bacteremia, neonatal infections, central nervous system, abdominal, pelvic, and endodontic infections [1, 3].

Enterococci are also involved as etiological agents of infections in veterinary medicine such as mastitis in cattle, enteritis in swine and cattle, as well as endocarditis, septicemia, spondylitis, and amyloid arthropathy in poultry [4, 5].

Companion animals are often reservoirs of zoonotic pathogens for their owners. In dogs, enterococci may not only be involved in the urinary tract infections, but also in diarrhea, endocarditis, post-surgical, and periodontal infections [6, 7]. However, domestic dogs, even though without clinical forms, may excrete enterococci in their feces contaminating the environment shared by humans.

Enterococci are of particular concern for their intrinsic antibiotic resistance, particularly to cephalosporins and aminoglycosides, or acquired resistance to many other antimicrobials [8]. Moreover, these bacteria in the gastrointestinal habitat are in a suitable position to acquire antimicrobial resistance genes from other commensals, which may further transfer to other more pathogenic bacteria [9].

The aim of the present study was to investigate the antimicrobial resistance profiles of *Enterococcus* spp. strains isolated from feces of healthy domestic dogs, with particular attention to vancomycin, ampicillin, and high-level aminoglycoside resistance (HLAR).

Materials and Methods

Sampling

Fecal swabs were collected from 287 clinically healthy dogs, which were regularly kept indoor, strictly in contact with their owners. Samples were collected during routine visits to local veterinarians and kept at 4 °C until bacteriological examinations.

Bacterial isolation

Within 24 h from collection, swabs were cultured directly on Kanamycin Aesculin Azide Agar (KAAA, Oxoid Ltd., Basingstoke, UK) and incubated at

42 ± 1 °C for 18–24 h. From plates with growth of colonies typical for enterococci, at least one colony was subcultured on KAAA.

Isolates were stored at −80 °C in Brain Hearth Infusion Broth (Oxoid Ltd.) for further investigations.

Antimicrobial susceptibility testing

Disk diffusion method. Isolates were tested by the standard disk diffusion method of Kirby–Bauer [10] on Mueller–Hinton Agar (Oxoid Ltd.) incubated at 35 ± 1 °C for 18–24 h. The following antimicrobials (Oxoid Ltd.) were tested: amoxicillin–clavulanic acid (30 μg), ampicillin (10 μg), cefalotin (30 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), clindamycin (2 μg), enrofloxacin (5 μg), erythromycin (10 μg), gentamicin (10 μg), linezolid (30 μg), neomycin (10 μg), nitrofurantoin (300 μg), oxacillin (1 μg), quinupristin–dalfopristin (15 μg), rifampicin (30 μg), streptomycin (10 μg), teicoplanin (30 μg), tetracycline (30 μg), tigecycline (15 μg), trimethoprim (5 μg), and vancomycin (30 μg). Results were interpreted following EUCAST breakpoint tables and, where not possible, according to National Committee for Clinical Laboratory Standards (NCCLS) [11, 12]. Reference strains *E. faecalis* ATCC 29212 and *E. faecium* ATCC 19434 were used as controls.

Minimum inhibitory concentration (MIC) determination. MIC for vancomycin and ampicillin were performed on microplates [13]. Concentrations from 0.5 to 256 μg/ml were used to test vancomycin MIC and concentrations from 8 to 256 μg/ml were used for ampicillin MIC. Microplates were incubated at 37 ± 1 °C in a humid chamber. Breakpoint values are 32 μg/ml for vancomycin and 64 μg/ml for ampicillin.

HLAR. As indicated by CLSI Performance Standards for antimicrobial susceptibility tests, isolates that showed resistance to gentamicin and/or streptomycin by disk diffusion method, were tested for resistance to high concentration of gentamicin (500 μg/ml) and streptomycin (1,000 μg/ml) [13].

