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SPECIES DISTRIBUTION, ANTIBIOTIC RESISTANCE AND VIRULENCE TRAITS IN CANINE AND FELINE ENTEROCOCCI IN TUNISIA

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In order to investigate the possible role of dogs and cats in the carriage and potential dissemination of resistant enterococci, seventy faecal samples from dogs and cats were tested for enterococci. Fifty-eight enterococci were recovered. Isolates were identified as *Enterococcus faecium* (n = 31) and *E. faecalis* (n = 14) *E. durans* (n = 6), *E. casseliflavus* (n = 2), *E. hirae* and *E. gallinarum* (2 isolates each). Enterococcal isolates showed resistance to ciprofloxacin (n = 35), erythromycin (n = 31), tetracycline (n = 25), kanamycin (n = 15), streptomycin (n = 13), pristinamycin (n = 11), gentamicin (n = 10), chloramphenicol (n = 8), and linezolid (n = 6). The gene *erm*(B) was detected in 22 out of 31 erythromycin-resistant enterococci. All tetracycline-resistant enterococci carried *tet*(M) and/or *tet*(L) genes. The gene *aac*(6')-Ie-*aph*(2")-Ia was identified in five of high-level gentamicin-resistant isolates, the genes *aph*(3')-IIIa and/or *aac*(6')-Ie-*aph*(2")-Ia in eleven high-level kanamycin-resistant isolates and the gene *ant*(6)-Ia in eleven high-level streptomycin-resistant isolates. Only one strain harboured *cat*(A) gene, and five strains contained *vat*(E) or *vat*(D) genes. Virulence genes *gel*(E) (21 strains), *esp* (11 strains) and *cylA/cylB* (5 strains) were detected. High genetic diversity was demonstrated among *E. faecium* isolates by pulsed-field gel electrophoresis (PFGE). Dogs and cats can be carriers of antibiotic-resistant enterococci in their faeces that could shed into the household environment.

Key words: *Enterococcus*, pets, resistance genes, virulence genes, linezolid resistance

Antibiotic-resistant bacteria have been increasingly detected in different habitats, mainly as a result of the intensive use of antimicrobial agents in human and veterinary medicine (Leite-Martins et al., 2015).

Enterococci are part of the normal intestinal microbiota of humans and animals, although they can also be opportunistic pathogens, causing different

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types of infections (Fisher and Phillips, 2009; Byappanahalli et al., 2012). Enterococci can easily acquire antimicrobial resistance through mutations or acquisition of antimicrobial resistance genes included in plasmids and transposons (Da Costa et al., 2013; Gilmore et al., 2013).

Cats and dogs are companion animals that have been in close contact with humans since ancient times, which makes possible the transfer of bacteria between these animals and their owners (Guardabassi et al., 2004; Lloyd, 2007). There are numerous reports that describe pet animals (cats and dogs) as reservoirs of antimicrobial resistance determinants for many pathogens and commensals, including staphylococci, enterococci, *Escherichia coli,* and *Salmonella* (Rodrigues et al., 2002; Guardabassi et al., 2004; Buma et al., 2006; Lloyd, 2007; Leite-Martins et al., 2014). The widespread use of antimicrobials in these animals favours the increase of resistance and exerts a selection pressure on commensal microorganisms of their intestinal tract (Guardabassi et al., 2004; Lloyd, 2007).

Some previous studies have analysed the presence of enterococci in healthy dogs and cats (Poeta et al., 2006; Delgado et al., 2007; Jackson et al., 2009; Kataoka et al., 2014; Leite-Martins et al., 2015). However, none of these studies have been performed in African countries. The objective of this study was to determine the carriage rate of enterococci in the faeces of a population of healthy dogs and cats in Tunisia (at a single point in time), by analysing the antimicrobial resistance phenotypes and genotypes as well as the virulence factors of the isolates recovered.

Materials and methods

Samples and bacterial strains

Faecal swabs were collected from 70 healthy pets (20 cats and 50 dogs) from March through June 2016. Samples were taken at three veterinary clinics located in the northern suburbs of Tunis during routine consultations, and they were immediately transported under refrigeration to the University of Tunis for analysis. The owners of the dogs and cats gave their informed consent to their animals' participation in the study. Samples were obtained by veterinarians who wore gloves during sample collection, following the local regulations. All healthy animals that attended the veterinary clinics during the four-month period, whose owners gave their informed consent to their animals' participation, were included in this study. None of the animals had suffered from infections or had taken antibiotics during the three months prior to the sample collection. The age of the animals varied from 3 weeks to 10 years. All the animals tested were living in different urban areas of Tunisia.

