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Multidrug resistance in *Enterococcus* species of faecal origin from commercial dairy lactating cattle: Public health concern

Godfred Ngu Tanih[1*], Anthony Ifeanyin Okoh[2,3], Roland Ndip Ndip[4], Uchechukwu Uchechukwu Nwodo[2,3], Ezekiel Green[1]

[1]*Department of Biotechnology and Food Technology, Faculty of science, University of Johannesburg, P.O Box 17011, Doornfontein Campus, Gauteng, South Africa*

[2]*SAMRC Microbial Water Quality Monitoring Centre, University of Fort Hare, Private Bag X1314, Alice 5700, Eastern Cape, South Africa*

[3]*Applied and Environmental Microbiology Research Group, Department of Biochemistry and Microbiology, University of Fort Hare, Alice 5700, Eastern Cape, South Africa*

[4]*Department of Microbiology and Parasitology, Faculty of Science, University of Buea, P.O. Box 63, Buea, Cameroon*

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ABSTRACT

Objective: To evaluate the prevalence of *Enterococcus* species in cattle faeces, their corresponding drug resistant patterns as well as the genes coding for resistance in the isolates.
Methods: Two hundred and ninety rectal swabs were cultured for the isolation of *Enterococcus*. Presumptive isolates were confirmed by PCR, targeting the *tuf* gene, and confirmed isolates were identified to species level, using species-specific primers aimed at targeting six different species. Additionally, antibiogram was performed by disc diffusion and genes implicated in resistance were evaluated using molecular methods.
Results: All presumptive isolates were confirmed as *Enterococcus* and speciated as: *Enterococcus hirae* (82%), *Enterococcus faecium* (5%), *Enterococcus durans* (5%), *Enterococcus faecalis* (2%) and 6% of unidentified species. Resistance to various antimicrobials ranged from 16.4% for penicillin to 69.6% for erythromycin. Among the tetracycline and erythromycin-resistant isolates, *tet* M (100%) and *erm* B (29%) were the only amplified genes known to mediate resistance respectively. Other detected genes included *van B* (25%), *van C1* (21%) and *bla Z* (11%).
Conclusions: A high prevalence of multidrug resistant *Enterococcus* species was observed in this study, accentuating the need to improve on animal farming practices to prevent the dissemination of this microorganism to the environment.

1. Introduction

The use of antibiotics to control bacterial diseases in animals has been undermined by the rise of antibiotic-resistant bacteria and the possible transfer of resistance genes through plasmid-mediated conjugation to related bacteria. Naturally, antibiotic-resistant bacteria exist in environments at minimal levels, even in areas where very little or no anthropogenic activities of antibiotics have been performed[1]. However, the abuse of antibiotics in dairy farms to enhance growth and treat animal diseases has contributed immensely to the occurrence of resistant and multidrug resistant bacteria species worldwide[2]. One such bacteria is the enterococci, which nowadays constitute a major clinical challenge to both animals and humans. This is owing to their inherent abilities to acquire and transfer resistant determinants horizontally to other bacteria and the difficulties in treating individuals infected by resistant strains. They are also intrinsically resistant to various groups of antibiotics such as cephalosporins, aminoglycosides and clindamycin; in contrast, resistance to chloramphenicol, macrolides, tetracyclines, fluoroquinolones and glycopeptides is acquired[3].

Enterococcus constitutes an integral part of the gastrointestinal microflora of humans, animals and insects. Despite their existence as commensals in the gastrointestinal tract, some strains exist as opportunistic pathogens. Of particular interest is the recent vancomycin-resistant *Enterococcus* (VRE), which has become a major cause of hospital acquired infection reported globally[4-6]. *Enterococcus* have been implicated in many livestock diseases such as diarrhoea in swine and cattle, and endocarditis, septicaemia, paralysis, lameness arthritis, osteomyelitis, and spondylitis in poultry[7-9]. *Enterococcus* may also occur as an opportunistic

*Corresponding author: Godfred Ngu Tanih, Department of Biotechnology and Food Microbiology, Faculty of Science, Doornfontein Campus, Gauteng, South Africa.
Tel: +27739833344
E-mail: tanihngu@gmail.com
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pathogen in immunocompromised individuals or persons with prolonged broad-spectrum antimicrobial therapy[10].