Classification of acquired resistance

To classify isolated strains for expression of acquired resistance, a standardized international terminology proposed by Magiorakos et al. [14] has been used in this study. For enterococci, aminoglycosides, carbapenems, fluoroquinolones, glycopeptides, glycylcyclines, lipopeptides, oxazolidinones, penicillins, streptogramins, and tetracycline categories should be tested. Criteria for defining acquired resistance are: multidrug-resistant (MDR) strain when it is

non-susceptible to ≥1 agent in ≥3 antimicrobial categories, extensively drug-resistant (XDR) strain when it is non-susceptible to ≥1 agent in all but ≤2 categories, and pandrug-resistant (PDR) strain when it is non-susceptible to all antimicrobial agents listed.

Since no all proposed antibiotics have been tested in our study, only MDR or possibly XDR strains could be detected.

Species identification

Enterococcus spp. isolates classified as MDR or possibly XDR were examined for species identification with API 20 STREP (Bio Mérieux Italia, Bagno a Ripoli, FI, Italy). Apiweb V 1.1.0 software was used as interpretative criteria.

Results

Bacterial isolation

Two hundred and twenty-two *Enterococcus* spp. isolates were obtained from the 287 examined fecal samples, with a 77.3% prevalence of shedding dogs.

Antimicrobial susceptibility testing

Disk diffusion method. All 222 strains were tested for antimicrobial susceptibility by disk diffusion method and the results were reported in Table I.

The isolates were more frequently non-susceptible to aminoglycosides category (92.3% of isolates were non-susceptible to neomycin and 94.1% to streptomycin), trimethoprim (97.3%), tetracycline (88.7%), fluoroquinolones (80.2% to enrofloxacin and 77.9% to ciprofloxacin), clindamycin (71.2%), and oxacillin (71.6%).

A moderate percentage of isolates were non-susceptible to erythromycin (64%), gentamicin (56.8%), cefalotin (49.5%), quinupristin–dalfopristin (45.5%), linezolid (43.2%), and tigecycline (40.5%).

A limited number of isolates were non-susceptible to glycopeptides (vancomycin 9.5% and teicoplanin 7.7%), ampicillin (12.2%) and association of amoxicillin–clavulanic acid (6.8%).

A total of 165 resistance patterns were identified and all *Enterococcus* spp. isolates were resistant to at least two different categories of antibiotics, with 182 (81.9%) isolates being resistant to five or more antibiotics.

Table I. Antimicrobial resistance expression of *Enterococcus* spp. isolates by disk diffusion method and the results

Antibiotics		Susceptible (No. of isolates)	%	Intermediate (No. of isolates)	%	Resistant (No. of isolates)	%	Non-susceptible: intermediate + resistant (No. of isolates)	%
Aminoglycoside	Neomycin (N)	17	7.7	26	11.7	179	80.6	205	**92.3**
	Gentamicin (CN)	96	43.2	40	18.0	86	38.7	126	**56.8**
	Streptomycin (S)	13	5.9	12	5.4	197	88.7	209	**94.1**
Cephalosporins	Cefalotin (KF)	112	50.5	46	20.7	64	28.8	110	**49.5**
Fluoroquinolones	Ciprofloxacin (CIP)	49	22.1	96	43.2	77	34.7	173	**77.9**
	Enrofloxacin (ENR)	44	19.8	68	30.6	110	49.5	178	**80.2**
Glycopeptide	Teicoplanin (TEC)	205	92.3	10	4.5	7	3.2	17	**7.7**
	Vancomycin (VA)	201	90.5	13	5.9	8	3.6	21	**9.5**
Penicillins	Amoxicillin + clavulanic acid (AMC)	207	93.2	10	4.5	5	2.3	15	**6.8**
	Ampicillin (AMP)	195	87.8	0	0	27	12.2	27	**12.2**
	Oxacillin (OX)	63	28.4	8	3.6	151	68.0	159	**71.6**
Tetracycline	Tetracycline (TE)	25	11.3	43	19.4	154	69.4	197	**88.7**
	Tigecycline (TGC)	132	59.5	47	21.2	43	19.4	90	**40.5**
Macrolides	Erythromycin (E)	80	36.0	66	29.7	76	34.2	142	**64.0**
Lincosamides	Quinupristin + dalfopristin (QD)	121	54.5	46	20.7	55	24.8	101	**45.5**
Streptogramins	Clindamycin (DA)	64	28.8	34	15.3	124	55.9	158	**71.2**
Others	Rifampicin (RD)	151	68.0	23	10.4	48	21.6	71	**32.0**
	Chloramphenicol (C)	155	69.8	29	13.1	38	17.1	67	**30.2**
	Linezolid (LZD)	126	56.8	31	14.0	65	29.3	96	**43.2**
	Nitrofurantoin (F)	148	66.7	19	8.6	55	24.8	74	**33.3**
	Trimethoprim (W)	6	2.7	61	27.5	155	69.8	216	**97.3**

