FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 4 No. 3, September, 2020, pp 1 – 9
DOI: https://doi.org/10.33003/fjs-2020-0403-343

DISTRIBUTION AND ANTIBIOTICS SUSCEPTIBILITY PATTERNS OF *ENTEROCOCCUS* SPP. FROM A SELECTED HOSPITAL IN INDIA

[1,2]Muhammad I. Getso, [2]Subha Sundaramoorthy, [2]Kanishka, H.D, [1]Mansur Aliyu, [3]Ibrahim Yusuf, [1]Azeez-Akande O., [1]Isah M. Daneji, [*1]Alhassan A. Sharif

[1]Department of Medical Microbiology and Parasitology, College of Health Sciences, Bayero University Kano, Kano-Nigeria.
[2]Department of Microbiology, SRM Medical College and Research Centre, SRM University, Kattankulathur -603203, Tamil Nadu-India.
[3]Department of Microbiology, Faculty of Science, Bayero University Kano, P.M.B. 3011, Kano, Nigeria

[*]Corresponding Author's Email: sharifosis@gmail.com

ABSTRACT
Enterococci cause recurrent infections, especially among hospitalized patients. Their potential for resistance to multiple antibiotics and incumbent treatment failure constitutes a significant cause of morbidity and mortality. In this study, we aimed to determine the distribution of *Enterococcus* species from clinical samples and their antibiotic resistance profiles to supporting patients' treatments based on informed-decision. We conducted a cross-sectional study at SRM Medical College Hospital, Tamil Nadu, India, from January to December 2014. Sixty *Enterococcus* isolates, from different clinical samples, were included in the study. The isolates were identified to species level based on sugar fermentation and biochemical reactions. The antibiotic susceptibility profile was determined using disk diffusion and agar dilution methods based on CLSI guidelines. The majority of *Enterococcus* isolates were recovered from urine samples (51.67%) and pus (38.33%). The predominant isolates were *E. fecalis* (55%) and *E. fecium* (33.30 %,). Others were *E. avium* (3.3%) and 1.7 % each for *E. durans* and *E. raffinosus*. Overall, the isolates demonstrated the highest frequency of resistance to high-level gentamicin (33.30%), and one-third (33.30%) of the isolates were multidrug-resistant. Because the majority of the drug-resistant isolates were from urine and pus samples, we concluded that suspected cases of UTIs, wound infections, and sepsis need critical evaluation for possible enterococcal infection. Clinical use of gentamicin, among other antibiotics, shall be closely monitored while treating infections.

Keywords: Enterococcus, High-level gentamicin resistance, Multidrug resistance, Antibiotic Susceptibility, India.

INTRODUCTION

Enterococci are a natural component of human intestinal normal flora but are also important pathogens responsible for healthcare-associated and community-onset infections (Silverman *et al*., 1998). These infections present a serious problem in terms of medical and socio-economic costs as well as a significant cause in morbidity and mortality (EARSS, 2009).

Enterococci exceptionally cause disease in healthy individuals. The disease is mainly acquired endogenously and may disseminate via cross-infection among hospitalized patients (Mims *et al*., 1998). Under conditions where host's resistance is compromised, or where the integrity of the gastrointestinal or genitourinary tract has been disrupted, for example by instrumentation, *Enterococci* can spread to normally sterile sites, causing urinary tract infections, bacteremia, sepsis, subacute bacterial endocarditis, biliary tract infection, or intra-abdominal abscesses (Richard *et al*., 2001; Mims *et al*., 1998). Common sources of *Enterococcus* isolates are the penetrating injuries of the abdominal cavity, urinary tract infections, prostate infections, and infections of damaged or compromised skin, especially in burns and surgical wounds (Gary, 2011). With the inherent and continuous acquisition of resistance to multiple antibiotics, researchers recognize *Enterococci* as salient nosocomial pathogens that can be challenging to treat (Susan, 2013; Ibrahim, 2015).

Species of Enterococci implicated with human infections include *E. faecalis, E. faecium Enterococcus avium, Enterococcus gallinarum, Enterococcus casseliflavus, Enterococcus durans, Enterococcus raffinosus and Enterococcus mundti* (Devriese *et al*., 1987; Gorbatch *et al*., 2001). The emergence of *E. faecalis* and *E. faecium* as species with significant medical importance paralleled the increased usage of glycopeptides and high-level aminoglycosides for the treatment of human infections (Guzman Prieto *et al*., 2016).
Biotyping and antibiogram of *Enterococci* isolated from clinical specimens is an essential tool for epidemiologists and hospital policymakers to get useful information for hospital

antibiotic stewardship and overall treatment and prevention of the infections. The aim of this study is to biotype and evaluate antimicrobial susceptibility patterns of *Enterococci* isolated from clinical specimens in a tertiary care hospital in Tamil Nadu, India.

