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Antimicrobial Resistance Profiles of Enterococcus Faecalis and Enterococcus Faecium Isolated from Clinical Specimens at Kitwe Teaching Hospital in Zambia

Grace Mwikuma[1], Francis Musonda[2], Seke Kazuma[3], Victor Sichone[4], Abidan Chansa[5], Chie Nakajima[6], Yasuhiko Suzuki[7], Bernard Mudenda Hang'ombe[8]

[1]PhD student, Department of Paraclinical Studies, School of Veterinary Medicine, University of Zambia, Lusaka 10101, Zambia.
[8]Head of Department, Department of Paraclinical Studies, School of Veterinary Medicine, University of Zambia, Lusaka 10101, Zambia.
[2]Head of department, Department of Pathology, Kitwe Teaching Hospital, Kitwe 10101, Zambia
[3]Head Clinical Care, Department of Surgery, Kitwe Teaching Hospital, Kitwe 10101, Zambia.
[4]Senior Medical Superintendent, Department of Obstetrics and Gynaecology, Kitwe Teaching Hospital, Kitwe 10101, Zambia
[5]Consultant Physician, Department of Medicine, Ndola Teaching Hospital, Ndola, Zambia.
[6,7]Division of Bioresources, International Institute for Zoonosis Control, Hokkaido University, 8 Sapporo 060-0808, Japan.
[6,7]International Collaboration Unit, International Institute for Zoonosis Control, Hokkaido 19 University, Sapporo 060-0808, Japan.
[6,7]Hokkaido University Institute for Vaccine Research and Development, Hokkaido 19 University, Sapporo 060-0808, Japan.

Abstract

This research examined the occurrence of *Enterococcus faecalis* and *Enterococcus faecium* in clinical samples processed at Kitwe Teaching Hospital Laboratory, along with their resistance to antimicrobials and presence of virulence determinants. A total of 230 clinical specimens were analyzed to identify suspected *Enterococcus* strains. Standard laboratory techniques were employed to isolate, characterize, and confirm the species of *Enterococcus*, followed by testing their susceptibility to antimicrobials and detection of resistance and virulence genes using polymerase chain reaction. Among the 230 cultured specimens, 89 were stool samples, 3 were swabs, and 138 were urine specimens. Out of these, 71 isolates were confirmed *Enterococcus* by PCR using genus specific primers. The resulting prevalence rate of 30.9% was thus obtained. The most prevalent species was *Enterococcus faecalis*, accounting for 56.3% of the 71 *Enterococcus* isolates. The prevalence of *E. faecium* was 14.1%. All tested *E. faecalis* isolates displayed resistance to ciprofloxacin and erythromycin. Furthermore, high rates of resistance were observed among *E. faecalis* isolates for chloramphenicol and tetracycline (97.5%), vancomycin (90.0%),

nitrofurantoin (75.0%), penicillin (75.0%) and ampicillin (67.5%). Only two *E. faecium* isolates exhibited susceptibility to tetracycline and vancomycin, while the remaining isolates were 100% resistant to all other tested antimicrobials. Multidrug resistance was detected in all *E. faecium* isolates. In addition, various antibiotic resistance genes (*aac(6')-Ie-aph(2")-LA*, *ermA*, *ermB*, *tetK*, *tetL*, *tetM*, *tetX*, *vanA*) were identified in both *E. faecalis* and *E. faecium* isolates. A positive association between phenotype and genotype was found for tetracycline and erythromycin.

Keywords: antimicrobial susceptibility, antimicrobial resistance genes, *Enterococcus faecalis*, *Enterococcus faecium*, prevalence, clinical specimens, Zambia