Samples were inoculated in 3 ml of sterile saline solution, and an aliquot of 100 µl was seeded onto Slanetz-Bartley (SB) agar plates. These plates were incubated for 48 h at 37 °C. In order to study the diversity of enterococci in the

samples, we isolated one or two colonies per sample with a typical enterococcal morphology, they were identified by both classical biochemical methods and by PCR using primers specific for the different enterococcal species (Klibi et al., 2013). If the two colonies belonged to the same species and presented the same phenotype of resistance, we selected only one for this study. All PCR reactions included positive and negative controls from the strain collection of the University of Tunis (Tunisia).

Pulsed-field gel electrophoresis (PFGE)

Genomic DNA of *Enterococcus faecium* strains was prepared as previously described (Klibi et al., 2013); one colony of the tested isolate was incubated on 1 ml of Brain Heart Infusion Agar (BHIA) at 37 °C for 24 h, 200 µl of the suspension was centrifuged for 5 min at 8,000 rpm/min, and one ml of Tris-EDTA (TE) was added to the precipitate. The suspension was then mixed with an equal volume of 2% pulsed-field certified agarose (Bio-Rad Laboratories) and poured into a mould to obtain the block. For restriction endonuclease digestion of the whole genomic DNA, small slices of agarose blocks were placed in a mixture containing 88 µl of distilled water, 11 µl of 10 × reaction buffer and 10 U of SmaI (New England Biolabs). The preparations were incubated overnight at 25 °C. After digestion and washing, the blocks were placed in wells containing 1.2% pulsed-field-certified agarose gel made from 0.5 × Tris-borate-EDTA buffer. The gel was electrophoresed with a clamped homogeneous electric field using a CHEF-DR-III apparatus (Bio-Rad Laboratories). The total run time was 23 h, the switch time was 5 to 40 sec, and the voltage for the run was 6 V/cm. The gel was stained with ethidium bromide and photographed using a UV light source (Bio-rad XR^{+}). The resulting restriction patterns were analysed by visually and by GelCompar II software using the UPGMA algorithm and the Dice similarity coefficients (tolerance 1%) (Turabelidze et al., 2000). PFGE patterns were performed twice for reproducibility. Lambda Ladder (BioLabs) was used as a PFGE marker.

Antibiotic susceptibility testing

Susceptibility testing was performed by the disc diffusion method for the following antimicrobial agents (µg per disc): vancomycin (30), teicoplanin (30), ampicillin (10), chloramphenicol (30), ciprofloxacin (5), tetracycline (30), erythromycin (15), pristinamycin (15), linezolid (30), gentamicin (GEN, 500), kanamycin (KAN, 1000) and streptomycin (STR, 500) (CLSI, 2015). Minimal inhibitory concentrations (MICs) of vancomycin, teicoplanin and linezolid were also determined by E-test method. The strain *E. faecalis* ATCC 29212 was used as quality control for all susceptibility tests.

Detection of antibiotic resistance genes by PCR

The presence of antibiotic resistance genes was analysed by PCR in all enterococcal isolates using specific primers as previously described (Torres et al., 2003; Klibi et al., 2013). The genomic DNA of enterococci was obtained with a commercial system (Instagene matrix, Biorad). The genes studied were as follow: *aac*(6')-Ie-*aph*(2'')-Ia, *aph*(3')-IIIa, *ant*(6)-Ia, *erm*(A), *erm*(B), *erm*(C), *mef*A/E, *msr*A, *tet*(M), *tet*(L), *tet*(K), *vat*(D) and cat_{pIP501}.

To characterise the mechanism of linezolid resistance, domain V region of the 23S rRNA gene, *cfr* gene and ribosomal proteins L3 and L4 were amplified and sequenced using primers previously described (Patel et al., 2013). Mutations affecting ribosomal proteins L3 and L4 were analysed by comparing the obtained sequences to those from wild-type *S. epidermidis* ATCC 12228, in addition to the ones of a linezolid-susceptible *E. faecalis* strain isolated during the same period.

Production of gelatinase and haemolysin

For the detection of gelatinase activity, enterococci were inoculated on tryptic soy agar plates containing 3% gelatin (Difco, Detroit, Michigan) which were then incubated at 37 °C for 24 h. Gelatinase activity was observed as a transparent halo around the colonies after the plate was flooded with Frazier solution (Klibi et al., 2007).

To investigate haemolysin production, isolates were streaked onto fresh horse blood agar plates and were grown overnight at 37 °C. A clear zone of beta haemolysis around the streak was considered to be a positive reaction for haemolysin production.