Table II. Resistance patterns of strains classified as possibly XDR

Resistance patterns	Strain	Species
C; LZD; F; N; KF; CIP; ENR; TEC; E; QD; DA; AMP; TE; TIG	E239	*E. faecium*
RD; C; LZD; N; S (HLAR); KF; CIP; ENR; E; QD; DA; AMP; TE; TIG	E233	*E. faecalis*
RD; C; LZD; N; S; KF; CIP; ENR; TEC; E; QD; DA; TE; TIG	E218	*E. faecium*
RD; C; LZD; F; N; KF; CIP; ENR; TEC; E; QD; AMP; TE; TIG	E160	*E. faecium*
RD; C; LZD; F; N; KF; CIP; ENR; TEC; E; QD; DA; AMP; TE; TIG	E177	*E. faecium*
RD; C; LZD; F; N; CN (HLAR); S (HLAR); KF; CIP; ENR; TE; E; QD; DA; TE; TIG	E234	*E. faecalis*

MIC determination. All isolates characterized by a non-susceptibility to vancomycin and/or ampicillin with Kirby–Bauer test were tested for determination of MIC of these antibiotics.

Vancomycin MIC showed the following results: ≥256 μg/ml (1 isolate), 4 μg/ml (2 isolates), 2 μg/ml (9 isolates), 1 μg/ml (8 isolates), and 0.5 μg/ml (1 isolate).

Considering 32 μg/ml as breakpoint, one isolate was confirmed resistant to the antibiotic.

Ampicillin MIC showed the following results: >256 μg/ml (5 isolates), 256 μg/ml (2 isolates), 128 μg/ml (7 isolates), 64 μg/ml (4 isolates), 32 μg/ml (5 isolates), 16 μg/ml (2 isolates), and <8 μg/ml (3 isolates). Considering 64 μg/ml as breakpoint, 18 isolates resulted resistant to ampicillin.

HLAR. One hundred and twenty-six (56.8%) isolates that were non-susceptible to gentamicin and 209 (94.1%) to streptomycin with Kirby–Bauer test, were tested for the HLAR. Fifteen (6.7%) tested isolates showed a high level resistance to gentamicin, whereas 42 (18.9%) isolates showed a high level resistance to streptomycin. Six (2.7%) of these isolates had HLAR to both antibiotics. Table II shows the resistance patterns of the HLAR strains.

Classification of acquired resistance and species identification

Following MDR, XDR, and PDR classification, 136 (61.2%) strains were classified as MDR and 6 (2.7%) strains as possibly XDR bacteria. MDR isolates were distributed among the species *E. faecium* (63 strains), *E. faecalis* (56 strains), *E. durans* (13 strains), *E. casseliflavus* (3 strains), and *E. avium* (1 strain). XDR isolates belonged to the species *E. faecium* (4 strains) and *E. faecalis* (2 strains). Table III reports the resistance patterns of the six XDR isolates.