MATERIALS AND METHODS

Study design and data collection

We conducted this descriptive cross-sectional study at the Microbiology Department, SRM Medical College Hospital and Research Centre, Kattankulathur, Tamil Nadu, India, from January to December 2014. Sixty (60) *Enterococcus* isolates were obtained from different clinical samples submitted to the Microbiology Laboratory of SRM Medical College Hospital. They included urine samples, pus samples, blood samples, bronchial wash, and gastric fluid. Ethical approval for the study (Approval No: 592/IEC/2014) was obtained from the Institutional Ethical Committee of SRM MCH & RC, Tamil Nadu, India, before the commencement of the study.

Isolation and identification of Enterococcus

Nutrient agar, MacConkey agar, and 5% Sheep Blood agar plates (all prepared from dehydrated powder, HiMedia, India) were used to isolate *Enterococci* from blood, pus, and body fluids. Urine samples were inoculated on CLED (cysteine-lactose-electrolyte-deficient) agar, incubated overnight at 35^0C, and sub-cultured on Nutrient agar. Enterococci were presumptively identified on Blood agar as non-hemolytic, 0.5-1mm size *Streptococci*-like colonies; on MacConkey agar as small dark red magenta colonies and CLED agar as small yellow colonies (due to fermentation of lactose). We confirmed the colonies as *Enterococcus* based on their biochemical reactions such as positive Gram staining, negative Catalase test, positive Bile Aesculin test, and ability to grow in 6.5% NaCl broth (Collee *et al.,* 2012; Cheesbrough *et al.,* 2009). The isolates were further identified to species level by specific sugar fermentation reactions according to standard protocols (Facklam *et al*., 1970; Facklam *et al*., 1989; Collee *et al.,* 2012). We used microtitre 96-wells plates to test for acid production from various sugars (pyruvate, arabinose, mannitol, sorbitol, and sorbose) obtained from HiMedia India.

Antimicrobial Sensitivity Test

We determined the susceptibility to antimicrobial agents by the Kirby-Bauer disc diffusion method, according to Clinical Laboratory Standards Institute (CLSI) guidelines (CLSI, 2014). We used the following antibiotics discs (HiMedia, India): Ampicillin [AMP] (10 µg), Vancomycin [VAN] (30 µg), Teicoplanin [TEI] (30 µg), Amikacin [AK] (30 µg), Erythromycin [E] (15 µg), Tetracycline [TE] (30 µg), Linezolid [LZ] (15 µg), and Chloramphenicol [C] (30 µg). Besides, we used Nitrofurantoin disc [NIT] (300 µg) on isolates from urine samples. Further, we evaluated the high-aminoglycosides resistance using High-level Gentamicin disc [HLG] (120 µg) and Agar Dilution Method. We determined the high-level gentamicin resistance using agar dilution by spotting 10µL of 0.5 McFarland suspension of test strain on to the surface of Brain Heart Infusion Agar (BHIA) containing different concentrations of gentamicin. We regarded the presence of growth greater than one colony of the test strain in BHIA containing gentamicin at the concentration of 500 µg/mL as resistance (CLSI, 2014). We used *E. fecalis* (ATCC 29212) as a control strain.

Minimum Inhibitory Concentration (MIC) for strains that showed glycopeptides resistance (by disc diffusion method) was determined using Vancomycin and Teicoplanin Ezy MIC^{TM} strips (HiMedia India). Each of the E-strips was placed on a separate lawn culture made from 0.5 McFarland turbidity of the test strain incubated overnight at 37 ^{0}C.

Data analysis

Data obtained were analyzed by SPPS software version 22 (IBM, Chicago, IL, USA). The prevalence of *Enterococcus* species from clinical samples was expressed in simple proportions or percentages.

RESULTS

Sixty isolates recovered from clinical specimen were included in the current study; the majority from urine (51.67%, 31/60) followed by pus (38.33%, 23/60) samples [Table 1].