Detection of virulence genes by PCR

Specific primers were used in this study for amplification by PCR of the genes which encode virulence factors (Klibi et al., 2007): *esp* [enterococcal surface protein that plays a role in biofilm formation and adherence to abiotic surfaces (Shepard and Gilmore, 2002)]; *ace* [a collagen binding protein belonging to the microbial surface components recognising adhesive matrix molecules (Koch et al., 2004)]; *cyl*A/*cyl*B (cytolysin activator, cellular toxin capable of lysing a range of prokaryotic and eukaryotic cells), *gel*E (gelatinase, a zinc metalloprotease with hydrolytic capacity of gelatin, collagen, casein, haemoglobin, and other biological peptides), and *hyl* (glycoside hydrolase, which acts on hyaluronic acid and increases bacterial invasion) (Kayaoglu and Ørstavik, 2004).

Results and discussion

A total of 58 enterococcal isolates were recovered from 18 of 20 cats and 37 of 50 dogs, corresponding to one or two enterococci per positive sample. The distribution of species among the 58 enterococcal isolates was as follows: *E. faecium* (31 isolates), *E. faecalis* (14 isolates), *E. durans* (6 isolates), *E. hirae* (2 isolates), *E. casseliflavus* (3 isolates), and *E. gallinarum* (2 isolates). The detection of *E. faecium* and *E. faecalis* as the predominant enterococcal species shows strong similarities with data previously reported in the gut enterococcal microbiota of pets and other animals (Poeta et al., 2007; Jackson et al., 2009; Silva et al., 2012; Kataoka et al., 2014; Iseppi et al., 2015).

Only two enterococcal species were detected in cats (*E. faecium* and *E. faecalis*). Other authors have detected *E. faecium*, *E. hirae* or *E. avium* as the predominant species in cats (Rodrigues et al., 2002; Jackson et al., 2009). For dogs, *E. faecium* was also the most prevalent species, followed by *E. faecalis*, *E. durans*, *E. casseliflavus*, *E. gallinarum*, and *E. hirae*. These findings are in accordance with results obtained in dogs in Portugal (Rodrigues et al., 2002; Poeta et al., 2006), although other studies performed in Denmark, the United States or Japan found *E. faecalis* as the most predominant enterococcal species in dogs (Damborg et al., 2008; Jackson et al., 2009; Kataoka et al., 2014). The number of animals included in the present study is limited, and more extensive studies should be performed in the future.

The genetic diversity of the 31 *E. faecium* isolates recovered in this study from dogs and cats (the predominant species) was analysed by PFGE, using the SmaI restriction enzyme. Macrorestriction analysis by PFGE revealed a genomic diversity of the *E. faecium* under study, although there were four strains clonally related showing a similarity > 85% and differentiated in two PFGE groups, each consisting of two strains isolated from dogs or cats (Fig. 1).

Table 1 shows the number of antimicrobial-resistant strains detected in our series of enterococcal isolates according to their origin. A large number of the 58 tested strains showed resistance to erythromycin (n = 31) and tetracycline (n = 25). These antibiotics are used in dogs and cats for the treatment of a variety of infections, what could act as a selective pressure for this resistance phenotype (Jackson et al., 2009). Most of our erythromycin-resistant enterococci carried the *erm*(B) gene (22 of 31 isolates), which is in agreement with the findings of other studies, as *erm*(B) appears to be widespread among enterococci of animal origin (Jackson et al., 2010; Klibi et al., 2014). The erythromycin-resistant genes *erm*(A), *erm*(C), *mef*A/E and *msr*A were not detected in our erythromycin-resistant isolates.

The resistance to tetracycline was entirely explained by the presence of *tet*(M) gene in five isolates, by the combination of the *tet*(M) and *tet*(L) genes in 16 isolates and by the detection of *tet*(L) in four isolates. The *tet*(M) gene was

the most frequent genetic determinant found in tetracycline-resistant enterococci by other authors (Jackson et al., 2010) and ourselves (Klibi et al., 2014).

