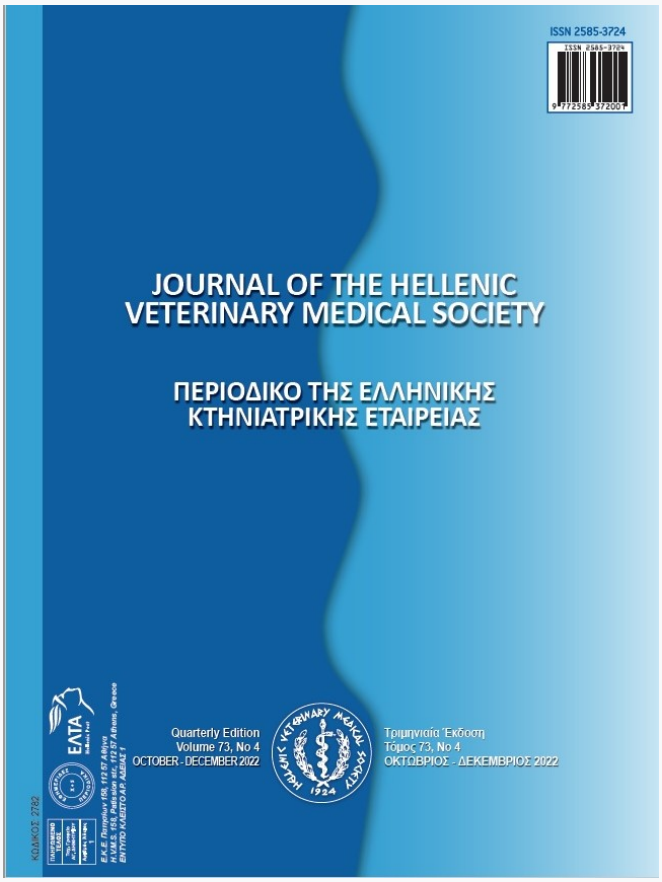
Journal of the Hellenic Veterinary Medical Society

Vol 73, No 4 (2022)



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doi: 10.12681/jhvms.27783

To cite this article:

Anyanwu, M., Eze, U., Nnamani, J., Ikenna-Ezeh, N., Akpa, P., Okorie-kanu, O., & Anene, B. (2023). Occurrence, antibiogram, high-level vancomycin and aminoglycosides resistance and potential virulence factors of enterococci in dogs in Nigeria. *Journal of the Hellenic Veterinary Medical Society*, *73*(4), 4689–4696. https://doi.org/10.12681/jhvms.27783

J HELLENIC VET MED SOC 2022, 73 (4): 4689-4696
ΠΕΚΕ 2022, 73 (4): 4689-4696

Research article
Ερευνητικό άρθρο

Occurrence, antibiogram, high-level vancomycin and aminoglycoside resistance and potential virulence factors of enterococci in dogs in Nigeria

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ABSTRACT: This study was conducted to isolate enterococci from dogs in Nigeria, and to determine the potential virulence, antibiotic susceptibility, phenotypic vancomycin (VAN), high-level ampicillin (AMP) and aminoglycoside susceptibility profile of the isolates. Rectal swabs were collected from 295 randomly-selected, clinically-healthy dogs. The isolation of enterococci was done using Slanetz and Bartley enterococcal selective medium. The resistance of 150 non-repetitive isolates was determined using disc diffusion method. VAN resistance was assessed by high-level disc diffusion and agar-screening methods. High-level AMP and aminoglycoside (gentamicin and streptomycin) resistance was determined by agar-screening method. Potential virulence factors were assayed using phenotypic methods. Out of 295 samples, 234 (80.7%) gave positive growth. From these, 250 enterococcal isolates comprised 229 (91.6%) non-pigmented and 21 (8.4%) pigmented strains, were obtained. Resistance of the isolates was 89% to erythromycin, 92% to rifampicin, 77% to chloramphenicol, 83% to tetracycline, 64% to ciprofloxacin, 32.7% to VAN, 24.7% to high-level streptomycin (HLS) and 6% to high-level gentamicin (HLG). Among 150 non-repetitive resistant isolates, 144 (96%), including all the VAN-, HLS- and HLG-resistant strains, exhibited resistance to at least 3 classes of antibiotics. Of these 150 isolates, 94 (62.7%), including all the VAN-, HLS- and HLG-resistant strains, displayed virulence potentials as biofilm (44.7%), surface-layer (13.8%), haemolysin (21.3%), gelatinase (40.4%), caesinase (10.6%) and deoxyribonuclease (12.8%) activities. This study showed that dogs in Nigeria are potential reservoirs and disseminators of potentially-virulent, multiple drug-resistant-, VAN- and high-level aminoglycoside-resistant enterococci.

Keywords: aminoglycoside resistance, canine, enterococci, vancomycin resistance, virulence.

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Date of initial submission: 09-08-2021
Date of acceptance: 04-03-2022

INTRODUCTION

Enterococci are normal inhabitants in the gut of humans and animals, including dogs (Osman et al., 2019; Oguttu et al., 2021). They are released in large quantities in faeces thus they profoundly contaminate environmental ecosystems (soil and water), food (animal/fermented food and plants) and human/animal skin (Pillay et al., 2018; Osman et al., 2019). Enterococci is established as one of the principal causes of multidrug-resistant nosocomial (human and veterinary) and community-associated infections (Beshiru et al., 2017). In dogs, enterococci have been associated with diarrhoea, urinary tract and skin/wound infections, otitis externa, cholangiohepatitis, pancreatitis, hepatic abscesses, peritonitis, endocarditis, and discospondylitis (Bertelloni et al., 2017). The capacity of enterococci to cause these infections is due to their virulence attributes (such as biofilm formation, production of cytolysin, proteases, gelatinase, lipases, deoxyribonucleases, and so on), tolerance to harsh environmental conditions, and a high rate of acquisition/transfer of resistance genes even from/to unrelated bacteria such as staphylococci and *Listeria* (Leite-Martins et al., 2015; Oguttu et al., 2021). The virulence factors (VFs) are essential for establishment of infection, invasion of host and persistence in colonized niche (Pillay et al., 2018). Studies have shown that *Enterococcus* that possessed and expressed virulence factors cause a more serious problem than strains that lack virulence factors (Chajecka- Wierzchowska et al. 2017; Pillay et al., 2018). Phenotypic assessment of VFs is important since the presence of a virulence-associated gene does not imply expression, especially where more than one gene is required for a VF to be expressed (Pillay et al., 2018). Although enterococci are naturally-resistant to low-levels of important antibiotics used in treating associated infections such as β-lactams (particularly ampicillin), last-resort glycopeptide vancomycin (VAN) and aminoglycoside (streptomycin and gentamicin), resistance to high-levels of these antibiotics jeopardizes therapy potentially complicating the treatment of enterococcal infections in humans and animals (Oguttu et al., 2021). This results in increased health cost, morbidity and mortality due to limited therapeutic options (Leite-Martins et al., 2015; Anyanwu et al., 2019). Unfortunately, the prevalence of VAN-resistant, high-level β-lactam- and aminoglycoside-resistant enterococci in human and veterinary settings are rising globally (Wada et al., 2020; 2021). Hence, the World Health Organization recommended increased surveillance of enterococci, especially the vancomycin-resistant and high-level β-lactam- and aminoglycoside-resistant strains (highest-priority pathogens) (WHO, 2017).