This genus comprises about fifty-four species, of which only two species [*Enterococcus faecalis* (*E. faecalis*) and *Enterococcus faecium* (*E. faecium*)] are the frequent cause of enterococcal diseases in humans. *E. faecalis* account for about 85%–90% of reported cases of infections, whereas most of the residual (10%–15%) are attributed to *E. faecium*. Occasionally, other species are reported in disease conditions, including *Enterococcus avium* (*E. avium*), *Enterococcus casseliflavus* (*E. casseliflavus*), *Enterococcus durans* (*E. durans*), and *Enterococcus gallinarum*, *Enterococcus hirae* (*E. hirae*), *Enterococcus mundtii* and *Enterococcus malodoratus*[11].

Rising over the last few years and to date, these bacteria have shown increasing resistance to multiple antimicrobial drugs, thus limiting therapeutic options available to the clinicians. Resistance to clinically relevant drugs such as vancomycin, among others, has been mentioned in several studies[6,12]. This has been ascribed to the use of antibiotics in animal feed to enhance growth and productivity in animals[13]. These bacteria are eventually disseminated into the surroundings through excretion of urine, faeces, use of dung as manure, and accidental spilling of sewage. Information gathered from other studies further proves that hospitals and animal farms are prime sources for the occurrence of VRE[14-16].

In Europe and other western countries, the prevalence of *Enterococcus* spp is well established and stringent policies are being enforced to mitigate dissemination. In South Africa, there is paucity of information about the prevalence of these bacteria and their contribution toward diseases in this country. Resistant enterococci were first reported in South Africa by von Gottberg *et al.*, isolated from patients in a critical care unit at a hospital in Johannesburg[17]. Thereafter, no published reports were found until 2015–2016, when a few findings were reported on the occurrence of antimicrobial resistant enterococci (ARE) from hospital settings, piggery farms and wastewater treatment plants[3,5,18]. Although no outbreak of resistant enterococcal infections has been reported in the country in the last decade, accurately, up to date information on the prevalence of *Enterococcus* spp and the corresponding antimicrobial resistant pattern is imperative.

Among the 9 provinces found in South Africa, the Eastern Cape Province is largely agrarian and rural, containing several commercial dairy farms. On these farms, animal productivity is managed by the use of antibiotics, and the impact of any resistance would be widespread into the environment through faecal material. This lack of information poses an epidemiological problem, considering that the province has one of the highest HIV prevalence in the country[19]. This is the first study to investigate the existence of enterococci from commercial dairy farmlands, which forms part of a larger study, titled the "distribution of potential disease causing microorganisms and their susceptibility profiles in different farms located in the Eastern Cape". The purpose of this work was to evaluate the prevalence of *Enterococcus* spp in terms of species distribution and corresponding antimicrobial susceptibility patterns in dairy farmlands in the Eastern Cape Province.

2. Materials and methods

2.1. Description of the study location

Three commercial, dairy farms located in the Nkonkobe and Amahlathi local municipalities were selected for this study, and these farms were designated A, B and C. The selection of the farms was based on their milk production capacity and the close proximity of these farms to the University of Fort Hare to ensure that samples were able to be analyzed within 6 h of collection. Farms A and B are located in Nkonkobe local municipality (32.7833° S, 26.6333° E) whereas Farm C is located in Keiskammahoek in the Amahlathi local municipality (32.5719° S, 27.4264° E). The cattle population of these farms are Farm A (900), Farm B (600) and Farm C (400) respectively. All three farms have similar farming management style, in that water is sourced from a nearby running stream (usually positioned downhill) used for irrigation farming, washing of the cows before milking and drinking, while wastewater from the washing process is contained in a sewage catchment located uphill. Farms A and C use a rotary parlour for milking whereas Farm B uses a line system.