Table 1: Distribution of isolates in different clinical specimens

Type of Sample	Frequency (%)
Urine	31(51.67)
Pus	23 (38.33)
Bronchial wash	3 (5.00)
Gastric fluid	1 (1.67)
Blood	1 (1.67)
Post-op drain	1 (1.67)
Total	**60 (100)**

Table 2 shows the distribution of clinical isolates of *Enterococcus* recovered from clinical specimens by the respective wards. The majority of the samples were from surgical wards (46.3%, 25/60) and ICUs (24.97%, 15/60).

Table 2: Ward-wise distribution of isolates by clinical specimen

	Medical wards No. (%)	**Surgical wards No. (%)**	**Obs & Gyne No. (%)**	**ICUs No. (%)**	**Pediatric ward No. (%)**
Urine	7 (11.69%)	11 (18.37%)	2 (3.3%)	10 (16.7%)	1 (1.7)
Blood				1 (1.7)	
Bronchial wash	1 (1.7)	1 (1.7)		1 (1.7)	
Pus	3 (5%)	14 (23.38%)	2 (3.3%)	4 (6.6%)	
Post-op drain		1 (1.7)			
Gastric Fluid	1 (1.7)				
Total	**12 (20%)**	**27 (45%)**	**4 (6.7%)**	**16 (26.7%)**	**1 (1.7%)**

Obs&Gyne = Obstetrics and Gynecology ward, ICUs = Intensive Care Units, Post-op drain = Post-operative drain, No. = Numbers

Table 3 shows the distribution of *Enterococcus* species in various clinical specimens. The predominant isolates identified were *Enterococcus fecalis (55%)* followed by *E. fecium* (33.3%). We could not identify three of the isolates (5%) to species level based on the sugars and the biochemical characteristics.

Table 3: Distribution of *Enterococcus species* isolated from various clinical samples

	Number of isolates from clinical samples (%)						Total Frequency (%)
	Urine	Pus	Bronchial Wash	Gastric fluid	Blood	Post-operative drain	
E. fecalis	18 (30)	13 (21.7)	1 (1.7)	1 (1.7)	-	-	33/60 (55%)
E. fecium	12 (20)	5 (8.3)	1 (1.7)	-	1 (1.7)	1 (1.7)	20/60 (33.3%)
E. avium	1 (1.7)	1 (1.7)	-	-	-	-	2/60 (3.3%)
E. durans	-	1 (1.7)	-	-	-	-	1/60 (1.7%)
E. raffinosus	-	1 (1.7)	-	-	-	-	1/60 (1.7%)
E. spp	1 (1.7)	1 (1.7)	1 (1.7)	-	-	-	3/60 (5%)

The frequency of resistance to single and multiple antibiotics among the clinical Enterococcus isolates is shown in Figure 1, while the distribution of antibiotic resistance by different *Enterococcus* species is presented in Table 4. Out of the sixty isolates in our study, 63.3% showed resistance to at least one antibiotic. One-third (33.3%) of the isolates in our study were multidrug-resistant (Fig 1). Resistance to high-level gentamicin (HLG) was the most noticeable (33.33%) among the isolates examined (Table 4). Figure 2 shows the distribution of *Enterococcus species* that were resistant to HLG. Also, a ward-wise distribution of the antibiotic-resistant *Enterococcus* isolates was shown in Figure 3.