Inappropriate use of antibiotics is a known risk factor for antibiotic resistance emergence (Leite-Martins et al., 2015). Nigeria has an estimated dog population of 23 million https://rabiesalliance.org/resource/nigeria-animal-health-sectorgarc-wap-workshop-country-progress-update-2019. There are currently no enforced regulations on the use of antimicrobials, including last-resort ones like VAN in management of canine diseases in Nigeria, and often dog owners treat dogs with antibiotics without the supervision of a veterinarian. Even the veterinarians themselves rarely treat animals based on the result of antibiotic susceptibility testing. Most Nigerian households keep dogs as pets, guard and/or as hunting dogs (Anyanwu et al., 2017). And a sizeable population of dogs in Nigeria is stray dogs and some Nigerians consume dog meat unhygienically processed, as a source of animal protein (Anyanwu et al., 2017; Eze et al., 2009). It is established that the closeness between humans and their dogs provides opportunities for the exchange of potentially pathogenic and resistant organisms (Wada et al., 2021). Thus, presence in the gut/faecal discharge of pathogenic and resistant (especially VAN-, high-level β-lactam and aminoglycosides-resistant strains) enterococci by dogs in Nigeria, is a putative risk of infection of the public especially dog handlers (such as caretakers/owners, veterinarians, dog meat processors) and handlers/consumers of raw/half-cooked dog meat, and environmental contamination.

Therefore, monitoring antibiotic-resistant (especially those resistant to last-resort drugs) and pathogenic bacteria in dogs in Nigeria is important for public health and veterinary medicine. Data from such surveillance is useful for guiding the use of antibiotics in clinical canine medicine and evaluation of trends in devising mitigation strategies for curbing the spread of multidrug-resistant pathogens. Healthy dogs have been screened as potential reservoirs/disseminators of antimicrobial-resistant/pathogenic enterococci in Europe (Devriese et al., 1996; De Graef et al., 2004; De Leener et al., 2005; Turkyilmaz et al., 2010; Aasmäe et al., 2015; Bertelloni et al., 2017; Wada et al., 2021), Asia (Zhao et al., 2012; Kataoka et al., 2013; Wu et al., 2019; Wada et al., 2021), USA (Jackson et al., 2009) and Africa (Ben Said et al., 2017; Pillay et al., 2018; Oguttu et al., 2021). In Nigeria, chickens (Amaechi

et al., 2015; Ayeni et al., 2016; Ngbede et al., 2016; Ngbede et al., 2017), cattle (Anyanwu and Obetta, 2015; Ngbede et al., 2017a; Ngbede et al., 2017b), horses (Anyanwu et al., 2019) and pigs (Amaechi et al., 2015; Beshiru et al., 2017) have been screened as potential reservoirs of antimicrobial-resistant enterococci. In the same country, VAN-resistant enterococci (VRE) has been isolated from poultry (Ayeni et al., 2016) and horses (Anyanwu et al., 2019); high-level ampicillin- and aminoglycoside-resistant enterococci were recovered from poultry and cattle (Ngbede et al., 2016, Ngbede et al., 2017), and VFs have been detected in enterococcal isolates from pigs, poultry and cattle (Ngbede et al., 2016; Beshiru et al., 2017). No study has been conducted to assess dogs in Nigeria as potential reservoirs and disseminators of pathogenic and/or antimicrobial-resistant enterococci. The objectives of this study were, therefore, to determine the occurrence of enterococci in dogs in Enugu State Southeast Nigeria, and assess the antibiotic susceptibility profile, phenotypic VAN and high-level ampicillin and aminoglycoside resistance, and potential virulence factors of the isolates.

MATERIALS AND METHODS

Study area

Enugu is the capital of Enugu State Southeastern Nigeria. It comprises three Local Government Areas (L. G. As) namely: Enugu South, Enugu North and Enugu East. Nsukka Agricultural Zone (NAZ) is made up of Nsukka metropolis (a L. G. A on its own), which is a University town with Veterinary Teaching Hospital and other towns (Orba and Obollo-Afor, and Ibagwa, and Enugu-Ezike) in Udenu and Igbo-Eze North L. G. As. Enugu and NAZ are geographically located at coordinates approximately 6°27'9.60''N 7°30'37.20''E and 6°51'24''N 7°23'45''E, respectively. Enugu and NAZ are the two most populated areas in Enugu State with a sizeable dog population.

Sampling

Clinically-healthy dogs of both sexes, varying ages and breeds sold at markets, kept in households and/or visited the veterinary hospitals between February and August 2019, were sampled. Two hundred and ninety dogs were selected using random sampling technique. Non-duplicate rectal swab was collected from each of the dog using a sterile swab stick and distinguishing body marks were noted to avoid re-sampling. The samples were transported aseptically with ice packs and processed on the day of collection in the Microbiology Laboratory, Department of Veterinary Medicine, University of Nigeria, Nsukka.

Isolation and generic identification of enterococci from dogs

The swabs were inoculated into tryptone soy broth with 6.5% salt and incubated at 37°C for 48 h in ambient air. A loopful of the broth cultures was sub-cultured by streaking onto Slanetz and Bartley agar and then incubated at 37°C for 48 h for selective isolation of enterococci. Suspected enterococcal colonies (reddish, pinkish or maroon-coloured tiny colonies) were noted and recorded appropriately. One colony of distinct morphotype per sample was picked and purified by sub-culturing on nutrient agar plates and incubated at 37°C for 48 hours. Pure cultures of the isolates were then inoculated onto nutrient agar slants, incubated at 37°C for 48 hours and stored in refrigerator at 4°C as stock cultures until needed for further analysis. Phenotypic characterization of the isolates was done by subjecting them to various tests such as Gram staining, catalase, bile esculin, haemolysis, motility, mannitol fermentation, pigmentation, growth in 6.5% salt, growth at 10°C, 45°C, and tolerance of 60°C for 30 min following standard methods (Manero and Blanch, 1999; Vu et al., 2012).

Antibiotic susceptibility testing (AST) of enterococcal isolates from dogs

Antibacterial susceptibility profiles of 150 non-repetitive (one isolate per dog) enterococcal isolates was determined by disc diffusion and agar dilution methods (CLSI, 2020), using 9 antibiotics in 8 classes: macrolides - erythromycin (ERY, 15 µg), ansamycins - rifampicin (RIF, 5 µg), tetracycline (TET, 30 µg), phenicols - chloramphenicol (CHL, 30 µg), glycopeptides - vancomycin (VAN, 30 µg), and fluoroquinolones - ciprofloxacin (CIP, 5µg). High-level aminoglycoside (high-level gentamicin [HLG] and high-level streptomycin [HLS]) resistance was determined using gentamicin (GEN; 500 µg/mL) and streptomycin (STR; 2000 µg/mL) agar screening/dilution method. High-level ampicillin (AMP, 16 µg/mL) resistance was also assessed by agar-screening method (CLSI, 2020). *Enterococcus faecalis* ATCC 29212 (American Type Culture Collection, USA) was used as the reference strain. Results of the susceptibility testing were interpreted according to the CLSI (2020) criteria for enterococcal isolates. Isolate was classified as multidrug-resistant (MDR) when it is non-susceptible to at least one agent in ≥ 3 antibiotic

classes/categories (Magiorakios et al., 2012).