1. Introduction

Enterococci are bacteria commonly found as normal flora in the gastrointestinal tracts of animals and humans (Lebreton, Willems, and Gilmore, 2014, Soodmand, *et al*, 2018). *Enterococcus* has spread widely as a hospital and a community acquired pathogen on a global scale (Zhou, *et al*., 2020; Guzman Prieto, *et al*., 2016). It has gained clinical relevance due to its implication in many clinical syndromes including urinary tract infections, bacteraemia, endocarditis, wound infections, endophthalmitis and root canal (NI and Huycke, 2014; Abat, *et al*., 2016; Todokoro, *et al*., 2017). *Enterococcus faecium* and *Enterococcus faecalis* are responsible for the majority of human enterococcal infections (Georges, *et al*., 2022; Horner, *et al*., 2021). *Enterococci* have also earned recognition due to their ability to acquire and transfer virulence and antimicrobial resistance determinants, from and to other commensal and pathogenic bacteria in animals and humans (Krawczyk, *et al*., 2021; Ramos, *et al*., 2020). There have been reports of genetic similarities between animal strains and those causing infections in humans (Lee, et al., 2021; Ahmed, *et al*., 2018; Zischka, *et al*., 2015). Cases of human infections caused by animal strains, as well as the transfer of virulence and resistance traits from animals to humans, have been documented (Ngbede, *et al*., 2017; Iseppi, *et al*., 2020; Miranda, *et al*., 2021). Such reports and cases are of significant concern for public health. Studies conducted in other parts of the world have demonstrated that the prevalence of antimicrobial resistance and virulence traits among *Enterococcus* species varies depending on geographical location and antimicrobial usage (Barbosa-Ribeiro, *et al*., 2016; Shridhar, and Dhanashree, 2019). Few studies have investigated the prevalence, characteristics and antimicrobial resistance of *Enterococci*. These include studies on *Enterococci* from poultry in some districts on the Copperbelt and Lusaka Provinces (Mwikuma, *et al*., 2023; Mudenda, *et al*, 2022), from clinical samples at the University Teaching Hospital (Mutalange, *et al*., 2021) and at cattle interface areas of Kafue basin in Zambia (Mubita, *et al*., 2008). However, there is a lack of information on the occurrence and antimicrobial resistance of *Enterococcus faecalis* and *Enterococcus faecium* isolated from clinical specimens at Kitwe Teaching Hospital, Copperbelt Province, Zambia. Considering the potential risk of harmful enterococcal strains; *Enterococcus faecalis* and *Enterococcus faecium* possibly being transmitted in the hospital environment and the role of *Enterococcus* in the spread of antimicrobial resistance genes, it is crucial to assess the prevalence and antibiotic resistance in clinical *Enterococcus faecalis* and *Enterococcus faecium*, as they may contribute to outbreaks of hospital acquired infections. It is therefore important to monitor the occurrence of resistant *Enterococcus* species in clinical specimens to prevent nosocomial infections. The current study aims to provide insight into the occurrence, species diversity and antibiotic resistance potential of *Enterococcus faecalis* and *Enterococcus faecium* isolated from clinical specimens at Kitwe Teaching Hospital in Zambia.

2.0 Materials and Methods

2.1 Study design, site and period

A cross-sectional study was conducted at Kitwe Teaching Hospital on the Copperbelt Province in Zambia from February to March, 2021.

2.2 Study Population

All pus swabs, urine and stool samples which were received in the laboratory from 1st February to 16th March, 2021 i.e., from first sample until the 230th specimen was reached were conveniently included in the study. The samples included in this study were from both hospitalized and non-hospitalized male and female patients ranging from 2 to 86 years old. Only 2 patients had their age not indicated on the request forms.

2.3 Sample Size and Sampling Frame

For determination of sample size prevalence "p" of 18.03% (Mpinda, *et al*., 2019), Z statistic of 1.96 at 95% confidence and acceptable error of 0.05 (Pourhoseingholi, *et al.,* 2013) were employed.

Formula:

$$n = \frac{\mathrm{z}^2 \text{ x } \mathrm{p}(1 - \mathrm{p})}{e^2}$$

$$n = \frac{1.96^2 \text{x } 0.18(1 - 0.18)}{0.05^2}$$

n = 227

Rounding up it gives us 230.

2.4 Sample Collection

From 1st February to 16th March, 2021 all stool and urine samples which were sent to laboratory were included in study. This was done from starting with first sample until the number 230 specimens was reached. After which sampling was discontinued.