2.2. Sample collection

Briefly, 290 swab samples were collected from the rectum of healthy dairy cattle during the milking process from the three selected farms (A = 97, B = 96 and C = 97). The samples were collected from each cattle using sterile cotton swabs labelled with the corresponding tags of each cow to avoid duplication of data. Samples were collected at regular intervals every fortnight from July to September 2014. Samples were kept in appropriate ice boxes and transported immediately to the microbiological laboratory at the University of Fort Hare for analysis.

2.3. Isolation of Enterococcus species

Isolation was performed by inoculating the tubes containing sterile nutrient broth with the rectal swab sticks, which were incubated at 37 ˚C for 24 h. Tubes showing positive growth (turbidity) were streaked on bile aesculin azide agar plates and incubated for a further 24 h at 37 ˚C. Distinct black colonies from each plate were considered presumptive *Enterococcus* spp after Gram staining and oxidase tests were performed. Glycerol stocks were prepared by placing a loopful of the bacteria into nutrient broth and then incubated at 37 ˚C for 18 h. The isolates were then preserved in 20% glycerol at –80 ˚C for future use.

2.4. DNA extraction

Genomic DNA was extracted from *Enterococcus* isolates previously stored in glycerol using the method described by Iweriebor *et al.*[18]. Briefly, the isolates were revived in 5 mL of sterile Todd Hewitt broth and incubated at 37 ˚C for 20 h. This was followed by the transfer of 2 mL of the broth (recovered cells) into sterile Eppendorf tubes and centrifuged at 13000 r/min for 10 min and the supernatant was discarded. The pellets were washed twice with normal saline and resuspended in 200 μL of sterile distilled water. The suspension was well mixed by vortexing, using a mini shaker (Digisystem Laboratory Instruments Inc., New Taipei City, Taiwan). The cells were then lysed by heating for 10 min at 100 ˚C in an MS2 Dri-Block DB.2A (Techne, SA). The bacteria suspensions were centrifuged at 13000 r/min for 5 min to pellet the cell debris. The lysate supernatant was removed and transferred into sterile microcentrifuge tubes and used for PCR amplification.

2.5. Molecular confirmation

Presumptive isolates were molecularly identified using genus specific primer (F–5' TACTGACAAACCATTCATGATG-3' and R–5'-AACTTCGTCACCAACGCGAAC-3') previously described by Ke *et al.*[20] for the amplification of the *tuf* gene (elongation factor Tu). *E. hirae* ATCC 8043 was used as a positive control. The PCR reaction was prepared to a final volume of 25 μL reaction mix. This consisted of 12.5 μL master mix, 0.5 μL of each primer (forward and

reverse), 6.5 μL of nuclease free water and 5 μL of bacterial DNA. Reaction conditions consisted of initial denaturation at 94 ˚C for 2 min, followed by 30 cycles of amplification at 94 ˚C for 30 s, each annealing at 55 ˚C for 15 s, extension at 72 ˚C for 30 s and a final extension at 72 ˚C for 4 min. PCR products were resolved on 1.5% agarose gel electrophoresis at 100 V for 40 min, after staining with 5 μL ethidium bromide, then visualized on a transilluminator (Alliance 4.7 XD-79 System, Uvitec, Cambridge, UK).

2.6. Species identification

Confirmed isolates were speciated using PCR targeting seven *Enterococcus* species with the aid of specific primers and PCR conditions as previously described by Jackson *et al.*[21], with modifications (Table 1). PCR ingredients included 12.5 μL of KAPA ready mix (Lasec Group, Cape Town, South Africa), 1 μL of each primer (forward and reverse) and 5.5 μL nuclease free water and 5 μL DNA template. The PCR conditions were as follows: initial denaturation at 95 ˚C for 4 min closely followed by 30 cycles of denaturation at 95 ˚C for 30 s, annealing at 52 ˚C for 1 min (*E. faecalis*, *E. durans* and *E. casseliflavus*) or 48 ˚C for 1 min (*E. faecium* and *E. hirae*), extension at 72 ˚C for 1 min and final extension at 72 ˚C for 7 min. Amplicons (7 μL) were subjected to electrophoresis on 1.5% agarose gel (Separations, South Africa). The gels were stained with 5 μL ethidium bromide in 1× TAE buffer and run at 100 V for 50 min. Subsequently, the gels were visualized and photographed under the transilluminator (Alliance 4.7 XD-79 System, Uvitec, Cambridge, UK). A 100 base pair molecular weight marker was included on each gel as a DNA size marker.