Table III. Resistance patterns of *Enterococcus* strains resulted high-level aminoglycoside resistance (HLAR) to gentamicin and/or streptomycin

Resistance patterns	Species
N; ENR; E; QD; DA; TE; CN (HLAR); S (HLAR)	*E. faecium* MDR
RD; C; LZD; F; N; KF; E; QD; DA; AMC; TE; CN (HLAR); S (HLAR)	*E. faecium* MDR
RD; N; KF; ENR; E; QD; DA; TE; CN (HLAR); S (HLAR)	*E. faecalis* MDR
C; N; CIP; ENR; E; DA; TIG; S (HLAR)	NT
N; CIP; TE; S (HLAR)	*E. faecalis* MDR
LZD; N; CIP; ENR; E;QD; TE; S (HLAR)	*E. durans* MDR
N;CIP; ENR; E; DA; TE; S (HLAR)	*E. faecium* MDR
RD; N; KF; E; QD; DA; TE; S (HLAR)	*E. faecium* MDR
N; CIP; ENR; TE; S (HLAR)	*E. faecium* MDR
N; CIP; ENR; DA; TE; S (HLAR)	*E. faecium* (2 strains) MDR
C; F; N; KF; CIP; ENR; QD; DA; AMC; AMP; TE; TIG; S (HLAR)	*E. faecalis* MDR
C; F; KF; E; QD; DA; TE; S (HLAR)	*E. faecium* MDR
RD; C; LZD; N; KF; CIP, ENR; E; QD; DA; TE; TIG; S (HLAR); CN (HLAR)	*E. faecium* (2 strains) MDR *E. faecalis* (1 strain) MDR *E. durans* (1 strain) MDR
RD; C; LZD; F; N; CIP, ENR; E; QD, DA, TE; TIG; CN (HLAR)	*E. faecalis* MDR
N; CIP; ENR; QD; TE; TIG; CN (HLAR)	*E. faecalis* MDR
RD; C; LZD; N; KF; CIP, ENR; TEC; E; QD; D; TE; TIG; CN (HLAR)	*E. faecium* MDR
RD; C; LZD; F; N; CIP; ENR; E; QD; DA; TE; TIG; CN (HLAR)	*E. faecium* MDR
C; LZD; F; N; KF; CIP; ENR; E; QD; DA; TE; TIG; CN (HLAR)	*E. faecium* MDR
N, KF, CIP; E; QD; DA; TE; TIG; S (HLAR)	*E. faecalis* MDR
N; CIP; ENR; E; QD; DA; TE; S (HLAR)	*E. faecium* MDR
RD; C; LZD; N; KF; CIP; ENR; E; QD; DA; TE; S (HLAR)	*E. faecium* MDR
C; LZD; N; CIP; ENR; TEC; E; DA; TE; S (HLAR)	*E. faecium* MDR
N; ENR; E, DA; TE; S (HLAR)	*E. faecium* MDR
RD; LZD; N; KF; CIP, ENR; E; QD, DA; TE; TIG; S (HLAR)	*E. faecium* (1 strain) MDR *E. casseliflavus* (1 strain) MDR
RD; C; LZD; N; KF; CIP; ENR; TEC; E; QD; DA; TE; TIG; S (HLAR)	*E. faecium* XDR
RD; C; LZD; F; N; KF; CIP; ENR, E; QD; DA; TE; TIG; S (HLAR)	*E. faecalis* MDR
RD; C; LZD, F; N; KF; CIP; ENR; E; QD; DA; AMC; TE; TIG; S (HLAR)	*E. durans* MDR
RD; C; LZD; N; KF; CIP; ENR; E; QD; DA; AMP; TE; TIG; S (HLAR)	*E. faecalis* XDR
RD; C; LZD; F; N; KF; CIP; ENR; TE; E; QD; DA; TE; TIG; CN (HLAR); S (HLAR)	*E. faecalis* XDR
RD; N, KF; CIP; ENR, E; DA; TE; TIG; S (HLAR)	*E. durans* MDR
RD; C; LZD; N; CN (HLAR); KF; CIP, ENR, E;QD; DA; AMP, TE; TIG	*E. faecium* MDR
N; DA; TE; S (HLAR)	NT
RD; N; CN; KF; CIP; ENR; E; QD; DA; TE; S (HLAR)	*E. faecium* MDR
C; LZD, F; N; KF; CIP; ENR; E; DA; AMC; AMP; TE; TIG; S (HLAR)	*E. faecalis* MDR

Table III. (cont.)