2.5 Laboratory Investigations

2.5.1 Culture of *Enterococcus*

To detect and characterize *Enterococcus* species, standard Microbiological methods were employed to as described by Facklam and Collins (1989) with a few modifications. In brief, 1g of faecal specimens was suspended in 9ml buffered peptone water (BPW) (HIMEDIA, India), while swabs were placed in 5ml of BPW and incubated at 37°C for 24hrs. 1ml of the overnight suspension was put into 5ml Trypticase Soy broth (TSB) (HIMEDIA, India), mixed and incubated at 37°C for 24 hours. For urine, 1ml of urine samples was dispensed into 5ml Trypticase Soy broth (TSB), mixed and incubated at 37°C for 24 hours. A loopful of the TSB suspension was streaked on Bile Esculin Agar (BEA) (HIMEDIA, India) and incubated at 37^0c for 24 hours. A total of 230 clinical specimens comprising of swabs, urine and stool were collected and processed at Kitwe Teaching Hospital Laboratory.

2.5.2 Phenotypic Characterization of *Enterococcus* Species

Species identification was based on phenotypic characteristics including colonial morphology, Gram Stain (Gainland Chemicals Company, United Kingdom), catalase test and biochemical tests. A total of 124 suspect bacterial colonies (small black shiny) were stored in 20% glycerol at -20℃ pending subsequent

experiments.

2.5.3 DNA Extraction

Colonies of overnight growth on a blood agar plate were put in a test tube containing 0.5ml of molecular grade water, vortexed and boiled at 95°C for 10 minutes, and then centrifuged for 5 minutes at 1500*xg*. The supernatant was pipetted into cryo-vials and stored at -20°C for further analysis

2.5.4 Molecular Identification of *Enterococci*

Confirmation of the genus *Enterococcus* was done by PCR using genus-specific primers (table 1) as described previously by Li and colleagues (2012). Extracted DNA PCR amplification of *elongation factor* (*tuf*) and *D -Ala- D -Ala ligase* (*ddl*) was done using Phusion Flash High-Fidelity PCR Master Mix (Thermofisher Scientific, US) in the thermal cycler (Applied Biosystems, Chiba, Japan) under the following PCR conditions; initial denaturation at 98°C for 2 minutes followed by 30 cycles of denaturation at 98°C for 5 seconds, annealing at 56°C for 5 seconds, extension at 72°C for 30 seconds, and final extension at 72°C for 1 minute. PCR amplicons were run on 1.5% agarose gels. The expected band width for *tuf* and *ddl* PCR products was 112bp and 475bp, respectively. For species identification, species-specific primers (Table 1) targeting the *superoxide dismutase* (*sodA*) gene of *E. faecalis* and *E. faecium* were used. No other primers for species were available. The PCR conditions were as described above for genus except the annealing temperature which was 52°C for both.

Table 1. Primers for Genus and Species identification of *Enterococci*

IDENTIFICATION PRIMERS				
Target gene	**Primer name**	**Primer sequence 5'-3'**	**Amplicon Size bp**	**References**
Tuf	*tuf-F*	TAC TGA CAA ACC ATT CAT GAT G	112	Ke, *et al*, 1999
	tuf-R	AAC TTC GTC ACC AAC GCG AAC		
Ddl	*ddlF*	CAC CTG AAG AAA CAG GC	475	Vilela, *et al*, 2006
	ddlR	ATG GCT ACT TCA ATT TCA CG		
sodAEfm	*sodAEfm1*	CAG CAA TTG AGA AAT AC	190	Bensalah, Flores and Mouats, 2006
	sodAEfm2	CTT CTTTTATTTCTCCTGTA		
sodAEfs	*sodAEfs1*	CTGTAG AAG ACC TAA TTT CA	209	Bensalah, Flores and Mouats, 2006
	sodAEfs2	CAG CTG TTT TGA AAG CAG		

bp = base pair

2.5.5 Determination of Levels of Antimicrobial Resistance

Susceptibility to vancomycin (30 µg), erythromycin (15 µg µg), ampicillin (10 µg), penicillin (10U), tetracycline (30 µg), nitrofurantoin (300 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg), and gentamicin (120 µg) was determined by disk diffusion method according to the Clinical and Laboratory Standards Institute guidelines (2012). Diameters of zones of inhibition were recorded in millimeters and interpreted according to Clinical and Laboratory Standards Institute (2012) as susceptible or intermediate or resistant. All intermediate results were taken as resistant. A reference strain, *Enterococcus faecalis* 29212 was used as control strain.