Table 1

List of primer sequences, targeted genes, expected PCR amplified product size of *Enterococcus* species.

Strains/target gene	Sequence (5' – 3')	Product size (bp)	Reference
E. faecalis ATCC 19433	ACTTATGTGACTAACTTAACC TAATGGTGAATCTTGGTTTGG	360	[21]
E. durans ATCC 19432	CCTACTGATATTAAGACAGCG TAATCCTAAGATAGGTGTTTG	295	[21]
E. casseliflavus ATCC 25788	TCCTGAATTAGGTGAAAAAAC GCTAGTTTACCGTCTTTAACG	288	[21]
E. hirae ATCC 8043	CTTTCTGATATGGATGCTGTC TAAATTCTTCCTTAAATGTTG	187	[21]
E. faecium ATCC19434	GAAAAAACAATAGAAGAATTAT TGCTTTTTTGAATTCTTCTTTA	215	[21]
E. avium ATCC 14025	GCTGCGATTGAAAAATATCCG AAGCCAATGATCGGTGTTTTT	368	[21]
Enterococcus gallinarum ATCC 49673	GCTAGTTTACCGTCTTTAACG TTACTTGCTGATTTTGATTCG	173	[21]
tet M	AGTGGAGCGATTACAGAA CATATGTCCTGGCGTGTCTA	158	[22]
tet K	GTAGCGACAATAGGTAATAGT GTAGTGACAATAAACCTCCTA	360	[22]
erm A	AAGCGGTAAACCCCTCTGA TTCGCAAATCCCTTCTCAAC	190	[22]
erm B	CTATCTGATTGTTGAAGAAGGATT GTTTACTCTTGGTTTAGGATGAAA	142	[22]
bla Z	ACTTCAACACCTGCTGCTTTC TGACCACTTTTATCAGCAACC	173	[22]
van C1	ATCCAAGCTATTGACCCGCT TGTGGCAGGATCGTTTTCAT	402	[23]
van B	AGACATTCCGGTCGAGGAAC GCTGTCAATTAGTGCGGGAA	220	[23]

2.7. Antimicrobial susceptibility testing

This test was performed using the standard Kirby-Bauer disc diffusion method recommended by Clinical and Laboratory Standards Institute (CLSI)[24]. The antibiotics chloramphenicol (10 μg), tetracycline (30 μg), erythromycin (15 μg), penicillin (10 μg), ciprofloxacin (5 μg), vancomycin (30 μg), linezolid (30 μg), nitrofurantoin (300 μg) and quinupristin/dalfopristin (15 μg) were purchased from Mast Diagnostics for this assay. Bacterial inoculum was prepared by emulsifying four to five discrete colonies, from an overnight pure culture of the bacteria, into normal saline solution and the turbidity of the suspension was adjusted to 0.5 McFarland standard. Sterile cotton swabs were used to inoculate the bacterial suspension evenly on Mueller-Hinton agar (Laboratorios Conda, Pronadisa, South Africa) plates. With the aid of a disc dispenser machine, antibiotic discs were dispensed onto the Mueller-Hinton agar plates and incubated at 37 ˚C in an inverted position for 18 h. After incubation, zones of inhibition were measured with a ruler and the results interpreted following the recommended guidelines of CLSI[24].