Resistance patterns	Species
C; LZD; N; E; DA; TE; CN; S (HLAR)	*E. faecalis* MDR
RD; C; F; N; ENR; E; QD, DA; TE; TIG; S (HLAR)	*E. faecalis* MDR
N; E; QD; DA; TE; S (HLAR)	*E. faecium* MDR
N; E; DA; TE; S (HLAR)	NT
LZD; F; N; CIP; ENR; TE; QD; DA; S (HLAR)	*E. faecium* MDR
C; LZD; N; KF; E; DA, TE; TIG; CN (HLAR); S (HLAR)	*E. durans* – MDR

Note: NT = not typed since MDR or XDR is not detected.

Discussion

During the present investigation, *Enterococcus* spp. strains were isolated from almost all tested dogs. *E. faecium* and *E. faecalis* were most frequently isolated, in agreement to the results obtained by other studies [15–17].

High degrees of antimicrobial activity were observed among penicillins. The association of amoxicillin and clavulanic acid resulted active against 93.2% of strains and ampicillin against 87.8% of strains. Other studies found higher values of resistance in enterococci from pets [17].

Most of the isolates showed resistance to aminoglycosides, particularly streptomycin (94.1% non-susceptible) and neomycin (92.3% non-susceptible). These results could be related to the intrinsic resistance of enterococci to clinically achievable concentrations of aminoglycosides due to the inability to enter the cell and for enzyme-mediated resistance or sterically hindered ribosome target site [18].

A low percentage of isolates showed resistance to vancomycin with the Kirby–Bauer test, and MIC determination confirmed this resistance only in one case. This result, in agreement to other studies [16], suggests that dogs are not an important reservoir of vancomycin-resistant enterococci (VRE), even though VRE have been isolated from canine feces hypothesizing dogs as a source of infection for their owners [15].

Considering the tetracyclines class, 88.7% and 40.5% of isolates resulted non-susceptible to tetracycline and tigecycline, respectively. Resistance to tetracycline is common among enterococci, because of their large employment in human and veterinary medicine [19].

Tigecycline is a bacteriostatic antibiotic active against a broad range of bacteria, with only few naturally resistant exceptions [20]. Instead, our result suggests that resistance to tigecycline is not so uncommon and could be a new threat in the therapy of infections.

Diffuse resistance to fluoroquinolones was detected: this result seems to be related to their large employment in human and veterinary medicine due to their effectiveness against both Gram-positive and Gram-negative bacteria [21].

Forty-five percent of isolates were non-susceptible to quinupristin–dalfopristin. Thirty-five isolates showed concomitant resistance to erythromycin, clindamycin, and quinupristin–dalfopristin, suggesting an acquired macrolide–lincosamide–streptogramin (MLS_B) resistance. MLS_B resistance is well characterized in enterococci and three mechanisms of acquired resistance have been described: methylation of 23S rRNA, active efflux, or inactivating enzymes [22].

The percentage of linezolid-resistant isolates found in the present study shows the increasing resistance to this antibiotic, that has been often used against complicated glycopeptide-resistant enterococci infections, mainly human endocarditis [18].

HLAR has been tested on the isolates with a non-susceptible phenotype in the Kirby–Bauer test for streptomycin and gentamicin. Forty-two (18.9%) isolates showed an HLAR to streptomycin and 15 (6.7%) to gentamicin, and among them six (2.7%) to both.

Recommended therapy for serious infections like endocarditis, meningitis, or possibly other serious infections in immunodeficiency human patients includes a cell-wall-active agent such as penicillin or vancomycin, combined with an aminoglycoside like gentamicin or streptomycin. This combination is synergistic in action. However, when an *Enterococcus* strain is resistant to the cell-wall-active agent or has HLAR, there is no synergism and the combination therapy is likely to be unsuccessful [23].