2.5.6 Detection of Antimicrobial Resistant Genes (ARG)

Detection of genes conferring resistance to glycopeptides (*vanA*), tetracyclines [*tet(M), tet(L), tet(K),* and *tet(X)*], macrolides [*erm(A)* and *erm(B)*] and aminoglycosides [*aac(6')-Ie-aph(2' ')-Ia*] was performed using PCR with gene-specific primers (Table 2). One Taq Quick-load 2X Master Mix (Biolabs, Durham, North Carolina, USA) was used for amplification using a thermal cycler (Applied Biosystems, Chiba, Japan). The following PCR conditions were employed; Initial denaturation at 93°C for 3 minutes. The amplification cycles consisted of denaturation at 93°C for 60 seconds, and annealing at 52°C for 60 seconds, elongation at 72°C for 60 seconds. After 35 cycles amplification cycles, the final elongation step was performed at 72°C for 5 minutes. PCR amplicons were run on 1.5% agarose gels. Expected PCR products were different for each gene (Table 2).

Table 2 Primers used for Detection of Resistance Genes

PRIMERS FOR RESISTANCE GENES				
Target gene	**Primer name**	**Primer sequence 5'-3'**	**Amplicon size bp**	**References**
aac(6′)-Ie-aph(2″)-LA	*aacF*	CAG GAA TTT ATC GAA AAT GGT AGA AAA G	369	Sabouni, *et al.*, 2016
	aacR	CAC AAT CGA CTA AAG AGT ACC AAT C		
***erm*A**	ermAF	TAT CTT ATC GTT GAG AAG GGA TT	139	Goudarzi, *et al.*, 2016
	ermAR	CTA CAC TTG GCT TAG GAT GAA A		
***erm*B**	ermB-1	GAA AAG TAC TCA ACC AAA TA	639	Sutcliffe, *et al.*, 1996
	ermB-2	AGT AAC GGT ACT TAA ATT GTT TA		
***tet*K**	tetK-1	TTA GGT GAA GGG TTA GGT CC	697	Aarestrup, *et al.*, 2000
	tetK-2	GCA AAC TCA TTC CAG AAG CA		
***tet*M**	tetM-1	GTT AAA TAG TGT TCT TGG AG	576	Aarestrup, *et al.*, 2000
	tetM-2	CTA AGA TAT GGC TCT AAC AA		
***tet*L**	tetL-1	CAT TTG GTC TTA TTG GAT CG	456	Aarestrup, *et al.*, 2000
	tetL-2	ATT ACA CTT CCG ATT TCG G		
***tet*X**	*tet*XF	CAA TAA TTG GTG GAC CC	468	Ng, et al., 2001
	tetXR	TTC TTA CCT TGG ACA TCC CG		
***van*A**	*vanAF*	CTG CAA TAG AGA TAG CCG CTA ACA	751	Sting, *et al.*, 2013
	vanAR	TGT ATC CGT CCT CGC TCC TC		

bp = base pair

3.0 RESULTS

3.1 Patient Demographic Characteristics and Isolate Identification

Specimens from 230 patients; 150 from out-patients (non-hospitalized) and 80 from in-patients (hospitalized) were included in this study. More than half of the specimens 55.2% (127/230) were from female patients. Specimens from male patients accounted for 44.8% (103/230). The age of patients ranged from 2 to 86 years old. Two had no age indicated on their request forms. The 230 specimens comprised

of 138 urine, 89 stool and 3 pus swabs. During the study period, a total of 71 *Enterococcus* species were isolated, 59 from urine, 11 from stool and 1 from pus swab. Table 4 shows the species identities in relation to age range and gender, along with specific sources of the isolates, which consisted of *E. faecalis* (40, 56.3%), *E. faecium* (10, 14.1%), a combination of *E. faecalis* and *E. faecium* (5, 7.0%) and other *Enterococcus* species (16, 22.5%) which were not identified to species level due to unavailability of other species-specific primers other than those for *E. faecalis* and *E. faecium* as well as inadequate DNA sequencing reagents.