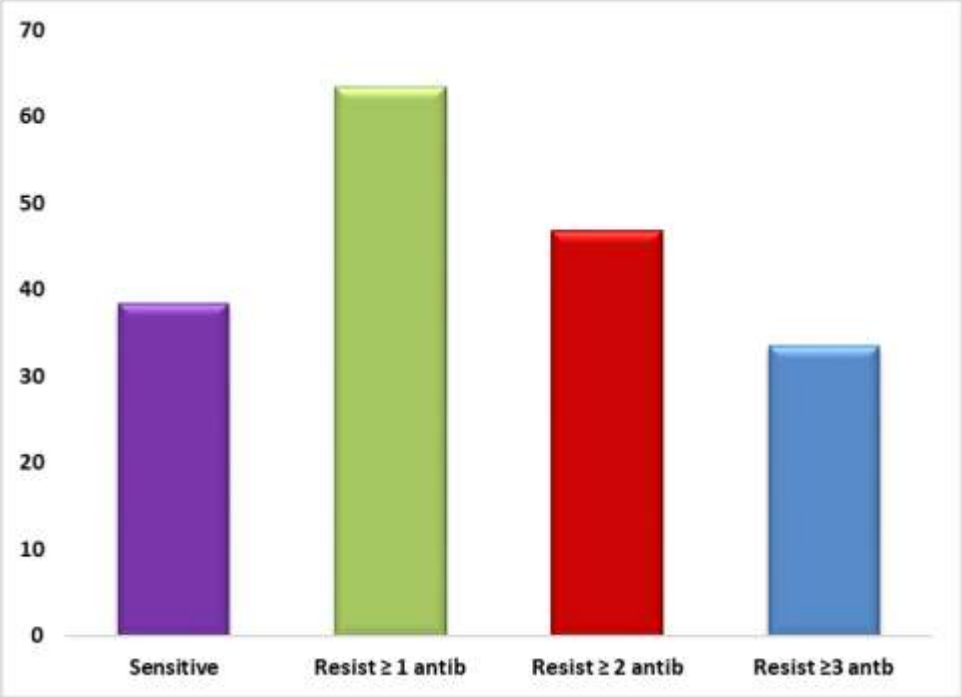

Figure 1: Frequency of resistance to multiple antibiotics among the clinical Enterococcus isolates
Sensitive = No resistance recorded, Resist ≥ 1 antib = resistant to one group of antibiotics, Resist ≥ 2 antib = resistant to two groups of antibiotics, Resist ≥3 antb = resistant to at least three groups of antibiotics (MDR).

Table 4: Antibiotic resistance profiles of Enterococcus isolates from clinical samples

	E. fecalis **(n=33)**		***E. fecium*** **(n=20)**		***E. avium*** **(n=2)**		***E. durans*** **(n=1)**		***E. raffinosus*** **(n=1)**		***E. spp*** **(n=3)**		**Total n= 60**	
Antibiotics	S n (%)	R n (%)	S n (%)	R n (%)	S n (%)	R n (%)	S n (%)	R n (%)	S n (%)	R n (%)	S n (%)	R n (%)	S n (%)	R n (%)
LZ	31 (51.67)	2 (3.33)	19 (31.67)	1 (1.67)	2 (3.33)	0 (0)	1 (1.67)	0 (0)	1 (1.67)	0.(0)	2 (3.33)	1 (1.67)	56 (93.33)	4 (6.67)
NIT	28 (46.67)	5 (8.33)	14 (23.33)	6 (10.00)	2 (3.33)	0 (0)	0 (0)	1 (1.67)	0.(0)	1 (1.67)	1 (1.67)	2 (3.33)	45 (75.00)	15 (25.00)
AMP	27 (45.00)	6 (10.00)	13 (21.67)	7 (11.67)	1 (1.67)	1 (1.67)	0 (0)	1 (1.67)	1 (1.67)	0.(0)	2 (3.33)	1 (1.67)	44 (73.33)	16 (26.67)
HLG	26 (43.33)	7 (11.67)	8 (13.33)	12 (20.00)	2 (3.33)	0 (0)	0 (0)	1 (1.67)	1 (1.67)	0.(0)	3 (5.00)	0.(0)	40 (66.67)	20 (33.33)
VAN	33 (55.00)	0 (0)	19 (31.67)	1 (1.67)	2 (3.33)	0 (0)	1 (1.67)	0 (0)	1 (1.67)	0.(0)	3 (5.00)	0.(0)	59 (98.33)	1 (1.67)
TEI	33 (55.00)	0 (0)	18 (30.00)	2 (3.33)	2 (3.33)	0 (0)	1 (1.67)	0 (0)	1 (1.67)	0.(0)	3 (5.00)	0.(0)	58 (96.67)	2 (3.33)
E	27 (45.00)	6 (10.00)	13 (21.67)	7 (11.67)	0 (0)	2 (3.33)	0 (0)	1 (1.67)	1 (1.67)	0.(0)	2 (3.33)	1 (1.67)	43 (71.67)	17 (28.33)
AK	29 (48.33)	4 (6.67)	14 (23.33)	6 (10.00)	1 (1.67)	1 (1.67)	1 (1.67)	0 (0)	0.(0)	1 (1.67)	2 (3.33)	1 (1.67)	47 (78.33)	13 (21.67)
C	33 (55.00)	0 (0)	19 (31.67)	1 (1.67)	2 (3.33)	0 (0)	1 (1.67)	0 (0)	1 (1.67)	0.(0)	2 (3.33)	1 (1.67)	58 (96.67)	2 (3.33)
TE	31 (51.67)	2 (3.33)	16 (26.67)	4 (6.67)	1 (1.67)	1 (1.67)	1 (1.67)	0 (0)	0.(0)	1 (1.67)	1 (1.67)	2 (3.33)	50 (83.33)	10 (16.67)