Confirmation of phenotypic high-level vancomycin resistance

Phenotypic HLV resistance of 49 isolates resistant to VAN in disc test was determined using VAN (6 µg/mL) agar-screening method (CLSI, 2020). *Enterococcus faecalis* ATCC 29212 was also used as the reference strain. Results of the susceptibility testing were interpreted according to the CLSI (2020) criteria for enterococcal isolates.

Phenotypic detection of virulence factors

All the 150 isolates subjected to AST underwent testing for potential virulence factors.

Cytolysin (haemolysin production) activity

Blood agar (tryptic soy agar with 5% v/v sheep blood) was inoculated with the isolates and incubated at 37°C for 24 h in ambient air as per Osman et al. (2019). Clear transparent zone (β-hemolysis) or greenish zone (partial or α-haemolysis) surrounding the colonies indicated cytolytic activity. The positive control used was *Staphylococcus aureus*ATCC 25923.

Proteinase (gelatinase) activity

Isolates were inoculated on gelatin agar (tryptic soy agar with 3% w/v gelatin) and incubated at 37°C for 48 h in ambient air (Igbinosa and Beshiru, 2019). The presence of transparent halo surrounding the colonies following the flooding of the agar with Frazier solution (mercuric chloride 15g, hydrochloric acid 37%, 20mL distilled water, 100mL) indicated the gelatinase production. *Staphylococcus aureus*ATCC 25923 was used as the positive control.

Protease activity (casein hydrolysis)

The isolates were spotted on skimmed milk agar (tryptic soy agar with 3% w/v skimmed milk) and incubated at 37°C for 24 h (Issepi et al., 2015). The presence of clear transparent zone surrounding the growth on flooding of the plate with Frazier solution indicated protease (caseinase) production. *Enterococcus faecalis*ATCC 29212 served as the positive control.

Congo red agar biofilm assay

The isolates were inoculated on Congo red agar (tryptic soy agar with 0.8 g/L of Congo red dye and 50 g/L of sucrose) and incubated at 37°C for 24-48 h aerobically and microaerobically (Solati et al., 2015). Isolates whose colonies were black with dry crystalline consistency were considered potential biofilm-producers whereas isolates with reddish or creamy colonies were considered non-biofilm-producers (Solati et al., 2015).

Surface layer assay

The presence of surface-layer (S- layer) was assessed following the method described by Igbinosa and Beshiru (2019). Isolates were repeatedly streaked on Coomassie brilliant blue agar (tryptic soy agar with 0.1 mg/mL Coomassie brilliant blue R 250 [Merck, Darmstadt Germany]) and incubated at 37°C for 24 h. Isolates with bluish colonies were considered S-layer-producers whereas those isolates whose colonies appeared whitish or creamy were considered non-S-layer-producers (Igbinosa and Beshiru, 2018).

Deoxyribonuclease (DNAse) activity assay

Ability of the isolates to degrade deoxyribonucleic acid was determined following the protocol described by Igbinosa and Beshiru (2019). Isolates were spot-inoculated on DNAse agar and incubated at 37°C for 24-48 h. The plates were flooded with Frazier solution and allowed to stand on the bench (with the petri-dish lids uppermost) for 5 minutes. Plates were examined in a dark background, and the presence of a clear zone surrounding isolate indicated DNA-degrading activity (Igbinosa and Beshiru, 2019).

Lipase activity assay

The ability of the isolates to break down lipids was determined as per Ramnath et al. (2017). Tween 20 agar (tryptic soy agar with 1% v/v tween 20 and 0.01% phenol red) and tween 80 agar (tryptic soy agar with 1% v/v tween 80 and 0.01% phenol red indicator) were spot-inoculated with the isolates and incubated at 37°C for 48 hours. An isolate with yellowish zone around it in the tween 20 agar was considered an esterase-producer while an isolate with yellowish zone surrounding the growth in tween 80 agar was regarded as a lipase-producer (Ramnath et al., 2017).

Data analysis

The frequencies of occurrence of enterococci, resistance of isolates to antibiotics and virulence factors were entered into Microsoft Excel TM (Microsoft Corp., Redmond, WA, USA). Data on the frequency of occurrence of enterococci and resistance were exported to SPSS v.15.0 (SPSS Inc., Chicago, IL, USA) and Graph- Pad Prism statistical package v.8.3.1

(GraphPad Software Inc., La Jolla, CA, USA) and the frequency, percentage and 95% Confidence Interval of variables were calculated as appropriate. The $\chi 2$ test was used to determine the possible association between variables (VAN and high-level aminoglycosides resistance) and expression of phenotypic virulence factors.

RESULTS

Occurrence of enterococci in dogs

Out of 290 rectal swabs cultured, 234 (80.7%, 95% CI 76.5 - 85.5) gave positive growth of enterococci. From these, 250 isolates were obtained. Twenty-one (8.4%) of the 250 isolates were pigmented suggesting they were *E. cassealiflavus*, *E. gallinarum*, *E. flavescence*, *E. canis* or any other pigmented *Enterococcus* species while 229 (91.6%) were non-pigmented (had creamy/whitish colonies) suggesting they were *E. faecalis* or *E. faecium*. Sixty-nine (30.1%) of the 229 non-pigmented isolates were mannitol-fermenters suggesting they were *E. faecalis*.

Antibiogram of enterococcal isolates from dogs

Of 150 isolates, 134 (89%, 95% CI 84.1 - 93.9) were resistant to ERY, 138 (92%, 95% CI 87.7 - 96) to RIF, 115 (77%, 95% CI 70.3 - 84) to CHL, 125 (83%, 95% CI 76 - 89) to TET, 96 (64%, 95% CI 56.3 - 71.7) to CIP, 49 (32.7%, 95% CI 25.2 - 40.2) to VAN, 37 (24.7%, 95% CI 17.8 - 31.6) to HLS and 9 (6%, 95% CI 2.2 - 10.4) to HLG (Table 1). Of the 150 isolates, 46 (30.7%) isolates exhibited high-level aminoglycoside resistance, 21 (14%) were simultaneously resistant to VAN and high-level aminoglycoside, while all isolates were susceptible to the high-level (16 μg/mL) ampicillin.

Among these 150 resistant isolates, 6 (4%) were resistant to 2 classes of antibiotic while 144 (96%), including all 49 VAN-resistant and 46 high-level aminoglycoside-resistant (HLAR) strains, were resistant to one antibiotic in 3 or more classes (MDR) (Table 2). The isolates exhibited 29 multiple resistance (resistance to 2 or more antibiotics) patterns with ERY,CIP,RIF,CHL,TET (n = 35) being the predominant.