Table 4. Patient Demographic Characteristics and Isolate Identification

Age range	Total n of specimens processed (230)	*Total n of Enterococcus* isolates (71)	*E. faecalis* (40)	*E. faecium* (10)	*E. faecalis + E. faecium* (5)	Other *Enterococcus* species (16)
2-15	51	9	4	1	0	5
16-30	70	19	9	3	1	6
31-45	62	20	12	4	2	2
46-60	14	6	5	0	1	0
61-75	23	12	8	1	0	3
76-86	7	2	1	1	0	0
Not indicated	3	2	1	0	1	0
Gender						
Male	103	39	21	10	1	7
Female	127	32	19	0	4	9
Specimen type						
urine	138	59	37	7	3	12
Stool	89	11	3	2	2	4
Pus swab	3	1	0	1	0	0
Department						
Out-patient	150	51	30	9	1	11
In-patient	80	20	10	1	4	5

n = number

3.2 Prevalence of *Enterococcus faecalis* and *Enterococcus faecium*

The prevalence of *Enterococcus* was 30.9% (71/230, CI: 25.15-37.3). The prevalence of *Enterococcus faecalis* among all *Enterococcus* species was 56.3% (40/71, CI: 44.1-67.9). Table 5 shows summary of the prevalence of *E. faecalis* and *E. faecium* (occurring as single isolates as well as in combination) in clinical specimens at Kitwe Teaching Hospital in the Copperbelt Province.

Table 5. Prevalence of *E. faecalis and E. faecium*

Factor	Categories	*n* Tested	*n* Positive	Prevalence (%)	95% CI
Overall	Positivity	230	71	30.9	25.15-37.3
Enterococci isolates	*E. faecalis*	71	40	56.3	44.1-67.9
	E. faecium	71	10	14.1	7.3-24.8
	E. faecalis + *E. faecium*	71	5	7.0	2.6-16.3
	Other *Enterococcus* species	71	16	22.5	13.8-34.3

n = number, % = percent, CI = confidence interval

3.3 Antimicrobial Susceptibility of *E. faecalis and E. faecium*

All *E. faecalis* isolates were resistant to ciprofloxacin and erythromycin. Most of the *E. faecalis* isolates were resistant to chloramphenicol and tetracycline (97.5%), while 90.0% were resistant to vancomycin. Eighty percent of *E. faecium* isolates were resistant to vancomycin and tetracycline. All *E. faecium* isolates showed phenotypic resistance to ampicillin, ciprofloxacin, erythromycin, nitrofurantoin and penicillin. Generally, *E. faecium* isolates exhibited more resistance to the eight antimicrobials tested than *E. faecalis* isolates (Table 6).

Table 6. Antimicrobial Susceptibility of *E. faecalis and E. faecium*

Species (Total)	Susceptibility Test Result	AMP n (%)	CHL n (%)	CIP n (%)	ERY n (%)	NIT n (%)	PEN n (%)	TET n (%)	VAN n (%)
E. faecalis (40)	Resistant	27 (67.5)	39 (97.5)	40 (100)	40 (100)	30 (75.0)	30 (75.0)	39 (97.5)	36 (90.0)
	Susceptible	13 (32.5)	1 (2.5)	0	0	10 (25.0)	10 (25.0)	1 (2.5)	4 (10)
E. faecium (10)	Resistant	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)	8 (80)	8 (80)
	Susceptible	0	0	0	0	0	0	2 (20)	2 (20)

n = number, AMP = ampicillin, CHL = chloramphenicol, CIP = ciprofloxacin, ERY = erythromycin, NIT = nitrofurantoin, PEN = penicillin, TET = tetracycline, VAN = vancomycin

3.4 Number of *E. faecalis* and *E. faecium* which were resistant to one, two and more than two antimicrobial classes

The majority (97.4%) of *E. faecalis* isolate were multidrug resistant, while 2.6% were resistant only to one class of antibiotics (Table 7). All *E. faecium* isolates were MDR.