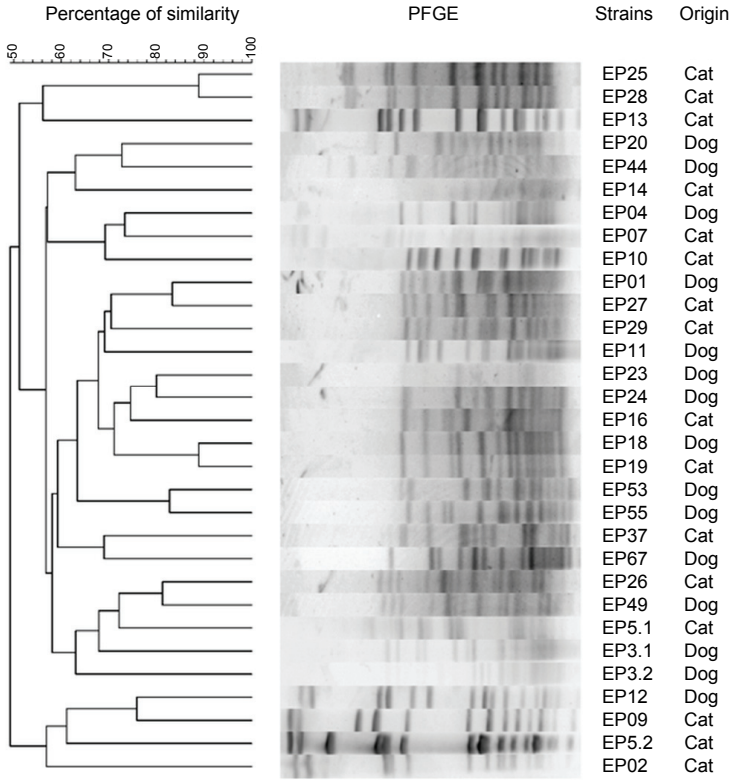

Fig. 1. Dendrogram based on SmaI-PFGE patterns. The GelCompar software (Applied Maths, Kortrijk, Belgium) was used to register macrorestriction patterns, and clustering analysis was performed using Dice similarity coefficient and the unweighted-pair group method with arithmetic mean (UPGMA) (tolerance 1% and optimisation 1%)

Ampicillin resistance was detected in one *E. faecium* isolate recovered from a dog. This resistance in enterococci might be attributed either to β-lactamase production or more probably to an increased production of penicillin-binding protein PBP5 (Poeta et al., 2007).

High-level resistance to streptomycin was ensured by the *ant*(6)-Ia gene in 11 strains. High-level resistance to gentamicin is conferred by the *aac*(6')-Ie-*aph*(2'')-Ia gene in 5 of 10 resistant strains, and high-level resistance to kanamycin by *aph*(3')-IIIa and/or *aac*(6')-Ie-*aph*(2'')-Ia genes in 11 of 15 resistant strains. These results correlate with the findings of similar studies in other regions in the world (Poeta et al., 2006; Jackson et al., 2010).

Table 1

Number of isolates showing antimicrobial resistance phenotypes in a series of 58 *Enterococcus* isolates from pets (dogs and cats)

Antimicrobial agent	Number of antimicrobial-resistant enterococci from pets						
	Dogs (n = 39)			Cats (n = 19)		Total (n = 58)	
	E. faecium (n = 16)	*E. faecalis* (n = 10)	Others (n = 13)	*E. faecium* (n = 15)	*E. faecalis* (n = 4)	Dogs (n = 39)	Cats (n = 19)
AMP	1	0	0	0	0	1	0
ERY	9	8	5	8	1	22	9
CIP	12	8	5	8	2	25	10
PT	3	4	2	1	1	9	2
STR	3	4	1	4	1	8	5
KAN	3	4	3	4	1	10	5
GEN	0	3	1	4	2	4	6
TET	5	6	4	7	3	15	10
CHL	2	1	1	2	2	4	4
VAN	0	0	5[c]	0	0	5	0
TEC	0	0	0	0	0	0	0
LZ	0	3[b]	0	0	3[d]	3	3

[a]AMP: ampicillin, ERY: erythromycin, CIP: ciprofloxacin, PT: pristinamycin, GEN: gentamicin, KAN: kanamycin, STR: streptomycin, TET: tetracycline, CHL: chloramphenicol, VAN: vancomycin, TEC: teicoplanin, LZ: linezolid; [b]one strain with linezolid MIC of 8 µg/ml and two isolates with linezolid MIC of 4 µg/ml; [c]intrinsic resistance to vancomycin: *E. casseliflavus* (n = 3) and *E. gallinarum* (n = 2); [d]three strains with linezolid MIC of 4 µg/ml

Eight strains showed resistance to chloramphenicol, but only one strain harboured the *cat*(A) gene. Most of the strains showed resistance to ciprofloxacin (35/58, 60%), this resistance being associated most frequently with *E. faecium*; this percentage of resistance is higher than the one previously reported by other authors (Poeta et al., 2006; Delgado et al., 2007; Kataoka et al., 2014; Iseppi et al., 2015). Four of eleven pristinamycin-resistant strains (two *E. faecium* and two *E. faecalis*) harboured the *vat*(E) while one *E. faecalis* strain contained the *vat*(D) gene.

Enterococcus gallinarum and *E. casseliflavus* isolates, with an intrinsic low-level vancomycin resistance and susceptibility to teicoplanin, were recovered from five of the healthy dogs included in this study. The detection of enterococci with this type of glycopeptide intrinsic resistance, located on the chromosome and not transferable to other bacteria, implicates a lower risk to public health. No acquired vancomycin resistance was identified among our isolates, which is similar to the findings of other studies (Damborg et al., 2008; Jackson et al., 2009; Kataoka et al., 2014; Iseppi et al., 2015). However, *van*A enterococci have been identified in dogs in a previous study in Spain (Torres et al., 2003). In

Tunisia, *van*A-mediated glycopeptide resistance has been sporadically reported in enterococci in the hospital setting (Abbassi et al., 2007; Elhani et al., 2014; Dziri et al., 2016), and also in birds (Klibi et al., 2015), but not in food-producing animals or healthy humans (Klibi et al., 2014; Ben Sallem et al., 2015).