Occurrence of phenotypic vancomycin-resistant enterococci in dogs

All 49 isolates that exhibited resistance to high-level (30 μg) VAN disc test were also VAN-resistant (MIC > 6 μg/mL) in agar screening.

Phenotypic virulence of enterococcal isolates from dogs

Of the 150 isolates, 94 (62.7%), including all the 49 VAN-, 37 HLS- and 9 HLG-resistant strains, phenotypically expressed virulence factors. Among these 94 isolates, 42 (44.7%) showed potential biofilm (bif) production, 38 (40.4%) displayed gelatinase (gel) activity, 20 (21.3%) showed β-haemolytic (β-haem) activity, 13 (13.8%) revealed surface layer (S-layer), 12 (12.8%) portrayed DNA-degrading activity while 10 (10.6%) displayed caseinase (cas) activity (Figure 1). No isolate produced lipase and none of the enterococci was simultaneously positive for the tested virulence factors. These 94 isolates expressed 17 potential virulence patterns with bif (n = 26) being the predominant pattern. (Table 3). Production of virulence factors was not significantly different (P > 0.05) between VAN-resistant/HLAR and nonresistant strains.

Table 1: Antibiotic susceptibility profile of enterococcal isolates from dogs

Antibiotic class	Antibiotic (concentration)	Number (Percentage) of isolates n = 150		
		Susceptible	Intermediately-susceptible	Resistant, 95% CI
Macrolides	Erythromycin (15 μg)	3 (2)	13 (9)	134 (89), 84.1 - 93.9
Ansamycins	Rifampicin (5 μg)	6 (4)	6 (4)	138 (92), 87.7 - 96
Phenicols	Chloramphenicol (30 μg)	35 (23)	0 (0)	115(77), 70.3 - 84
Tetracyclines	Tetracycline (30 μg)	19 (13)	6 (4)	125 (83), 76 - 89
Fluoroquinolones	Ciprofloxacin (5 μg)	9 (6)	45 (30)	96 (64), 56.3 - 71.7
Glycopeptides	Vancomcyin (30 μg)	95 (63.3)	6 (4)	49 (32.7), 25.2 - 40.2
β-lactam	Ampicillin (16 μg)	150 (100)	0 (0)	0 (0), 0.00 - 0.00
Aminoglycosides	Streptomycin (500 μg)	113 (75.3)	0 (0)	37 (24.7), 17.8 - 31.6
	Gentamicin (2000 μg)	141 (94)	0 (0)	9 (6), 2.2 - 10.4

CI: Confidence interval

Table 2: Antibiotic resistance patterns of 150 enterococcal isolates from dogs

Number of antibiotic classes	Resistance pattern (Number of isolates)	Total number of isolates (Percentage)
2	RIF,TET (3)	6 (4)
	ERY,RIF (3)	
3	ERY,CIP,CHL (3)	144 (96)
	VAN,RIF,TET (3)	
	VAN,TET,CHL (3)	
	ERY,CIP,RIF (3)	
	ERY,CHL,TET (6)	
	ERY,RIF,TET (3)	
	ERY,RIF,CHL (3)	
4	STR,ERY,CIP,RIF (3)	
	VAN,RIF,CHL,TET (3)	
	ERY,CIP,RIF,TET (7)	
	VAN,ERY,CIP,RIF (3)	
	ERY,RIF,CHL,TET (13)	
	STR,ERY,CHL,TET (3)	
	STR,ERY,RIF,TET (3)	
5	ERY,CIP,RIF,CHL,TET (35)	
	STR,ERY,CIP,RIF,CHL (3)	
	STR,VAN,ERY,CIP,RIF (3)	
	VAN,ERY,RIF,CHL,TET (3)	
6	VAN,ERY,CIP,RIF,CHL,TET (13)	
	STR,ERY,CIP,RIF,CHL,TET (7)	
	STR,VAN,ERY,RIF,TET,CHL (3)	
	STR,VAN,ERY,CIP,RIF,CHL (3)	
	STR,VAN,ERY,CIP,RIF,TET (3)	
	GEN,ERY,CIP,RIF,CHL,TET (3)	
7	STR,VAN,ERY,CIP,RIF,CHL,TET (3)	
	GEN,VAN,ERY,CIP,RIF,CHL,TET (3)	
	STR,GEN,VAN,ERY,CIP,RIF,CHL,TET (3)	

GEN: Gentamicin; VAN: Vancomycin; ERY: Erythromycin; TET: Tetracycline; RIF: Rifampicin; CHL: Chloramphenicol; STR: Streptomycin; CIP: Ciprofloxacin

Table 3: Phenotypic virulence pattern of enterococcal isolates from dogs

S/N	Phenotypic virulence pattern	Number of isolates *n* = 94	% Frequency
1.	gel, bif, β-haem	2	2.1
2	Bif	26	27.7
3.	gel, S-layer	5	5.3
4.	β-haem	13	13.8
5.	Gel	12	12.8
6.	gel, β-haem	2	2.1
7.	DNase	9	9.6
8.	gel, bif	8	8.5
9.	S-layer	5	5.3
10.	cas, bif	2	2.1
11.	cas, gel	2	2.1
12.	cas, gel, S-layer	2	2.1
13.	cas, gel, β-haem	2	2.1
14.	DNase, β-haem	1	1.1
15.	gel, DNase	1	1.1
16.	cas, DNase	1	1.1
17.	cas, gel, bif	1	1.1

cas: Caseinase; gel: Gelatinase; S-layer: Surface layer; bif: Biofilm; β-haem: β-haemolysin; DNase: Deoxyribonuclease

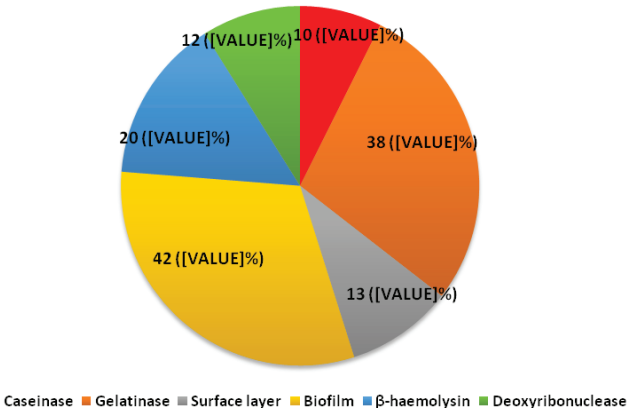

Figure 1: Distribution of potential virulence factors among 94 enterococcal isolates from dogs.

DISCUSSION

The fact that 234 (80.7%) of the 295 non-duplicate rectal swab samples cultured using Slanetz and Bartley agar gave positive growth, suggested that a high percentage (majority) of dogs in Nigeria is colonized by enterococci. This finding is not unexpected since enterococci is among the normal commensal microbiota in the intestine of dogs (Oguttu et al., 2021). However, the isolates could also be of exogenous origin in the sampled dogs. Possible exogenous sources of the isolates in the sampled dogs include contaminated environment since most dogs in the study area are allowed to stray and scavenge (even in refuse dump sites), formites, drinking water, commercial/domestic pet foods, and/or slaughterhouse waste products (internal organs and bones of animals used as source of protein for pets in Nigeria) fed to them. Enterococci tolerates harsh environmental conditions enabling them to survive for a long time in the environment (Oguttu et al., 2021), and it has been reported to be a common microbial contaminant in industrial dog foods (Finisterra et al., 2021). There is also a possibility that the dogs acquired organisms from licking their skin contaminated by their handlers/caretakers during grooming or petting and/or following the licking of their caretakers' skin/mouth (Ossiprandi and Zerbini, 2015; Pillay et al., 2018).