Table 7. Number of *E. faecalis* and *E. faecium* which were resistant to one, two and more than two antimicrobial classes

Isolate (Total	All susceptible	Resistant to one class of antibiotic,	Resistant to two classes	Resistant to more than two classes of

number)	n (%)	n (%)	of antibiotic, n (%)	antibiotic, n (%)
E. faecalis (102)	0 (0)	0 (0)	1 (2.5)	39 (97.5)
E. faecium (6)	0 (0)	0 (0)	0 (0)	10 (100)

n = number, % = percent

3.5 MDR patterns

3.5.1 *E. faecalis* MDR Patterns

Only one *E. faecalis* isolate was resistant to two tested antibiotics. Thirty nine isolates were resistant to more than two antibiotic classes. (Table 8). The MDR pattern exhibited by the majority of *E. faecalis* isolates (18, 45%) was AMP, CHL, CIP, ERY, NIT, PEN, TET, VAN combination.

Table 8. MDR patterns of *E. faecalis*

MDR PATTERNS	No. of isolates	No. of antibiotic classes
CIP, ERY	1	2
CHL, CIP, ERY, PEN, TET	1	5
CHL, CIP, ERY, NIT, TET, VAN	3	6
AMP, CHL, CIP, ERY, TET, VAN	2	6
CHL, CIP, ERY, PEN, TET, VAN	3	6
AMP, CHL, CIP, ERY, NIT, PEN, TET	3	6
AMP, CHL, CIP, ERY, NIT, TET, VAN	3	7
AMP, CHL, CIP, ERY, PEN, TET, VAN	2	6
CHL, CIP, ERY, NIT, PEN, TET, VAN	4	7
AMP, CHL, CIP, ERY, NIT, PEN, TET, VAN	18	7

AMP = ampicillin, CHL = chloramphenicol, CIP = ciprofloxacin, ERY = erythromycin, NIT = nitrofurantoin, PEN = penicillin, TET = tetracycline, VAN = vancomycin

3.5.2 *E. faecium* MDR patterns

None of the *E. faecium* isolates was susceptible to all tested antibiotics. Two isolates were resistant to two antibiotics and the rest were resistant to three or more antibiotics (Table 9).

Table 9. MDR patterns of *E. faecium*

MDR patterns	*No. of isolates*	*No. of antibiotic classes*
AMP, CHL, CIP, ERY, NIT, PEN	2	6
AMP, CHL, CIP, ERY, NIT, PEN, TET, VAN	*8*	*7*

AMP = ampicillin, CHL = chloramphenicol, CIP = ciprofloxacin, ERY = erythromycin, NIT = nitrofurantoin, PEN = penicillin, TET = tetracycline, VAN = vancomycin

3.6 Presence of Antimicrobial Resistance Genes in *E. faecalis* and *E. faecium*

The most commonly detected resistant gene in *E. faecalis* was *ermB*. This was followed by *tetL* and *aac(6')-Ie-aph(2")-LA.* TetX was absent in these isolates, while the occurrence of vanA gene was infrequent. In *E. faecium*, the most commonly detected resistance gene was *aac(6')-Ie-aph(2")-LA* and *tetM*, while *ermA* was seldomly detected and *tetX* and *vanA* were undetected (Table 10).

Table 10. The number of different resistance genes detected in *E. faecalis* and *E. faecium* isolates

Resistance gene	*E. faecalis* (40)			*E. faecium* (10)		
	Detected (n)	Proportion (%)	Undetected	Detected (n)	Proportion (%)	Undetected
aac(6')-Ie-aph(2")-LA	33	82.50%	7	9	90.00%	1
ermA	2	5.00%	38	1	10.00%	9
ermB	39	97.50%	1	5	50.00%	5
tetK	19	47.50%	21	4	40.00%	6
tetM	31	77.50%	9	8	80.00%	2
tetL	38	95.00%	2	5	50.00%	5
tetX	0	0	40	0	0	10
vanA	**1**	2.50%	39	0	0	10

n = number

3.7 Association between antimicrobials and resistance genes

Differences in antimicrobial resistance patterns and resistance genes in both *Enterococcus* species were analyzed to assess possible associations between resistance phenotypes and their corresponding genotypes. A positive association between phenotype and genotype was found for tetracycline ($p = 0.047$) and erythromycin ($p = 0.008$), but there was no association between genotype and the vancomycin resistance phenotype ($p = 0.051$) (Table 11).