It is of interest to mention the presence of five *E. faecalis* strains which showed an MIC for linezolid of 4 μg/ml (intermediate susceptibility according to CLSI human breakpoints, although in the susceptible category according to EUCAST, 2015), and one additional *E. faecalis* strain with a linezolid MIC of 8 μg/ml (resistant category in both CLSI 2015 and EUCAST 2015) (CLSI, 2015; EUCAST, 2015). Linezolid is an important antibiotic for the treatment of human infections by multidrug-resistant Gram-positive cocci. Our results indicate that enterococci with a low-level linezolid resistance might be emerging in Tunisia, after the drug has been licensed in human medicine and the surveillance of this resistance is required. Our sequence analysis results for the six *E. faecalis* isolates with linezolid MIC of 4–8 μg/ml were negative for the mutation at nucleotide position 2576 (G2576T) in domain V of the 23S rRNA. No other described mutations at positions 2503, 2504 and 2505 were found in these isolates (Patel et al., 2013). On the other hand, amino acid changes in ribosomal proteins L3 and L4, associated with decreased susceptibility to linezolid in staphylococci (Mendes et al., 2012), were not detected among our enterococci. Moreover, the *cfr* gene was also not detected in our enterococci with increased MICs for linezolid. Therefore, other mechanisms of resistance could be implicated in the low-level linezolid resistance phenotype of our strains. It has been reported that a decrease in antimicrobial uptake, due to alterations in the permeability of the bacterial membrane or overexpression of an efflux system, may be implicated in linezolid resistance (Sierra et al., 2009). Alterations in ribosomal protein L22 have also been associated with increased resistance to linezolid (Tian et al., 2014). These possibilities will be evaluated in the future in our isolates. Few reports have mentioned the susceptibility of enterococci to linezolid in dogs and cats (Delgado et al., 2007; Jackson et al., 2009; Ghosh et al., 2012).

Table 2 shows the different antimicrobial resistance phenotypes detected among our 58 enterococci from pets. In fact, multidrug resistance (MDR), defined as resistance to at least one agent in three or more antimicrobial categories, was observed in 8 strains (4 *E. faecium*, 2 *E. faecalis*, 1 *E. casseliflavus* and 1 *E. hirae*). This indicates that a small proportion of healthy pets could be carriers of antimicrobial-resistant enterococci. The transmission of MDR enterococci from companion animals might happen and the risk of this possibility should be assessed.

One of the main concerns about enterococci is the presence of virulence factors that may contribute to the severity of infections. Seventeen of our enterococcal isolates exhibited gelatinase activity and harboured the *gel*(E) gene. This gene was also detected in four gelatinase-negative isolates, which finding might be explained by a deletion in the *fsr* operon (Nakayama et al., 2002; Klibi et al.,

Table 2

Antimicrobial resistance patterns in enterococci isolated from dogs and cats

Pattern of antimicrobial resistance[a]	Species (no isolates/type of animal)[b]
LZ^I, GEN, KAN, STR, CHL, ERY, TET	*E. faecalis* (1/c)
LZ, GEN, KAN, ERY, TET, CIP	*E. faecalis* (1/d)
KAN, STR, CHL, ERY, TET, CIP, PT	*E. faecium* (1/d), *E. casseliflavus* (1/d)
GEN, KAN, STR, ERY, TET, CIP	*E. faecium* (1/c)
GEN, KAN, ERY, TET, CIP, PT	*E. hirae* (1/d)
KAN, STR, CHL, ERY, TET, PT	*E. faecium* (1/d)
KAN, STR, CHL, ERY, TET, CIP	*E. faecalis* (1/d)
LZ^I, GEN, CHL, TET, CIP	*E. faecalis* (1/c)
GEN, KAN, STR, ERY, PT	*E. faecium* (1/c)
KAN, STR, ERY, CIP, AMP	*E. faecium* (1/d)
STR, ERY, TET, CIP	*E. faecalis* (1/d)
LZ^I, GEN, KAN, STR, ERY	*E. faecalis* (1/d)
GEN, KAN, CHL, ERY	*E. faecium* (1/c)
GEN, KAN, STR, ERY, CIP	*E. faecium* (1/c)
GEN, KAN, STR, ERY, TET	*E. faecalis*(1/d)
STR, CHL, ERY, TET	*E. faecium* (1/c)
ERY, TET, CIP	*E. faecalis* (1/d)
LZ^I, ERY, TET	*E. faecalis* (1/d)
ERY, TET, CIP	*E. faecium* (2/d, 1/c)
ERY, CIP	*E. faecalis* (1/d)
LZ^I, TET	*E. faecalis* (1/c)
KAN, ERY	*E. durans* (1/d)
ERY, TET	*E. faecium* (1/c), *E. casseliflavus* (1/d)
ERY, CIP	*E. faecium* (3/d, 1/c), *E. durans* (1/d)
ERY, PT	*E. faecium* (1/d)
TET, CIP	*E. faecium* (1/d, 1/c), *E. durans* (1/d)
TET	*E. faecium* (2/c), *E. faecalis* (1)
CIP	*E. faecium*(6), *E. faecalis* (1/c), *E. durans* (3/d), *E. gallinarum* (1/d), *E. faecium* (1/c)