2.8. Screening for resistant genes

Enterococcus strains which expressed phenotypic resistance to penicillin, tetracycline, vancomycin and erythromycin were screened molecularly for the existence of putative resistance genes (*tet* M, *tet* K, *van* B, *van* C1, *erm* A, *erm* B and *bla* Z) using primers. The primer sequences and PCR conditions were obtained from Duran *et al.*[22], listed in Table 1, with minor modifications. The PCR amplifications were performed in a thermocycler manufactured by BioRad (CA, Foster City, USA), in a reaction volume of 25 μL consisting of 5 μL of genomic DNA, 12.5 μL master mix, 0.5 μL of forward and reverse primer each and 6.5 μL of nuclease free water. The amplification process was as follows: initial denaturation step at 95 ˚C for 3 min, 30 cycles of amplification (denaturation at 95 ˚C for 30 s, annealing at 54 ˚C for 30 s, and extension at 72 ˚C for 30 s) and a final extension at 72 ˚C for 4 min. Amplicons (7 μL aliquots) were resolved in 1.5% agarose gel containing 5 μL ethidium bromide in 1× TAE buffer (40 mmol/L Tris-HCl, 20 mmol/L Na-acetate, 1 mmol/L EDTA, pH 8) and run at 100 V for 50 min, before being visualized and photographed under the transilluminator (Alliance 4.7 XD-79 System, Uvitec, Cambridge, UK).

3. Results

3.1. Bacterial recovery

All two hundred and ninety cultured faecal samples collected from the three farms were positive for presumptive *Enterococcus* isolates, and all the isolates were confirmed and identified molecularly as *Enterococcus* (Figure 1). Of the 290 confirmed isolates, four *Enterococcus* species were identified, *E. hirae* (82%), *E. faecium* (5%), *E. durans* (5%), *E. faecalis* (2%). There were 6% other enterococcal species which could not be identified. Table 2 represents the distribution of species identified within each farm. *E. hirae* was the predominant species in all three farms. Two other targeted species in the study, *E. casseliflavus* and *E. avium*, were not detected.

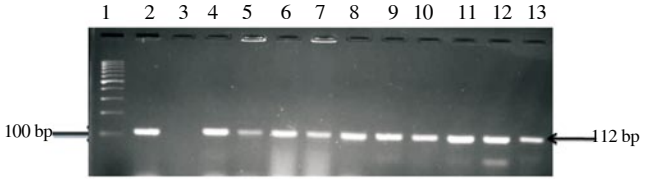

Figure 1. Agarose gel electrophoresis of PCR amplified products of the *tuf* genes.

Lane 1: Molecular weight maker (100 bp); Lane 2: Positive control (*E. faecium* ATCC19434); Lane 3: Negative control; Lanes 4 to 13: Amplicons of positive isolates.

Table 2

Instances of *Enterococcus* spp from cattle faeces in selected dairy farms [*n* (%)].

Farms	No. of samples	Isolates	*E. hirae*	*E. faecium*	*E. faecalis*	*E. durans*	Other species
Farm A	97	97	83 (86)	3 (3)	2 (2)	3 (3)	6 (6)
Farm B	96	96	80 (84)	7 (7)	3 (3)	4 (4)	2 (2)
Farm C	97	97	76 (78)	5 (5)	1 (1)	6 (6)	9 (10)
Total	290	290	239 (82)	15 (5)	6 (2)	13 (5)	17 (6)

3.2. Antimicrobial resistance

The drug resistance status of the 290 isolates isolated from dairy lactating cows was evaluated against nine antibiotics, and the results were interpreted in accordance with CLSI. Most of the isolates proved to be resistant to the antibiotics tested, with resistance ranging from 45.8% to 69.6% in linezolid, quinupristin/dalfopristin and erythromycin. However, a few isolates were also resistant to chloramphenicol, penicillin, nitrofurantoin and vancomycin (Table 3). All of the isolates demonstrated resistance to at least one of the antibiotics tested. Multidrug resistance patterns were also observed in some isolates with up to ten drug combination patterns obtained (Table 4). The majority of the isolates proved to be resistant to a combination of two to three drugs and the number decreased as the combination patterns increased. ERY SYN was the most predominant pattern observed in 144 isolates. Interestingly, only one isolate was resistant to a combination of eight antibiotics (PG CHL LZD NIT ERY VAN TET SYN).