Table 11. Association between antimicrobial results and their corresponding resistance genes.

Antibiotic	Genes	X^2—Value	*p*-Value
TET	*tet*	3.945	0.047 ***
ERY	*erm*	6.947	0.008 ***
VAN	*vanA*	3.795	0.051

X^2 = Chi-square value; *p*-Value = significant at <0.05; TET = Tetracycline; ERY = Erythromycin; VAN = Vancomycin; *tet* = all tetracycline genes (*tetM*, *tetL*, *tetK* and *tetX*); *erm* = both *ermA* and *ermB* genes

4.0 Discussion

Detection of *E. faecalis* and *E. faecium* in this study is remarkable because these two species are the leading causes of enterococcal infections world-wide (Zhou, *et al*., 2020; García-Solache and Rice, 2019). Of interest too is the detection of antimicrobial resistance genes as well as virulence genes in these *Enterococcus* species in clinical specimens. It is important to note that these findings are of public health importance whether they have caused infection in the host or are just colonizing. Patients harboring such

Enterococcus species have the potential to act as reservoirs from which the organisms can be transmitted to healthcare workers, the surrounding environment and other patients (Jackson, *et al*., 2019; Cassone, *et al*., 2020). The current research presents an assessment of the current trends in species distribution, antimicrobial susceptibility, and virulence trait profiles of clinical enterococcal isolates at Kitwe Teaching hospital in Zambia. The majority of *Enterococcus* isolates (59/71) were obtained from urine specimens. This is consistent with findings from similar studies conducted in India, Egypt and France (Mohanty and Behera, 2022; Sumangala, Sharlee and Sahana Shetty, 2020; Said and Abdelmegeed, 2019) which reported 80.1%, 89/111; 54.4%, 56/103 and 59.5%, 25/42; 54.4% respectively.

In this study prevalence of *Enterococcus* was 30.9%. This finding align with previous report which indicated prevalence of *Enterococcus* to be 32.6% by Lancu and others in Romania (2023) and also agreed with a study done earlier whose prevalence was 31.1% in poultry droppings (Mwikuma, *et al*., 2023). However our findings did not agree with an earlier study which reported higher prevalence of 43.42% in India (Sreeja, *et al*., 2012). The present study's prevalence was higher than reports from studies conducted in Ethiopia (2.1%), Asian pacific (3.6%) and in USA and Canada (18.0% and 21.2%, respectively) (Abera, *et al*., 2021; Paul, Nirwan and Srivastava, 2017; Low, *et al*., 2001).

The prevalence of *Enterococcus faecalis* among all *Enterococcus* species was 56.3% (40/71, CI: 46.24-70.41) making it the most prevalent species. *Enterococcus faecium* accounted for 14.1%. This distribution agrees with other studies which found *E. faecalis* to be higher than *E. faecium*; 69.2% and 11.3% respectively in Saudi Arabia (Salem-Bekhit, *et al*., 2012) and in Kuwait with 85.3% and 7.7% respectively (Udo *et al*., 2003). However, a study done by Jia, Li and Wang (2014) shows a higher distribution of *E. faecium* (58.7%) than that of *E. faecalis* (33.0%). The majority of *E. faecalis* and *E. faecium* isolates were from urine specimens and were from age ranging from 16 to 45 years old. In agreement with this, studies by Boccella and others (2021) and Salem-Bekhit and others (2012) indicated that *E. faecalis* and *E. faecium* were isolated at higher frequency from urine cultures (32.5% and 46.6% respectively). *E. faecium* was only recovered from males and mainly from urine of non-hospitalized patients.