Ampicillin = [AMP], Vancomycin = [VAN], Teicoplanin = [TEI], Amikacin = [AK], Erythromycin = [E], Tetracycline = [TE], Linezolid = [LZ], Chloramphenicol = [C], Nitrofurantoin = [NIT], High-level Gentamicin = [HLG]. S= sensitive; R= resistant; n= number of the isolate

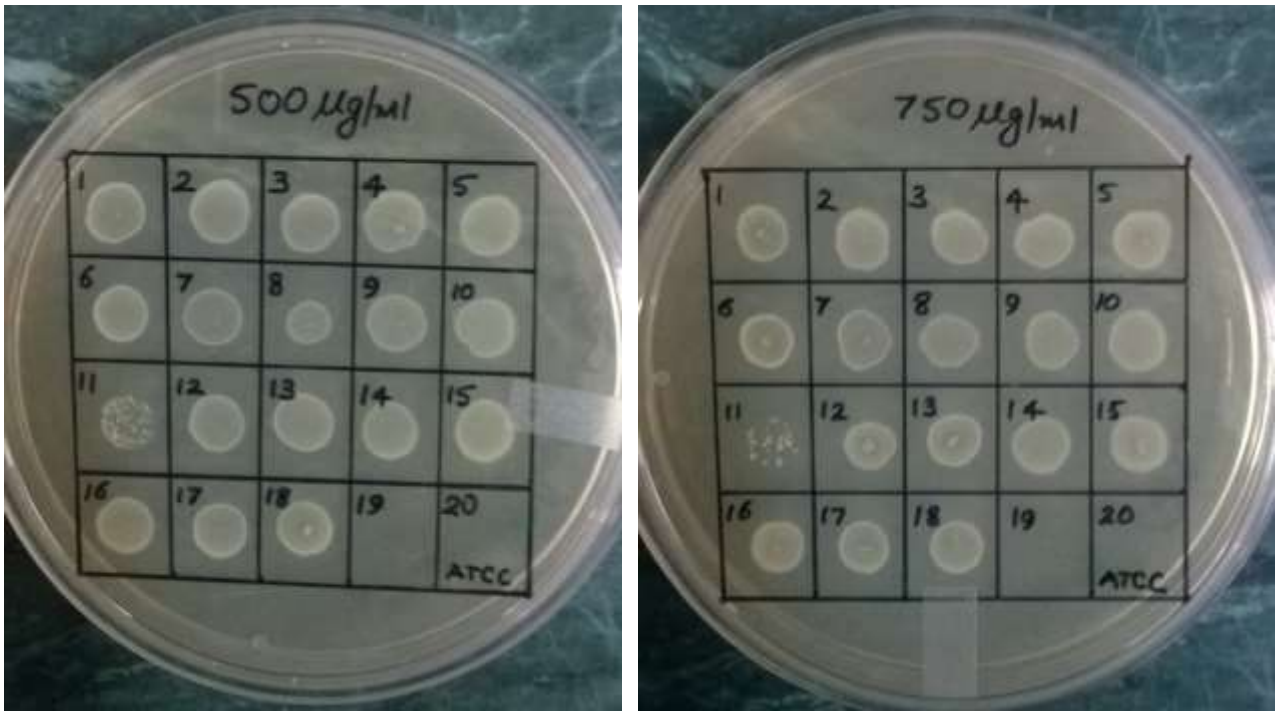

Plate 1: BHIA plates used to determine Minimum Inhibitory Concentration (MIC) of the HLG resistant isolates by agar dilution method.