Although the clinical use of streptomycin for enterococci has long been restricted due to intrinsic low level resistance, the present study revealed a relevant number of strains with HLAR to this antibiotic. The present investigation found a higher percentage of strains with HLAR to streptomycin, whereas previous studies indicated HLAR to gentamicin to be more common in all species of enterococci [24].

Conclusion

Infections caused by enterococci are not frequent in companion animals. However, antibiotic therapy against other infections in these animals allows the selection of antimicrobial resistant *Enterococcus* strains as well as other bacteria.

The presence of antimicrobial-resistant *Enterococcus* strains in the intestinal microflora of animals represents a severe risk of genetic linkage of resistance genes with other bacteria [25]. Moreover, companion animals, that often live in

close contact with their owners, may serve as reservoirs of antimicrobial resistance genes that can be transferred from pets to humans and within the environment.

In the past, the main threat related to enterococci antibiotic resistance was the circulation of VRE among humans and animals [26]. Currently, on the basis of more recent studies and our results, VRE seem to be not frequent in pet population, but other concerns have been added: the increasing spread of enterococci resistant to several other antimicrobials.

In light of these considerations, it is important to monitor the extent of antimicrobial resistant enterococci in companion animals, in order to determine the role of pets as reservoirs of bacterial strains with old and new antimicrobial resistance patterns.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Byappanahalli, M. N., Nevers, M. B., Korajkic, A., Staley, Z. R., Harwood, V. J.: Enterococci in the environment. Microbiol Mol Bio Rev **4**, 685–706 (2012).
2. Franz, C. M., Huch, M., Abriouel, H., Holzapfel, W., Galvez, A.: Enterococci as probiotics and their implications in food safety. Int J Food Microbiol **151**, 125–140 (2011).
3. Herczegh, A., Gyurkovics, M., Ghidan, A., Megyesi, M., Lohinai, Z.: Effect of dentin powder on the antimicrobial properties of hyperpure chlorine-dioxide and its comparison to conventional endodontic disinfecting agents. Acta Microbiol Immunol Hung **61**, 209–220 (2014).
4. Robbins, K. M., Suyemoto, M. M., Lyman, R. L., Martin, M. P., Barnes, H. J., Borst, L. B.: An outbreak and source investigation of enterococcal spondylitis in broiler caused by *Enterococcus cecorum*. Avian Dis **56**, 768–773 (2012).
5. Šeputienė, V., Bogdaitė, A., Ružauskas, M., Sužiedėlienė, E.: Antibiotic resistance genes and virulence factors in *Enterococcus faecium* and *Enterococcus faecalis* from diseased farm animals: Pigs, cattle and poultry. Pol J Vet Sci **3**, 431–438 (2012).
6. Abbot, Y., Kirby, B. M., Karczmarczk, M., Markey, B. K., Leonard, F. C., Fitzgerald, S.: High-level gentamicin-resistant and vancomycin-resistant *Enterococcus faecium* isolated from a wound in a dog. J Small Anim Pract **50**, 194–197 (2009).
7. Semedo-Lemsaddek, T., Tavares, M., São Braz, B., Tavares, L., Oliveira, M.: Enterococcal infective endocarditis following periodontal disease in dogs. PLoS One **11**, e0146860 (2016).
8. Tendolkar, P. M., Baghdayan, A. S., Shankar, N.: Pathogenic enterococci: New developments in the 21st century. Cell Mol Life Sci **60**, 2622–2636 (2003).
9. Hegstad, K., Mikalsenm, T., Coque, T. M., Werner, G., Sundsfjord, A.: Mobile genetics elements and their contribution to the emergence of antimicrobial resistant *Enterococcus faecalis* and *Enterococcus faecium*. Clin Microbiol Infect **16**, 541–554 (2010).