3.3. Detection of antibiotic resistance genes

In accordance with results obtained of phenotypic antibiotic resistance (Table 3), specific putative genes (*tet* M, *erm* B, *bla* Z, *van* B and *van* C1) tested were detected in some of the isolates. Genes amplified included those conferring resistance to tetracycline (*tet* M), erythromycin (*erm* B), penicillin (*bla* Z) and vancomycin (*van* B, *van* C1) as shown in Table 5. Figure 2 is a representation of some of the amplified *tet* M resistance genes. Surprisingly, *erm* A and *tet* K genes were not detected in any of the phenotypically resistant *Enterococcus* species to erythromycin and tetracycline respectively.

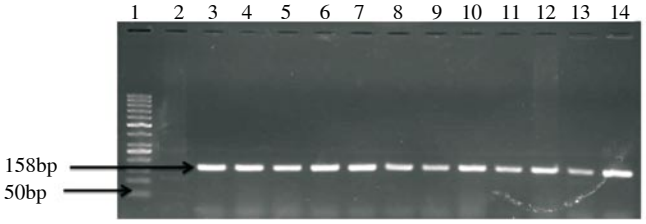

Figure 2. Agarose gel electrophoresis of PCR amplified products of the *tet M* genes.

Lane 1: Molecular weight maker (50 bp); Lane 2: Negative control; Lanes 3 to 14: Amplicons of positive isolates.

Table 4

Predominant phenotypic multi-resistance patterns of enterococci isolates.

Number of drugs	Drug combination patterns	Total number of isolates
2	ERY SYN	144
	LZD SYN	66
3	ERY TET SYN	24
	ERY VAN SYN	3
4	PG LZD ERY VAN	10
5	PG LZD ERY VAN SYN	4
6	PG LZD ERY VAN TET SYN	9
	PG LZD NIT ERY VAN SYN	4
7	PG CHL LZD ERY VAN TET SYN	2
8	PG CHL LZD NIT ERY VAN TET SYN	1

PG: Penicillin; CHL: Chloramphenicol; ERY: Erythromycin; NIT: Nitrofurantoin; SYN: Synercid (quinupristin/dalfopristin); TET: Tetracycline; CIP: Ciprofloxacin; LZD: Linezolid.

Table 5

Resistant genes detected in enterococci isolates.

Antibiotics	Resistant phenotype	Resistant genes	Isolates [*n* (%)]
Tetracycline	67	*tet* M	67 (100)
		tet K	0 (0)
Erythromycin	202	*erm* A	0 (0)
		erm B	58 (29)
Penicillin	47	*bla* Z	5 (11)
Vancomycin	53	*van* B	13 (25)
		van C1	11 (21)

4. Discussion

The rapid emergence of ARE and its increasing incidences in humans and animals has become a major concern to clinicians and other health personnel. This phenomenon poses an epidemiological challenge globally, and even more in South Africa due to the high prevalence of HIV/AIDS. Currently, considerable attention is been accorded to resistant bacteria occurrences, due to the exploitation of antibiotics in the management of farm animals and the subsequent dissemination of the bacteria into the environments. In South Africa, the prevalence of ARE in farm animals is largely unknown, with very few published reports[18]. The environment, animal farms and hospitals all serve as reservoirs for resistant enterococci, which are later disseminated to other ecological niches. Although *Enterococcus* is a natural member of the intestinal microfloral, some species are

Table 3

Antibiogram resistance profiles of *Enterococcus* species showing resistance to the tested antibiotics [*n* (%)].

Antibiotics	*E. hirae* (*n* = 239)	*E. faecium* (*n* = 15)	*E. durans* (*n* = 13)	*E. faecalis* (*n* = 6)	Other species (*n* = 17)	Total (*n* = 290)
Penicillin (10 IU)	34 (14.2)	5 (33.3)	3 (23.0)	0 (0.0)	5 (29.4)	47 (16.2)
Vancomycin (30 μg)	39 (16.3)	5 (33.3)	5 (38.5)	0 (0.0)	4 (23.5)	53 (18.2)
Ciprofloxacin (15 μg)	94 (39.3)	7 (46.7)	5 (38.5)	1 (16.7)	5 (29.4)	112 (38.6)
Chloramphenicol (10 μg)	11 (4.6)	1 (6.6)	0 (0.0)	0 (0.0)	1 (5.8)	13 (4.5)
Tetracycline (30 μg)	58 (24.3)	4 (26.6)	0 (0.0)	1 (16.7)	4 (23.5)	67 (23.1)
Erythromycin (15 μg)	168 (70.3)	13 (86.5)	11 (84.6)	2 (33.3)	8 (47.0)	202 (69.6)
Nitrofurantoin (300 μg)	16 (6.7)	0 (0.0)	0 (0.0)	0 (0.0)	2 (11.7)	18 (6.2)
Quinupristin/dalfopristin (15 μg)	157 (65.7)	9 (60.0)	8 (61.5)	1 (16.7)	6 (35.0)	181 (62.4)
Linezolid (30 μg)	111 (46.4)	8 (53.3)	4 (30.7)	4 (66.6)	6 (35.0)	133 (45.8)