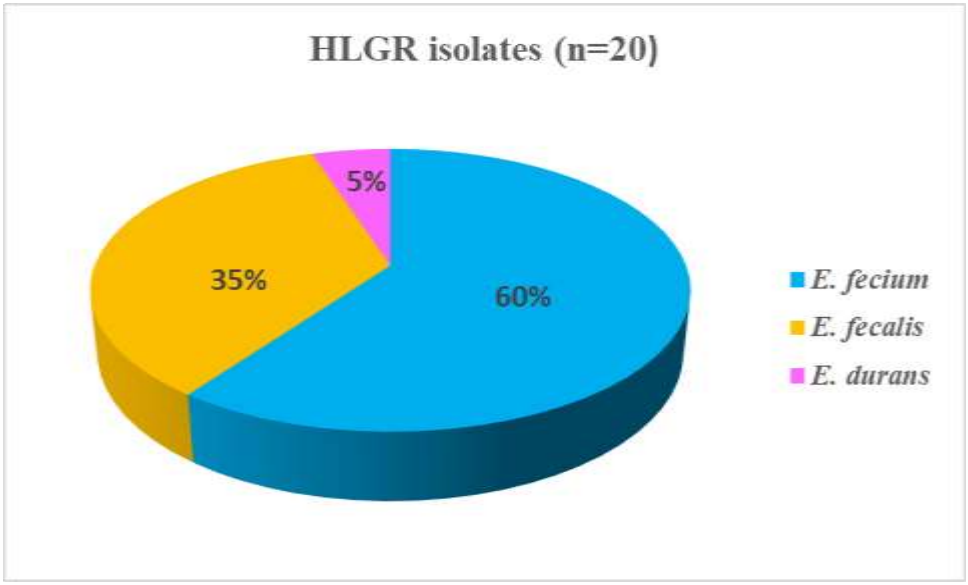

Figure 2: Distribution of High-level gentamicin (HLG) resistance among clinical *Enterococcus* isolates.

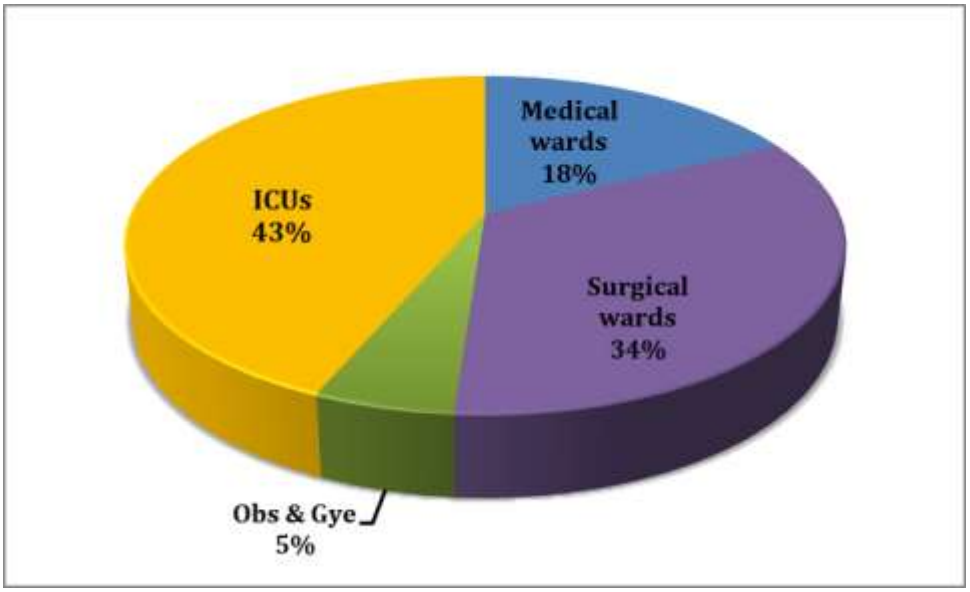

Figure 3: Distribution of antibiotic resistant *Enterococcus* isolates from different wards in the hospital.

DISCUSSION

The current study revealed that the majority of the *Enterococcus* isolates were from urine (51.67%, 31/60), followed by pus (38.33%, 23/60) samples. *Enterococci* are the normal resident of gastrointestinal tracts. The proximity of urethra and anus in the perineum might account for the high number of *Enterococcus* isolates from urine samples. The highest number of isolates from urine samples were also reported in previous studies (Praveen *et al*., 2012; Fernandes *et al*., 2013; Sharma *et al*., 2013; Tamanna *et al*., 2013; Golia *et al*., 2014; Padmasini *et al*., 2014) but this was not in agreement with reports from other countries where most of the isolates were from blood (Acharya *et al*., 2003) and pus (Salem-Bekhit *et al*., 2012). Similarly, isolates from pus were ranked second in some studies (Fernandes *et al*., 2013; Acharya *et al*., 2003). In this study, the majority of the samples were from surgical wards (46.3%, 25/60) and ICUs (24.97%, 15/60), as reported in Nepal (Acharya *et al*., 2003). However, predominant samples reported in other parts of India (Jain *et al*., 2011) and Saudi Arabia (Salem-Bekhit *et al*., 2012) were mainly from ICUs alone.