10. Bauer, A., Kirby, W. M. N., Sherris, J. C., Turk, M.: Antibiotic susceptibility testing by a standardized single disc method. Am J Pathol **45**, 493–496 (1966).
11. Clinical and Laboratory Standards Institute (CLSI): Performance standards for antimicrobial susceptibility testing, seventeenth informational supplement. Clinical and Laboratory Standards Institute document M100-S17, Clinical and Laboratory Standards Institute, Wayne, PA, USA, 2007.
12. The European Committee on Antimicrobial Susceptibility Testing (EUCAST). Breakpoint tables for interpretation of MICs and zone diameters. Version 3.1, 2013. Retrieved from http://www.eucast.org
13. Clinical and Laboratory Standards Institute (CLSI). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard, Seventh edition. Clinical and Laboratory Standards Institute document M7-A7. Clinical and Laboratory Standards Institute, Wayne, PA, USA, 2006.
14. Magiorakos, A. P., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M. E., Giske, C. G., Harbarth, S., Hindler, J. F., Kahlmeter, G., Olsson-Liljequist, B., Paterson, D. L., Rice, L. B., Stelling, J., Struelens, M. J., Vatopoulos, A., Weber, J. T., Monnet, D. L.: Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect **18**, 268–281 (2012).
15. Devriese, L. A., Cruz Colque, J. I., De Herdt, P., Haesebrouck, F.: Identification and composition of the tonsillar and anal enterococcal and streptococcal flora of dogs and cats. J Appl Bacteriol **73**, 421–425 (1992).
16. Ossiprandi, M. C., Bottarelli, E., Cattabiani, F., Bianchi, E.: Susceptibility to vancomycin and other antibiotics of 165 *Enterococcus* strains isolated from dogs in Italy. Comp Immunol Microbiol Infect Dis **31**, 1–9 (2008).
17. Iseppi, R., Messi, P., Anacarso, I., Bondi, M., Sabia, C., Condò, C., de Niederhausern, S.: Antimicrobial resistance and virulence traits in *Enterococcus* strains isolated from dogs and cats. New Microbiol **38**, 369–378 (2015).
18. Hollenbeck, B. L., Rice, L. B.: Intrinsic and acquired resistance mechanisms in *Enterococcus*. Virulence **5**, 421–433 (2012).
19. Guardabassi, L., Schwarz, S., Lloyd, D. H.: Pet animals as reservoirs of antimicrobial-resistant bacteria. J Antimicrob Chemother **54**, 321–332 (2004).
20. Sun, Y., Cai, Y., Liu, X., Bai, N., Liang, B., Wang, R.: The emergence of clinical resistance to tigecycline. Int J Antimicrob Agents **41**, 110–116 (2013).
21. Redgrave, L. S., Sutton, S. B., Webber, M. A., Piddock, L. J. V.: Fluoroquinolone resistance: Mechanism, impact on bacteria, and role in evolutionary success. Trends Microbiol **22**, 438–445 (2014).
22. Jaglic, Z., Vlkova, H., Bardon, J., Michu, E., Cervinkova, D., Babak, V.: Distribution, characterization and genetic bases of erythromycin resistance in staphylococci and enterococci originating from livestock. Zoonoses Public Health **59**, 202–211 (2012).
23. Adhikari, L.: High-level aminoglycoside resistance and reduced susceptibility to vancomycin in nosocomial enterococci. J Glob Infect Dis **2**, 231–235 (2010).
24. Padmasini, E., Padmaraj, R., Srivani Ramesh, S.: High level aminoglycoside resistance and distribution of aminoglycoside resistant genes among clinical isolates of *Enterococcus* species in Chennai, India. Sci World J **2014**, 329157 (2014).

25. Jackson, C. R., Fedorka-Cray, P. J., Davis, J. A., Barrett, J. B., Brousse, J. H., Gustafson, J., Kucher, M.: Mechanisms of antimicrobial resistance and genetic relatedness among enterococci isolated from dogs and cats in the United States. J Appl Microbiol **108**, 2171–2179 (2010).
26. Jovanović, M., Milošević, B., Tošić, T., Stevanović, G., Mioljević, V., Indić, N., Velebit, B., Zervos, M.: Molecular typing, pathogenicity factor genes and antimicrobial susceptibility of vancomycin resistant enterococci in Belgrade, Serbia. Acta Microbiol Immunol Hung **62**, 147–160 (2015).