opportunistic. To provide a better understanding of these bacteria, their prevalence in terms of species distribution and antimicrobial resistance in commercial dairy farmlands, this study was initiated with a focus on specific dairy farms in the Eastern Cape Province of South Africa.

Two hundred and ninety enterococci were isolated and further characterised as *E. hirae* (82%), *E. faecalis* (2%), *E. durans* (5%), *E. faecium* (5%) and other *Enterococcus* species (6%). *E. hirae* were the most predominant species isolated, with a distribution across the farms as Farm A (86%), Farm B (84%), and Farm C (78%). Despite the dissimilarity in the distribution of the species within the farms, our findings were in line with the reports of Jackson *et al.*[25] which also accounts for a greater number of *E. hirae* isolates, alongside other identified *Enterococcus* species from the same source[26,27]. However, our findings contradict those of Bekele and Ashenafi[27] which isolated *E. faecium* as the predominant species from cattle. The results are also in disagreement with the findings of Krause and Khafipour[28] which reported *E. casseliflavus* as the predominant species recovered, a species that was not isolated in this study.

Notably, results from antimicrobial sensitivity testing indicate that 4%–20% of the strains were resistant to chloramphenicol, nitrofurantoin, penicillin and vancomycin. More than 20% of the isolates were resistant to ciprofloxacin, tetracycline, erythromycin, linezolid, and quinupristin/dalfopristin. Erythromycin and quinupristin/dalfopristin resistances were the most prevalent in this study. The results were fairly similar to those of Iweriebor *et al.*[18] which also reported a high prevalence of multidrug resistant *Enterococcus* isolates to various antimicrobial agents from piggery in the Eastern Cape Province. Their findings were as follows: clindamycin (98.72%), penicillin G (91%), ciprofloxacin (77.5%), erythromycin (98.72%), neomycin (93.8%), amikacin (85%), and cephalothin (86.3%). None of the strains was resistant to all antimicrobials tested. Our data also show a high resistance of 69.6% (202/290) to erythromycin. Multiple antibiotic resistant patterns were also observed among the isolates, with a combination of erythromycin and quinupristin/dalfopristin (ERY SYN) as the most recurrent pattern.

Genetic analysis reveals *erm* B to be the lone amplified erythromycin resistance gene found in some of the isolates, with an incidence of 58%. These results are in partial agreement with the study of Zou *et al.*[29]. However, most of the isolates did not harbour either *erm* A or *erm* B genes, which are widely known to mediate resistance to erythromycin. *erm* B genes are the most widely distributed macrolide-resistant gene in enterococci, associated with conjugative plasmid or transposons Tn*916-1545*[30]. Considering that very few erythromycin-resistant isolates in this study harbour either of the genes (*erm* A and *erm* B), this suggests that other genetic determinants could be responsible for mediating resistance. Antimicrobial resistance in *Enterococcus* which are plasmid mediated is often regarded as hazardous to human and animal. This is because the bacteria harbouring these genes may transfer these genes not only to the same species but possibly to other pathogenic bacteria in the environment or zoonotic bacteria, which may pose a health threat to humans. Resistance to erythromycin is a matter of concern because this drug is sometimes used as a substitute for individuals with a penicillin allergy. Further experiments are required to identify the determinant responsible for this resistance.