Although, identifying enterococcal species using phenotypic methods is difficult, non-pigmentation (whitish/creamy colonies) of the majority (229/250, 91.6%) of isolates in this study, suggested that they may likely be *E. faecalis* or *E. faecium* (Cartwright *et al*., 1995; Manero and Blanch, 1999). These two have been the commonest *Enterococcus* species isolated from dogs elsewhere (Ben Said et al., 2017; Miranda et al., 2021). Furthermore, mannitol-fermentation exhibited by 69 (30.1%) of the 229 non-pigmented isolates in this study, suggested that these mannitol-fermenters are *E. faecalis* (Vu et al., 2012). This finding suggests that *E. faecalis* constitute at least one-third of the enterococcal species colonizing dogs in the study area. Elsewhere, *E. faecalis* dominated among enterococcal species isolated from dogs (De Leener et al., 2005; Delgado et al., 2007; Jackson et al., 2009; Zhao et al., 2012; Kataoka et al., 2013; Miranda et al., 2021). But in some other places, *E. faecium* predominated among canine enterococcal isolates (Rodrigues et al., 2002; Boynukara et al., 2002; Turkyilmaz et al., 2013; Iseppi et al., 2015; Kirkan et al., 2019). Alternatively, 21 (8.4%) of 250 isolates in this study were pigmented (yellow-coloured colonies) and could be *E. casseali flavus*, *E. gallinarum*, *E. flavesence*, or *E. canis* since these are the commonest pigmented enterococcal species isolated from dogs elsewhere (Cinquepalmi et al., 2013; Ossiprandi and Zerbini, 2015; Miranda et al., 2021). Nonetheless, they could also be any other pigmented *Enterococcus* species belonging to the 5 branches/groups of enterococcal species uncommonly recovered from dogs (Torres et al., 2018; Miranda et al., 2021). The findings of different phenotypic characteristics in this work, imply that a diversity of *Enterococcus* species is colonizing dogs in Nigeria.

The 80.7% *Enterococcus* occurrence in this study is similar to 80% enterococcal occurrence in pooled nasal/oral/rectal swabs of 155 dogs in United States (Jackson et al., 2009). But it is higher than 16.7 - 77.3% enterococcal occurrence in faecal/rectal swabs of varying numbers of randomly-selected clinically-healthy dogs in Europe (De Graef et al., 2004; Cinquepalmi et al., 2013; Turkyilmaz et al., 2013; Aasmäe et al., 2015; Bertelloni et al., 2017), China (Wu et al., 2019) and Tunisia (Said et al., 2017). However, the 80.7% occurrence in this study is lesser than 100% enterococcal occurrence in faecal samples/rectal swabs of varying numbers of randomly/systematically-selected clinically-healthy dogs in Europe (Rodrigues et al., 2002; Iseppi et al., 2015; Ossiprandi and Zerbini, 2015; Leite-Martins et al., 2015), and Asia (Zhao et al., 2012; Kataoka et al., 2013). The differences in the results of these studies may be related to the method of sampling, isolation/processing, rate of infection of sampled animals and management of dogs in the study areas.

Detection of 49 (32.7%) VAN-, 37 (24.7%)

HLS- and 9 (6%) HLG-resistant isolates among 150 non-repetitive isolates indicated that these dogs are potential reservoirs of VAN- and high-level aminoglycosides-resistant enterococci. It also suggested that either the dogs already harboured the organisms or that the isolates acquired the genes encoding VAN and aminoglycosides resistance from other organisms in the gut. The sale and use of antibiotics (including last-resort ones like VAN) in humans and animals, including companion animals, are not controlled in Nigeria (Anyanwu et al., 2019). Thus, the findings also suggested that these dogs could have been treated with VAN and/or aminoglycosides during previous visitations to hospital or by their caretakers/owners and nonprofessionals without veterinary supervision. In Nigeria, aminoglycosides (STR and GEN) have been tremendously abused in human and veterinary settings, including clinical canine medicine (Anyanwu et al., 2017). In the country, STR was used in combination with penicillin as a broad spectrum for treating several infections in humans and animals (Anyanwu et al., 2017). Even so, VAN is commonly used in human medicine in Nigeria where the selective pressure could also have emerged (Anyanwu et al., 2019). Dogs that strayed/scavenged possibly acquired VAN-resistant/HLAR enterococci following coprophagic behaviour (Leite-Martis et al., 2015). This acquisition following coprophagic behavior is plausible since human open-air defecation and improper disposal of human/animal wastes/sewages in refuse dump sites is common in Nigeria. Furthermore, avoparcin (VAN analogue used for growth enhancement in livestock) has never been available/used in Nigeria, but slaughterhouse wastes such as internal organs, bones and blood meal from food animals are often fed to dogs in the country. These food animals have been reported to be reservoirs of VRE in Nigeria (Amaechi et al., 2015; Anyanwu and Obetta, 2015; Ayeni et al., 2016; Ngbede et al., 2017a). Therefore, acquisition of HLAR enterococci from contaminated environment, formites, drinking water and/or food by the dogs could be postulated.

Since VAN and aminoglycosides are critically-important antibiotics used in management of severe Gram-positive bacterial infections in humans and animals, detection of enterococci resistant to high-levels of these drugs in this study calls for serious concerns. Acquisition of these dangerous organisms could jeopardize therapy resulting in increased health cost, morbidity and mortality. These dogs potentially serve as reservoirs and by faecal shedding disseminates these organisms into the environment, thereby posing health threat to individuals (humans and animals) who get in contact with them. Persons (such as veterinarians, dog owners/caretakers/handlers and children) who often make contact with these dogs, could acquire these organisms following contact with faeces from the dogs, formites or environment contaminated by the organisms. Those that butcher dogs and consume dog meat are also at risk of acquiring these organisms because poor hygienic practices are employed during animal slaughter in Nigeria. Being an intestinal organism, enterococci easily transfer resistance genes to other bacteria by horizontal transfer (Torres et al., 2018). Consequently, colonized individuals could serve as disseminators of these dangerous organisms to the public.Leite-Martins et al. (2015) and Boynukara et al. (2002) detected 1 and 98.9% VAN resistance among enterococcal isolates from dogs using HLV disc method in Portugal and Italy, respectively, whereas Deverisie et al. (1996) detected 4 HLV-resistant isolates out of 49 enterococcal isolates from dogs in USA using VAN (20 µg/mL)-supplemented medium. These findings are lower than that (32.7%) in this study. In contrast to this current study, however, some authors did not observe HLV resistance among enterococcal isolates from healthy dogs (Zhao et al., 2012; Rodrigues et al., 2002; Delgado et al., 2007; Iseppi et al., 2015; Jackson et al., 2009; Kataoka et al., 2013; Said et al., 2017). It is worth noting that in Nigeria, Anyanwu et al. (2019) and Ayeni et al. (2017) detected 29.7 and 65% HLV resistance among 30 and 60 enterococcal isolates from horses and poultry using agar dilution and HLV disc method, respectively. Variations in these studies is related to differences in the number samples analyzed, method of phenotypic VAN resistance detection, concentration of VAN in the detection medium, previous hospitalization and health status of sampled animals, degree of contamination of animal's environment and colonization, and degree of usage of avoparcin/VAN in the study area (Anyanwu et al., 2019). In the hereby study, HLV resistance was confirmed using agar dilution/screening (with 6 µg/mL of VAN), which is the recommended method for detecting phenotypic HLV resistance rather than the disc method (CLSI, 2020). Nevertheless, without the inclusion of teicoplanin, agar dilution method cannot indicate VAN resistance genotype (CLSI, 2020).