[a]LZ: linezolid, GEN: gentamicin, KAN: kanamycin, STR: streptomycin, CHL: chloramphenicol, ERY: erythromycin, TET: tetracycline, CIP: ciprofloxacin, PT: pristinamycin, AMP: ampicillin, LZ^I: intermediate resistance to linezolid. [b]Type of animal: dog (d), cat (c)

2007). The *esp* gene, that encodes an enterococcal surface protein associated with the ability to form biofilm onto abiotic surfaces (Klibi et al., 2007), was found in 6 *E. faecalis*, 3 *E. faecium* and *2 E. casseliflavus* strains. The *hyl* gene was absent in all the collection and this gene is located on a megaplasmid which is widely distributed among clinically associated *E. faecium* isolates, and seems to be restricted to this species (Laverde Gomez et al., 2010). Five *E. faecalis* isolates, which showed haemolytic activity, harboured the *cyl*A and *cyl*B genes. Haemolysin is a cytolytic protein capable of lysing human, horse and rabbit erythrocytes. Haemolysin-producing strains have been found to be associated

with increased severity of infections (Buma et al., 2006). The lack of apparent pathogenic potential of these isolates in healthy animals might be due to virulence being multifactorial and associated with different genes. Further studies are needed to investigate the pathogenic role of virulence factors in animals.

Enterococci are opportunistic organisms, and typically only cause clinical disease in patients when there is an underlying problem with local or systemic immunity, giving them the opportunity to thrive. It is possible that virulence factors would help cause disease in this type of situation, but not in healthy pets. We have very little evidence linking enterococcal virulence factors to disease in veterinary patients.

In conclusion, some pets in Tunisia may shed antimicrobial-resistant enterococci in their faeces, and more research is required to investigate the possibility of household contamination and risk of transmission to other household members. In addition, the close contact between pets and their owners highlights the importance of performing regular epidemiological surveillance in this setting.

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References

Abbassi, M. S., Znazen, A., Mahjoubi, F., Hammami, A. and Ben Hassen, A. (2007): Emergence of vancomycin-resistant *Enterococcus faecium* in Sfax: clinical features and molecular typing. Med. Mal. Infect. **37,** 240–241.

Ben Sallem, R., Klibi, N., Klibi, A., Ben Said, L., Dziri, R., Boudabous, A., Torres, C. and Ben Slama, K. (2016): Antibiotic resistance and virulence of enterococci isolates from healthy humans in Tunisia. Ann. Microbiol. **66,** 717–725.

Buma, R., Maeda, T., Kamei, M. and Kourai, H. (2006): Pathogenic bacteria carried by companion animals and their susceptibility to antibacterial agents. Biocontrol Sci. **11,** 1–9.

Byappanahalli, M. N., Nevers, M. B., Korajkic, A., Staley, Z. R. and Harwood, V. J. (2012): Enterococci in the environment. Microbiol. Mol. Biol. Rev. **76,** 685–706.

CLSI (2015): Performance Standards for Antimicrobial Susceptibility Testing. Twenty-fifth Informational Supplement. CLSI document M100-S25.

Da Costa, P. M., Loureiro, L. and Matos, A. J. (2013): Transfer of multidrug-resistant bacteria between intermingled ecological niches: the interface between humans, animals and the environment. Int. J. Environ. Res. Public. Health **10,** 278–294.

Damborg, P., Sørensen, A. H. and Guardabassi, L. (2008): Monitoring of antimicrobial resistance in healthy dogs: first report of canine ampicillin-resistant *Enterococcus faecium* clonal complex 17. Vet. Microbiol. **132,** 190–196.

Delgado, M., Neto, I., Correia, J. H. and Pomba, C. (2007): Antimicrobial resistance and evaluation of susceptibility testing among pathogenic enterococci isolated from dogs and cats. Int. J. Antimicrob. Agents **30,** 98–100.