In the present study, the highest levels of resistance observed in both *E. faecalis* and *E. faecium* was to ciprofloxacin (100%) and erythromycin (100%). Our findings revealed that *E. faecium* exhibited higher resistance rates to most antimicrobial agents used in clinical treatment compared to *E. faecalis*. For example; all *Enterococcus faecium* isolates were resistant to ampicillin, chloramphenicol, ciprofloxacin, erythromycin, nitrofurantoin and penicillin whereas all *E. faecalis* isolates were resistant to just two drugs namely ciprofloxacin and erythromycin. These results align with previous reports (Horner, *et al*., 2021; Tollu and Ekin, 2020; Cui, *et al*., 2020; Golob, *et al*., 2019) which supports the notion that *E. faecium* is generally more prone to develop resistance than *E. faecalis*. The infections caused by *E. faecium* pose significant clinical challenges for physicians due to their higher resistance to drugs commonly used in clinical practice thereby limiting treatment options (Zhou, *et al*., 2020). All (100%) *E. faecium* and 97.5% *E. faecalis* isolates were multidrug resistant (MDR). This is concurrent with a studies by Esmail, Abdulghany and Khairy (2019), and Tremblay and colleagues (2011) in which 100% of all *E. faecalis* and *E. faecium* isolates respectively were MDR. Selective pressure exerted by broad application of antibiotics in health care and animal husbandry enhance development of MDR. This leads to increase in prevalence of resistance and creation of reservoirs resistance genes in some *Enterococcus* species, especially *Enterococcus faecalis* and *E. faecium* (Ahmad, *et al*., 2011).

The most prevalent resistance gene in *E. faecalis* was *ermB* (97.5%), followed by *tetM* (95.0%) and *aac(6')-Ie-aph(2")-LA* (82.5%). In *E. faecium*, the most frequently detected resistance genes were *aac(6')-*

Ie-aph(2")-LA (90.0%) and tetL (80.0%). The detection of *aac(6')-Ie-aph(2")-LA* gene in 82.5% of *E. faecalis* and in 90.0% of *E. faecium* was in line with Niu and others (2016) who found that 89.3% of *Enterococcus* isolates in their study carried the *aac(6')-Ie-aph(2")-LA* gene. Although the gene *aac(6')-Ie-aph(2")-LA*, which encodes resistance to gentamicin was detected in both *Enterococci* species tested, an association between the phenotype and genotype could not be established as gentamycin discs containing high concentrations of gentamicin such as 120 µg or 500 µg, which are used for detection of high-level aminoglycoside resistance, were not available. Amongst the *erm* genes detected, *ermB* was the most prevalent gene in erythromycin resistant *Enterococcus faecalis* isolates. This finding concurred with the findings of earlier studies (Ahmadpoor , et al., 2021; Marghmalek, *et al*., 2021; Kim, *et al*., 2019; Tian, *et al*., 2019). *tetM* was the most frequently detected gene among the tetracycline resistant *Enterococcus faecalis* isolates. Our findings collaborated with the findings of Tian and colleagues (2019).
The *vanA* gene was only detected in one *E. faecalis* isolate and was not detected in *E. faecium* isolates. The phenotypic resistance exhibited by both *E. faecalis* and *E. faecium* were due to other *van* genes which were not tested. This shows that *vanA* was not common in *Enterococcus* species in our study and is in contrast with what others studies assert. For example, Daghighi and others (2014) shows that 89.3% samples had *vanA* gene. Haghi, Lohrasbi, and Zeighami (2019) detected *vanA* gene in all *E. faecium* isolates. Others studies which shows predominance of *vanA* over other *van* genes include Jahansepas and others (2018) and Kristich, Rice and Arias (2014).

5.0 Conclusion

In essence, this study underscores the clinical significance of detecting and understanding the prevalence, distribution, and resistance patterns of *E. faecalis* and *E. faecium*. The findings contribute to the broader understanding of antimicrobial resistance and the potential impact on patient care, healthcare practices, and public health strategies. The study further shed light on the challenges posed by multidrug resistance and genetic factors affecting resistance. Addressing the challenges posed by these organisms requires a multi-faceted approach, encompassing appropriate antibiotic stewardship, infection control measures, and further research into novel treatment strategies to mitigate the impact of antimicrobial resistance.

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