The 24.7% HLS resistance in this study is higher than 4.5 - 20.5 % HLS resistance recorded among enterococcal isolates from healthy dogs in Europe (Po-

eta et al., 2006; Damborg et al., 2009; Cinquepalmi et al., 2013; Bertelloni et al., 2017), USA (Jackson et al., 2009), and Tunisia (Ben Said et al., 2017). But it is lower than 42.5% HLS resistance reported among enterococci of canine origin in USA (Ghosh et al., 2011). The 6% HLG resistance in this study is similar to 6.3% HLG resistance recorded by Leite-Martins et al. (2015) among enterococcal isolates from dogs in Portugal. But it is lower than 15.1 - 79% HLG resistance reported by other authors among enterococcal isolates from dogs in Europe (De Graef et al. 2004; Turkyilmaz et al., 2010; Cinquepalmi et al., 2013; Iseppi et al., 2015), China (Zhao et al., 2012), USA (Jackson et al., 2009; Ghosh et al., 2011) and Tunisia (Ben Said et al., 2017). The differences in the results of these studies could be related to variation in the use of aminoglycosides (in humans, dogs and other animal species) in the study areas.

The high rates of resistance observed against ERY (89%), RIF (92%), CHL (77%), TET (83%) and CIP (64%) in this study suggested selection pressure. Some of these resistances may be intrinsic (for example against CIP) or due to acquisition of genes encoding resistance to the agents following use-selection pressure. By horizontal gene transfer, enterococci rapidly acquire and transfer genes encoding resistance to most antibiotics (Torres et al., 2018). It was not possible to trace the medical history of the dogs because the owners/sellers of almost all of them do not have records. Be that as it may, previous treatment of the dogs with the antibiotics could be postulated. Other potential sources of the selection pressures are as aforementioned. The 89% ERY resistance in this study is higher than 18 - 81.7% ERY resistance recorded among enterococcal isolates from dogs by other investigators (Boynukara et al., 2002; Poeta et al., 2006; Damborg et al., 2009; Jackson et al., 2009; Turkyilmaz et al., 2010; Ghosh et al., 2011; Cinquepalmi et al., 2013; Leite-Martins et al., 2015; Iseppi et al., 2015; Ben Said et al., 2017). High ERY resistance in this study calls for concern because ERY is a macrolide which may be important as an alternative therapy for enterococcal infections (Iseppi et al., 2015). The 92% RIF resistance noted in this study is higher than 32 - 60.8% RIF resistance observed by other investigators (Damborg et al., 2009; Zhao et al., 2012; Iseppi et al.; 2015; Leite-Martins et al., 2015). The 77% CHL resistance in this study is higher than 1.4 - 61.8% CHL resistance noted among enterococcal isolates from dogs elsewhere (Poeta et al., 2006; Turkyilmaz et al., 2010; Cinquepalmi et al., 2013; Iseppi et al., 2015; Ben Said et al., 2017), but it is lesser than 85% CHL resistance reported by Jackson et al. (2009). The 83% TET resistance in this study is higher than 2.2 - 65.7% (De Graef et al. 2004; Poeta et al., 2006; Cinquepalmi et al., 2013; Leite-Martins et al., 2015; Ben Said et al., 2017), but it is lesser than 84.1 - 100% observed elsewhere (Damborg et al., 2009; Ghosh et al., 2011; Zhao et al., 2012; Iseppi et al., 2015; Bertelloni et al., 2017). The 64% CIP resistance in this study is similar to 64% CIP resistance recorded by Ben Said et al. (2017) in Tunisia. But it is higher than 7.8 - 25% reported by other authors (Damborg et al., 2009; Kataoka et al., 2013; Iseppi et al., 2015). However, Jackson et al. (2009) and Boynukara et al. (2002) reported 90 and 79.2% CIP resistance which are higher than 64% CIP resistance recorded in this study. Differences in resistance recorded in these studies may be attributed to the use of some of these antibiotics in management of dogs and other animal species as well as in humans in the study areas.

High multidrug resistance observed in this study could be related to the fact that a sizeable percentage of the isolates were VAN-resistant and HLAR strains known to exhibit resistance to drugs in other classes of antibiotics (Torres et al., 2018; Wada et al., 2020). This calls for concern because of the impact of multiple drug-resistant enterococci on antibiotic therapy and epidemiology of antibiotic resistance. These dogs are potential disseminators of multiple drug-resistant enterococci (reservoirs of diverse resistance genes) in the environment where they can persist thereby posing threat (compromise therapy in colonized/infected individuals) to public health (Anyanwu et al., 2019).

Enterococci is currently among the commonest causes of hospital (human/veterinary)-associated infections worldwide; therefore, the presence of VFs in enterococci is a major public health concern (Ben Said et al., 2017; Pillay et al., 2018). In this study, 94 (62.7%) of 150 isolates tested possessed virulence potentials having produced one or more of the tested virulence factors. Fourty-two (44.7%) isolates in this study were potential biofilm-producers. Ghosh et al. (2011) also observed biofilm production by enterococcal isolates from dogs. Biofilm production enables the organism to colonize the gut, survive harsh environmental conditions, causes chronic infection, evade the immune system and resist the effect of antibiotics and disinfectants (Ngbede et al. 2017a; Osman et al., 2019). Gelatinase is a metalloproteinase