Out of the sixty isolates, the predominant isolates identified were *Enterococcus fecalis* (55%) followed by *E. fecium* (33.3%). *E. fecalis* has been the predominant *Enterococcus* species reported in various studies in India (Fernandes *et al*., 2013; Miskeen *et al*.,2002; Rahanghadale *et al.*, 2008; Deshpande *et al*., 2013; Palanisamy *et al*., 2013; Padmasini *et al*., 2013; Sharma *et al*., 2013; and Desai *et al*., 2001. Comparable results reported from other parts of the world include Europe (EARSS, 2009; Fisher *et al*., 2009), USA (Silverman *et al*., 1998; Madani *et al*., 1999), France (Monstravers *et al*., 2009), Kingdom of Saudi Arabia (Salem-Bakhit *et al*., 2012), Nepal (Acharya *et al*., 2003) and Nigeria (Olawale *et al.,* 2011). The dominance of *E. fecalis* among the isolates might be related to the fact that *E. fecalis* is as well the predominant luminal and gut mucosal microbiota than the rest of the species (Jandhyala *et al*., 2015). However, *E. fecium* was reported as predominant isolates from the blood of bacteraemic patients by Jain *et al*. (2011) and Randhawa *et al*. (2003).

The distribution of uncommon (non-fecalis non-fecium) *Enterococcus species* varies throughout the world. Our study reported *E. avium* in 3.3% of the isolates, 1.7 % each for *E. durans* and *E. raffinosus*. Studies in India (Desai *et al*., 2001; Praven *et al*., 2012; Sharma *et al*., 2013; Padmasini et al., 2014) reported the distribution of *E. avium* between 0.94% and 9.4% meanwhile; lesser proportions were reported from other parts of the world (Madani *et al.,* 1999). Similar to our findings, the distribution of *E. durans* in India and other parts of the world was between 0.6 and 4%. Similarly, our finding corroborates that of previous studies on the distribution of *E. raffinosus* (Sharma et al., 2013; Jain, 2011; Desai *et al*., 2011). The frequency of uncommon *Enterococci* in our study was 12%, which was comparable to that reported from some parts of India Fernandes *et al*. (2013) and Desai *et al*. (2011) but not in agreement with the findings of Despande *et al*. (2013) who did not report a single isolate of non-fecalis, non-fecium enterococci among clinical samples. Moreover, we could not identify three (5%) of the isolates in our studies to the species level based on the sugar and biochemical tests used.

One remarkable feature of enterococci is their resistance to a wide range of antibiotics, making efficient treatment of enterococcal infections highly challenging. The majority (63.33%) of isolates in our studies showed resistance to at least one antibiotic and were mainly recovered from pus. This result was comparable to those reported by other studies (Despande *et al*., 2013; Acharya *et al*., 2003) but another study (Lall *et al*., 2014) demonstrated that the majority of the drug-resistant isolates were recovered from urine samples. Resistance to high-level gentamicin (HLG) was the most noticeable (33.33%) among the isolates examined in this study. This finding corroborates the outcome of other studies from within India where HLG resistance among clinical *Enterococcus*

isolates was between 30% to 70% (Fernandes *et al*., 2013; Despande *et al*., 2013; Palanisamy *et al*., 2013, Padmasini *et al.,* 2013; Sharma *et al*., 2013;) and other parts of the world (Salem-Bakhit *et al*., 2012; Tamanna *et al.,* 2013; Acharya *et al*., 2003). The majority of the HLG resistant isolates were *E. fecium* (60%) and *E. fecalis* (35%) [figure 2]; 65% of the HLG resistant isolates were also resistant to ampicillin. The highlights mentioned above depicts the limited therapeutic options in treating enterococcal infections in the study centre. Resistance to at least three different groups of antibiotics is termed multidrug resistance (MDR). One-third (33.3%) of the isolates in our study were multidrug-resistant (shown in figure 1). MDR is a common phenomenon among clinical *Enterococcus* isolates; it's been reported in various studies around the world (Despande *et al*., 2013; Acharya *et al*., 2003; Jain *et al*., 2011; Madani *et al.,* 1999; Lall *et al*., 2014). In the current study, *E. fecium* showed more resistance to antibiotics compared to *E. fecalis*. Similar results were reported in other studies (Despande *et al.,* 2013; Palanisamy *et al.,* 2013; Jain *et al*., 2011; Madani *et al*., 1999). Isolates from ICUs showed the highest frequency (43%) of antibiotic resistance (figure 3), and the majority were from urine samples. This fact might not be surprising, because patients in ICUs are usually on the catheter, relatively immune-compromised, and prone to multiple antibiotic therapies. Similarly, reports from Saudi Arabia (Salem-Bakhit *et al*., 2013) showed that isolates from ICUs exhibited the highest frequency of antibiotic resistance. However, in Nepal, the highest antibiotic resistance was reported among isolates from a surgical ward (Acharya *et al.,* 2003). Among the HLG resistant isolates, one isolate was susceptible when evaluated for MICs by agar dilution method, as shown in Plate 1; other isolates confirmed to be resistant. Among the twenty HLG resistant isolates, the majority were *E. fecium* (60%) and *E. fecalis* (35%).