Dziri, R., Lozano, C., Ben Said, L., Bellaaj, R., Boudabous, A., Ben Slama, K., Torres, C. and Klibi, N. (2016): Multidrug-resistant enterococci in the hospital environment: detection of novel vancomycin-resistant *E. faecium* clone ST910. J. Infect. Dev. Ctries **10,** 799–806.

Elhani, D., Klibi, N., Dziri, R., Ben Hassan, M., Asli, M. S., Ben Said, L., Mahjoub, A., Ben Slama, K., Jemli, B., Bellaj, R., Barguellil, F. and Torres, C. (2014): *van*A-containing *E. faecium* isolates of clonal complex CC17 in clinical and environmental samples in a Tunisian hospital. Diagn. Microbiol. Infect. Dis. **79,** 60–63.

EUCAST (2015): European Committee on Antimicrobial Susceptibility Testing. 2015. 289 Edition V 1.0. 130 pages.

Fisher, K. and Phillips, C. (2009): The ecology, epidemiology and virulence of *Enterococcus*. Microbiology **155,** 1749–1757.

Ghosh, A., KuKanich, K., Brown, C. E. and Zurek, L. (2012): Resident cats in small animal veterinary hospitals carry multi-drug resistant enterococci and are likely involved in cross-contamination of the hospital environment. Front. Microbiol. **3,** 62.

Gilmore, M. S., Lebreton, F. and Van Schaik, W. (2013): Genomic transition of enterococci from gut commensals to leading causes of multidrug-resistant hospital infection in the antibiotic era. Curr. Opin. Microbiol. **16,** 10–16.

Guardabassi, L., Schwarz, S. and Lloyd, D. H. (2004): Pet animals as reservoirs of antimicrobial-resistant bacteria. J. Antimicrob. Chemother. **54,** 321–332.

Iseppi, R., Messi, P., Anacarso, I., Bondi, M., Sabia, C., Condò, C. and de Niederhausern, S. (2015): Antimicrobial resistance and virulence traits in *Enterococcus* strains isolated from dogs and cats. New Microbiol. **38,** 369–378.

Jackson, C. R., Fedorka-Cray, P. J., Davis, J. A., Barrett, J. B. and Frye, J. G. (2009): Prevalence, species distribution and antimicrobial resistance of enterococci isolated from dogs and cats in the United States. J. Appl. Microbiol. **107,** 1269–1278.

Jackson, C. R., Fedorka-Cray, P. J., Davis, J. A., Barrett, J. B., Brousse, J. H., Gustafson, J. and Kucher, M. (2010): Mechanisms of antimicrobial resistance and genetic relatedness among enterococci isolated from dogs and cats in the United States. J. Appl. Microbiol. **108,** 2171–2179.

Kataoka, Y., Umino, Y., Ochi, H., Harada, K. and Sawada, T. (2014): Antimicrobial susceptibility of enterococcal species isolated from antibiotic-treated dogs and cats. J. Vet. Med. Sci. **76,** 1399–1402.

Kayaoglu, G. and Ørstavik, D. (2004): Virulence factors of *Enterococcus faecalis*: Relationship to endodontic disease. Crit. Rev. Oral. Biol. Med. **15,** 308–320.

Klibi, N., Aouini, R., Borgo, F., Ben Said, L., Ferrario, C., Dziri, R., Boudabous, A., Torres, C. and Ben Slama, K. (2014): Antibiotic resistance and virulence of faecal enterococci isolated from food-producing animals in Tunisia. Ann. Microbiol. **65,** 695–702.

Klibi, N., Ben Amor, I., Rahmouni, M., Dziri, R., Gtari, D., Ben Said, L., Lozano, C., Boudabous, A., Ben Slama, K., Mansouri, R. and Torres, C. (2015): Diversity of species and antibiotic resistance among fecal enterococci from wild birds in Tunisia. Detection of *van*A-containing *Enterococcus faecium* isolates. Eur. J. Wild. Res. **61,** 319–323.

Klibi, N., Ben Slama, K., Sáenz, Y., Masmoudi, A., Zanetti, S., Sechi, L. A., Boudabous, A. and Torres, C. (2007): Detection of virulence factors in high-level gentamicin-resistant *Enterococcus faecalis* and *Enterococcus faecium* isolates from a Tunisian hospital. Can. J. Microbiol. **53,** 372–379.

Klibi, N., Said, L. B., Jouini, A., Slama, K. B., López, M., Sallem, R. B., Boudabous, A. and Torres, C. (2013): Species distribution, antibiotic resistance and virulence traits in enterococci from meat in Tunisia. Meat Sci. **93,** 675–680.