that can cleave haemoglobin, fibrinogen, fibronectin, gelatin, collagen, laminin, and numerous peptides/proteins thereby promoting dissemination of the organism and it also helps in biofilm formation (Ngbede et al., 2017a; Oliveira et al., 2016; Igbinosa and Beshiru, 2019). Thirty-eight (40.4%) isolates in this study were gelatinase-producers. Previous studies reported 25 - 29.3% phenotypic gelatinase production among enterococcal isolates from dogs (Iseppi et al., 2015; Oliveira et al. 2016; Said et al., 2017). Cytolysin is a hemolytic toxin that destroys certain red blood cells, eukaryotic cell types, including macrophages and neutrophils and also possesses bacteriocin activity against a broad range of Gram-positive bacteria (Oliveira et al., 2016). In the present study, 21.3% β-hemolytic phenotype was detected. Oliveira et al. (2016) detected 35% β-hemolytic isolates while some other authors (Gulhan et al., 2006; Iseppi et al., 2015) did not observe haemolysis among enterococcal isolates from dogs. The S-layer is a surface protein that protects against bacteriophages and phagocytosis, confers resistance to low pH, serves as barrier against lytic enzymes and has adherence characteristics (Igbinosa and Beshiru, 2019). In the hereby experiment, 13 (13.8%) isolates revealed the presence of S-layer. Interestingly, the isolates that produced S-layer were negative for biofilm. This finding is consistent with other studies that reported negative correlation between S-layer and biofilm production (Igbinosa and Beshiru, 2019). Proteases are significant class of biomolecules that cleaves peptide bonds (Igbinosa and Beshiru, 2019). Ten (10.6%) isolates in this study displayed caseinase (protease) activity whereas Iseppi et al. (2015) reported 86.96% casein hydrolysis among enterococci of canine origin. DNA degrading activity was showed by a sizeable percentage (12.8%) of isolates in this study.

More worrisome is that 17 virulence patterns were exhibited by the isolates in this study, meaning they could express a diversity of VFs. Worse still, they were multiple drug-resistant isolatesindicating they could potentially cause difficult-to treat diseases in individuals infected by them.

CONCLUSIONS

This study has shown that multiple drug-resistant (96.1%), VAN-resistant (32.7%) and HLAR (30.7%) enterococci are harboured by a high percentage of dogs in Enugu State Southeastern Nigeria. These organisms possess virulence potentials including biofilm, surface-layer, gelatinase, proteases, haemolysin and deoxyribonuclease activities. The enterococcal species are diversified dominated by non-pigmented strains that are possibly *E. faecalis* and *E. faecium*. Thus, these dogs are potential reservoirs and disseminators of virulent, multiple drug-resistant, VAN-resistant and HLAR enterococci and genes encoding resistance to many classes of antibiotics. This has huge impact on the ecology and epidemiology of antibiotic resistance. Therefore, attention should be paid on the use of antibiotics, including VAN, in dogs as well as humans and other animal species in Nigeria. However, further studies to determine the genes encoding VAN and high-level aminoglycoside resistance and VFs in the isolates are recommended.

CONFLICT OF INTEREST

None declared by the authors.

REFERENCES

Aasmäe B, Volkova J, Häkkinen L, Orro T, Tenson T, Kalmus P (2015). In vitro antimicrobial resistance of intestinal Escherichia coli and enterococci in clinically healthy dogs in Estonia. Veterinarija IR Zootechnika (Vet Med Zoot). T. 72 (94). 2015

Amaechi N., Nwankwo I.U. (2015) Evaluation of prevalence and antimicrobial resistance using enterococci isolates from pigs and poultry and birds in Abia State, Nigeria. Int. J. Curr. Microbiol. Appl. Sci., 4:825-833.

Anyanwu MU, Obetta TU (2015). Prevalence and antibiogram of generic enterococci in ready-to-slaughter beef cattle. Not Sci Biol, 7(4):390-399

Anyanwu MU, Okorie-Kanu OJ, Ogugua AJ, Ezenduka EV, Anidebe CO (2019). Occurrence, antibiogram and vancomycin resistance of generic enterococci in horses in Nigeria. Revue Méd. Vét., 170, 1-3, 46-52

Anyanwu MU, Ugwu IC, Ezekwelu MO, Okorafor ON (2017). Prevalence and antibiogram of generic extended-spectrum β-lactam-resistant enterobacteria in healthy dogs. Notulae Scientia Biologicae, 9(1): 22-33.

Ayeni F.A., Odumosu B.T., Oluseyi A.E., Ruppitsch W.: Identification and prevalence of tetracycline resistance in enterococci isolated from poultry in Ilishan, Ogun State, Nigeria. J. Pharm. Bioallied Sci., 2016, 8, 69-73.

Bertelloni F, Salvadori C, Lotti G, Cerri D, Ebani VV (2017) Antimicrobial resistance in Enterococcus strains isolated from healthy domestic dogs. Acta Microbiologica et Immunologica Hungarica 64 (3):301-312

Bertelloni F, Salvadori C, Lotti G, Cerri D, Ebani VV. (2017) Antimicrobial resistance in Enterococcus strains isolated from healthy domestic dogs. Acta Microbiologica et Immunologica Hungarica 64(3):301-312.

Beshiru A, Igbinosa IH, Omeje FI, Ogofure AG, Eyong MM, Igbinosa EO (2017) Multi-antibiotic resistant and putative virulence gene signatures in Enterococcus species isolated from pig farms environment. Microbial Pathogenesis 104 (2017) 90e96

Boynukara, B., Ekin, İ.H., Aksakal, A., Gulhan, T. (2002). Isolation and

antibiotic susceptibility of enterococci from human, dog and cat faeces. Vet. Hek. Mikrobiyol. Derg., 2:37-42.

Cartwright CP, Stock F, Fahle GA, Gill VJ (1995) Comparison of pigment production and motility tests with PCR for reliable identification of intrinsically vancomycin-resistant enterococci. J Clin Microbiol. 33(7): 1931-1933.

Cinquepalmi V, Monno R, Fumarola L, Ventrella G, Calia C, Greco MF, Danila de Vito, and Soleo L. (2013) Environmental contamination by dog's faeces: a public health problem? Int. J. Environ. Res. Public Health 2013, 10, 72-84; doi:10.3390/ijerph10010072

Clinical and Laboratory Standards Institute (CLSI). (2020). Performance Standards for Antimicrobial Susceptibility Testing. 30th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute.

Damborg P., Top J., Hendrickx A.P.A, Dawson S, Willems R.J.L, Guardabassi L (2009) Dogs are a reservoir of ampicillin-resistant Enterococcus faecium lineages associated with human infections. Applied and Environmental Microbiology, 75(8):, p. 2360-2365

De Graef, E.M., Decostere, A., Devriese, L.A. and Haesebrouck, F. (2004) Antibiotic resistance among fecal indicator bacteria from healthy individually owned and kennel dogs. Microb Drug Resist 10, 65-69.

De Leener E, Decostere A, De Graef EM, Moyaert H, Haesebrouck F (2005) Presence and mechanism of antimicrobial resistance among enterococci from cats and dogs. Microbial Drug Resistance, 11(4):395-403.

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.

Devriese, L. A., M. Ieven, H. Goossens, P. Vandamme, B. Pot, J. Hommez, and F. Haesebrouck. (1996). Presence of vancomycin-resistant enterococci in farm and pet animals. Antimicrob. Agents Chemother. 40:2285-2287.

Eze UU, Nwosu C, Dan-Jumbo SO, Ogbu KI, Anene BM, Okeke OS, Ogunkoya AB (2009). The role of dog markets in the spread of rabies in Enugu State, South Eastern Nigeria. Epizootiology and Animal Health in West Africa 5:35-42

Finisterra L, Duartea B, Peixe L, NovaiC, Freitas AR (2021). Industrial dog food is a vehicle of multidrug-resistant enterococci carrying virulence genes often linked to human infections. International Journal of Food Microbiology, https://doi.org/10.1016/j.ijfoodmicro.2021.109284

Ghosh A, Dowd SE, Zurek L (2011) Dogs leaving the ICU carry a very large multi-drug resistant enterococcal population with capacity for biofilm formation and horizontal gene transfer. PLoS ONE 6(7): e22451.