Of the fifty-three isolates which were phenotypically resistant to vancomycin, only a few were found to harbour the *van* B and van C1 genes. This appears to contradict other studies in which *van* A was the most predominant vancomycin resistant gene obtained from faecal samples[12]. Our findings were similar to that of Iweriebor *et al.*[31] in which some of the isolates were reported to harbour *van* B (19.7%) and *van* C1 (25%) genes. Vancomycin resistance in enterococci is mediated by several van gene clusters consisting of *van* A, *van* B, *van* C, *van* D, *van* E, *van* G, *van* L, *van* M, and *van* N. These clusters consist of three groups of genes encoding two-component system, which are enzymes necessary for the synthesis of new peptidoglycan precursors and enzymes that destroy the normal D-Ala-D-Ala ending precursors[32]. VRE have emerged in the last decade as one of the major causes of nosocomial infections, and the presence of VRE has been detected from several sources, especially in hospitals and animal farms. Although the origin of vancomycin-resistant enterococci in animal farms is not clearly defined, some researchers have suggested the use of avoparcin, an analogue of glycopeptides to promote growth in animals as possible cause[33]. This is contradictory to our findings, where no vancomycin or related drugs were used in all three farms, even though vancomycin-resistant strains were obtained. The presence of VREs in dairy farms is indicative of a major health threat, considering that vancomycin is a therapeutic remedy available in clinical medicine in cases of enterococcal infection. Resistance to vancomycin could be attributed to the acquisition of resistance determinants through plasmid mediated conjugation from related bacteria species.

Sixty-seven strains of *Enterococcus* species, with the exception *E. durans*, were resistant to tetracycline. Based on information gathered from farm management in all three farms, penicillin and tetracycline were the only drugs used in excess to promote growth and productivity in the animals. This suggests a reason why some isolates were resistant to these drugs. Following our genetic profiling, we detected *tet* M in all tetracycline-resistant isolates tested. This gene is associated with the Tn*916*-Tn*1545* family of conjugative transposon, which suggests reasons for its very high prevalence. Similarly, in a study conducted by Klibi *et al.*[34], *tet* M genes were most prevalent of antibiotic resistances in enterococci from meat. In enterococci, tetracycline resistance is mediated by the *tet* genes. These genes comprise two main groups: those which confer resistance by ribosomal protection (*tet* M, *tet* O and *tet* S) and those which mediate an energy-dependent efflux of tetracycline from cells (*tet* K and *tet* L)[35]. Only *tet* M and K were investigated in our research, considering the high-frequency occurrence in isolates reported in other studies.

Lastly, very high resistance to quinupristin/dalfopristin and linezolid (which are emerging drugs used in the treatment of enterococcal infection) was observed in 62.3% and 45.8% of the isolates from all three farms. The majority of the species were resistant to these antibiotics in comparison to nitrofurantoin, to which only *E. hirae* isolates and other unidentified species were resistant. These resistances should be treated as a matter of concern, considering that these are new emerging drugs. However, further research is necessary to determine the genetic mechanism responsible for this resistance in the isolates.

In conclusion, our results suggest a relatively high prevalence of antibiotic resistance in enterococci of faecal origin in all three commercial dairy farms, particularly to erythromycin, quinupristin/dalfopristin and linezolid. However, the source of antibiotic resistance in bacterial isolates is not clear. The results also indicate that healthy lactating cattle are potential sources of antimicrobial resistant enterococci, which could be transferred among cattle herds, cattle to workers, or directly to the community. It is of concern that the range of antibiotics to which resistance has been acquired over time is relatively wide, to the extent that it now includes new emerging antibiotics used for the treatment of enterococcal infection (vancomycin, quinupristin/dalfopristin and linezolid). This may have significant consequences in the health sector, most especially in a crisis situation if these resistant strains could be transferred to humans. Emerging ARE is a problem that requires attention and intensified measures to mitigate the dissemination of resistance by these bacteria. The findings in this study are relevant to public and environmental health as it highlights the need for good farming practices through the controlled and proper use of antibiotic on farm

animals.

Conflict of interest statement

We declare that we have no conflict of interest.

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