Koch, S., Hufnagel, M., Theilacker, C. and Huebner, J. (2004): Enterococcal infections: Host response, therapeutic, and prophylactic possibilities. Vaccine **22,** 822–830.

Laverde Gomez, J. A., Van Schaik, W., Freitas, A. R., Coque, T. M., Weaver, K. E., Francia, M. V., Witte, W. and Werner, G. (2010): Multiresistance megaplasmid pLG1 bearing a hyl_{Efm}

genomic island in hospital *Enterococcus faecium* isolates. Int. J. Med. Microbiol. **301,** 165–175.

Leite-Martins, L., Mahú, M. I., Costa, A. L., Bessa, L. J., Vaz-Pires, P., Loureiro, L., Niza-Ribeiro, J., de Matos, A. J. and Martins da Costa, P. (2015): Prevalence of antimicrobial resistance in faecal enterococci from vet-visiting pets and assessment of risk factors.Vet. Rec. **176,** 674.

Leite-Martins, L., Meireles, D., Bessa, L. J., Mendes, A., de Matos, A. J. and da Costa, P. M. (2014): Spread of multidrug-resistant *Enterococcus faecalis* within household setting. Microb. Drug. Resist. **20,** 501–507.

Lloyd, D. H. (2007): Reservoirs of antimicrobial resistance in pet animals. Clin. Infect. Dis. **45 (Suppl. 2: S1),** S148–S152.

Mendes, R. E., Deshpande, L. M., Costello, A. J. and Farrell, D. J. (2012): Molecular epidemiology of *Staphylococcus epidermidis* clinical isolates from U.S. hospitals. Antimicrob. Agents Chemother. **56,** 4656–4661.

Nakayama, J., Kariyama, R. and Kumon, H. (2002): Description of a 23.9-kilobase chromosomal deletion containing a region encoding *fsr* genes which mainly determines the gelatinase-negative phenotype of clinical isolates of *Enterococcus faecalis* in urine. Appl. Environ. Microbiol. **68,** 3152–3155.

Patel, S. N., Memari, N., Shahinas, D., Toye, B., Jamieson, F. B. and Farrell, D. J. (2013): Linezolid in *Enterococcus faecium* isolated in Ontario, Canada. Diagn. Microbiol. Infect. Dis. **77,** 350–353.

Poeta, P., Costa, D., Igrejas, G., Sáenz, Y., Zarazaga, M., Rodrigues, J. and Torres, C. (2007): Polymorphisms of the *pbp5* gene and correlation with ampicillin resistance in *Enterococcus faecium* isolates of animal origin. J. Med. Microbiol. **56,** 236–240.

Poeta, P., Costa, D., Rodrigues, J. and Torres, C. (2006): Antimicrobial resistance and the mechanisms implicated in faecal enterococci from healthy humans, poultry and pets in Portugal. Inter. J. Antimicrob. Agents **27,** 131–137.

Rodrigues, J., Poeta, P., Martins, A. and Costa, D. (2002): The importance of pets as reservoirs of resistant *Enterococcus* strains, with special reference to vancomycin. J. Vet. Med. B Infect. Dis. Vet. Public Health **49,** 278–280.

Shepard, B. D. and Gilmore, M. S. (2002): Differential expression of virulence-related genes in *Enterococcus faecalis* in response to biological cues in serum and urine. Infect. Immun. **70,** 4344–4352.

Sierra, J. M., Ortega, M., Tarrago, C., Albet, C., Vila, J., Terencio, J. and Guglietta, A. (2009): Decreased linezolid uptake in an *in vitro*-selected linezolid-resistant *Staphylococcus epidermidis* mutant. J. Antimicrob. Chemother. **64,** 990–992.

Silva, N., Igrejas, G., Gonçalves, A. and Poeta, P. (2012): Commensal gut bacteria: distribution of *Enterococcus* species and prevalence of *E. coli* phylogenetic groups in animals and humans in Portugal. Ann. Microbiol. **62,** 449–459.

Tian, Y., Li, T., Zhu, Y., Wang, B., Zou, X. and Li, M. (2014): Mechanisms of linezolid resistance in staphylococci and enterococci isolated from two teaching hospitals in Shanghai, China. BMC Microbiol. **14,** 292.

Torres, C., Tenorio, C., Portillo, A., García, M., Martínez, C., Del Campo, R., Ruiz-Larrea, F. and Zarazaga, M. (2003): Intestinal colonization by *van*A or *van*B2- containing enterococcal isolates of healthy animals in Spain. Microb. Drug Resist. **9,** 47–52.

Turabelidze, D., Kotetishvili, M., Kreger, A., Morris, J. G. and Sulakvelidze, A. (2000): Improved pulsed-field gel electrophoresis for typing vancomycin-resistant enterococci. J. Clin. Microbiol. **38,** 4242–4245.