Gulhan T, Aksakal A, Ekün UH, Savaþan S, Boynukara B (2006) Virulence factors of Enterococcus faecium and Enterococcus faecalis strains isolated from humans and pets. Turk. J. Vet. Anim. Sci. 30: 477-482

Igbinosa EO and Beshiru A (2019) Antimicrobial resistance, virulence determinants, and biofilm of Enterococcus species from ready-to-eat seafood. Front. Microbiol. 10:728.

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

Jackson CR, Fedorka-Cray PJ, Davis JA, Barrett JB, Frye JG (2009) Prevalence, species distribution and antimicrobial resistance of enterococci isolated from dogs and cats in the United States. Journal of Applied Microbiology, 107:1269-1278

Kataoka Y, Ito C, Kawashima A, Ishii M, Yamashiro S, Harada K, Ochi H, Sawada T. (2013). Identification and antimicrobial susceptibility of enterococci isolated from dogs and cats subjected to differing antibiotic pressures. J. Vet. Med. Sci. 75(6): 749-753

Kirkan S, Ugur Parin1, Gamze Balat. (2019) Antimicrobial resistance of Enterococcus faecium isolated from the urinary system of dogs. Mac Vet Rev 2019; 42 (1): i-vii

Leite-Martins L, Mahú MI, Costa AL, Bessa LJ, Vaz-Pires P, Loureiro L, Niza-Ribeiro J, de Matos AJF, Martins da Costa P. (2015) Prevalence of antimicrobial resistance in faecal enterococci from vet-visiting pets and assessment of risk factors. Vet. Rec. 176(26), 674 (2015).

Magiorakos A, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, 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. 2012; 18: 268-281

Manero A, Blanch AR (1999) Identification of Enterococcus spp. with a Biochemical key. Applied and Environmental Microbiology, 65(10): 4425-4430.

Miranda C, Vanessa Silva, Gilberto Igrejas, Patrıcia Poeta (2021) Impact of European pet antibiotic use on enterococci and staphylococci antimicrobial resistance and human health. Future Microbiol. (2021) 16(3), 185-201.

Ngbede E.O., Raji M.A., Kwanashie C.N., Kwaga J.K.P (2017a). Antimicrobial resistance and virulence profile of enterococci isolated from poultry and cattle sources in Nigeria. Trop. Anim. Health Prod., 2017, 49, 451-458.

Ngbede E.O., Raji M.A., Kwanashie C.N., Kwaga J.K.P., Adikwu A.A., Maurice N.A., Adamu A.M (2017b) Characterization of high level ampicillin- and aminoglycoside enterococci isolated from non-hospital sources. J. Med. Microbiol., 2017, 66, 1027-1032.

Oguttu JW, Qekwana DN and Odoi A (2021) Prevalence and predictors of antimicrobial resistance among Enterococcus spp. from dogs presented at a veterinary teaching hospital, South Africa. Front. Vet. Sci. 7:589439.

Oliveira M, Tavares M, Gomes D, Touret T, São Braz B, Tavares L, Semedo-Lemsaddek T. (2016). Virulence traits and antibiotic resistance among enterococci isolated from dogs with periodontal disease. Comparative Immunology, Microbiology and Infectious Diseases 46:27-31.

Osman, K. M., Badr, J., Orabi, A., Elbehiry, A., Saad, A., Ibrahim, M. D. S., et al. (2019). Poultry as a vector for emerging multidrug resistant Enterococcus spp.: first report of vancomycin (van) and the chloramphenicol-florfenicol (cat-fex-cfr) resistance genes from pigeon and duck faeces. Microb. Pathog. 128, 195-205.

Ossiprandi MC, Zerbini L. (2015) Antimicrobial susceptibility of enterococcal species isolated from Italian dogs. IntechOpen http://dx.doi.org/10.5772/61778

Pillay S., Zishiri O.T, Adeleke MA(2018). Prevalence of virulence genes in Enterococcus species isolated from companion animals and livestock. Onderstepoort J Vet Res., 85(1):1583.

Pillay, S., Zishiri, O.T, Adeleke M.A., (2018). Prevalence of virulence genes in Enterococcus species isolated from companion animals and livestock. Onderstepoort Journal of Veterinary Research 85(1):a1583.

Poeta, P, Costa D, Rodrigues J, 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.

Ramnath L, Sithole B, Govinden R (2017) Identification of lipolytic enzymes isolated from bacteria indigenous to Eucalyptus wood species for application in the pulping industry. Biotechnol Rep (Amst). 15:114-124.

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.

Said L, Dziri R, Sassi N, Lozano C, Ben Slama K, Ouzari I, Torres C, Klibi N (2017) Species distribution, antibiotic resistance and virulence traits in canine and feline enterococci in Tunisia. Acta Vet Hung, 65(2):173-184.

Solati SM, Tajbakhsh E, Khamesipour F, Gugnani HC (2015) Prevalence of virulence genes of biofilm producing strains of Staphylococcus epidermidis isolated from clinical samples in Iran. AMB Express, 5(1):134

Torres C., Alonso C.A., Ruiz-Ripa L., Leónsampedro R., Del Campo R., Coque T.M. (2018) Antimicrobial resistance in Enterococcus spp. of animal origin. Microbiol. Spectr., 2018, 6, doi:10.1128/ microbiolspec.ARBA-0032-2018.

Turkyilmaz S, Erdem V, Bozdoğan B (2010) Investigation of antimicro-

bial susceptibility for enterococci isolated from cats and dogs and the determination of resistance genes by polymerase chain reaction. Turk. J. Vet. Anim. Sci. 34(1): 61-68

Vu J, Carvalho J, Quiloan MLG (2012). Enterococcus faecalis can be distinguished from Enterococcus faecium via differential susceptibility to antibiotics and growth and fermentation characteristics on mannitol salt agar. Front Biol, 7(2):167-177.

Wada Y, Harun AB., Yean CY, Zaidah AR (2020). Vancomycin-resistant enterococci (VRE) in Nigeria: the first systematic review and meta-analysis. Antibiotics 9:565.

Wada Y, Irekeola A.A, E.A.R. E.N.S, Yusof W, Lih Huey L, Ladan Muhammad S, Harun A, Yean C.Y, Zaidah A.R. (2021). Prevalence of vancomycin-resistant Enterococcus (VRE) in companion animals: the first meta-analysis and systematic review. Antibiotics, 10:138.

World Health Organization (WHO) (2017). Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics http://www.who.int/medicines/publications/WHOPPL-Short_Summary_25FebET_NM_WHO.pdf.

Zhao J, W Xie, X Lin, AR Baloch and X Zhang. (2012). Antimicrobial resistance in enterococci isolates from pet dogs in Xi'an, China. Pak Vet J., 32(3): 462-464.