One isolate (1.7%), of *E. fecium,* recovered from blood was resistant to both vancomycin and Teicoplanin. The MIC of the isolate was determined by HiMediaEzy MIC strips (HiMedia India) and was found to be above 256µg/µL for both vancomycin and Teicoplanin; shows that the isolate was of the VanA phenotype. A low frequency of vancomycin-resistant *Enterococcus* (VRE) of VanA phenotype exhibiting a high level of vancomycin resistance above 256µg/µL was also reported in India (Maradia *et al.,* 2017). In addition to glycopeptides, the isolate was also resistant to Ampicillin, HLG, Erythromycin but susceptible to Linezolid and chloramphenicol. Resistance to vancomycin is relatively low throughout India (Fernandes *et al*., 2013; Despande *et al*., 2013; Golia *et al*., 2014; Randhawa *et al*., 2003; Maradia *et al*., 2017). Vancomycin-resistant *Enterococcus* (VRE) infections pose serious challenges to clinicians because they are usually susceptible to a limited number of antibiotics including Linezolid, making them barely untreatable.

CONCLUSION

The distribution and antibiotic resistance of *Enterococcus* isolates in urine and pus is higher than in any other clinical sample examined in the health facilities; suspected cases of UTI, wound infections, and sepsis need critical evaluation for possible enterococcal infection. One-third of the isolates were multidrug-resistant and were also resistant to HLG and ampicillin. Confirmed susceptibility to antibiotics shall be available before prescription against enterococcal infections for judicial drug use. Clinical use of gentamicin, among other antibiotics, should be closely monitored while treating infections.

Limitations of the study

Limited resources hinder our ability to investigate the molecular basis for identification and antibiotic resistance among the studied Enterococci.

Acknowledgment:

We sincerely acknowledge the inputs of the academic and laboratory staff of the Microbiology Department, SRM Medical College Hospital & Research Centre, SRM University, Kattankulathur, Tamil Nadu, India. We also acknowledge the contributions of the scholars whose articles we cited in this manuscript.

Source of funding:

There was no financial support from any institution to this research.

Conflict of interests:

The authors declare that they have no conflict of interest.

Authors' contributions: MIG, SS, and AAO conceived the idea and designed the study; MIG, KHD, and SS performed the laboratory work; MIG, AAS, MA, and AAO interpreted the data. MIG, IY, AAS, and IMD drafted the manuscript. All authors read and approved the final manuscript.

REFERENCES

Silverman J, Thal LA, Perri MB, Bostic G, Zervos MJ (1998). Epidemiologic evaluation of antimicrobial resistance in community-acquired enterococci. *Journal of clinical microbiology*.1;36(3):830-2.

European Centre for Disease Prevention and Control (2009). European Antimicrobial Resistance Surveillance Center EARSS Annual Report Available at http://ecdc.europa.eu/en/activities/surveillance/EARS-Net/Documents/2008_EARSS_Annual_Report.pdf

Mims C, Playfair J, Roitt I, Wakelin D, Williams R (1998). Medical Microbiology Textbook Second Edition. Saunders, USA.

Richard AH, Pamela AC (2001). Lippincott's Illustrated Review: Microbiology. Lippincott and Wilkins. The USA. p 154-156

Gary EK (2011). Organisms and Infections Identification: Isolation and Identification of Streptococci and Enterococci. BIOL 230 Lab Manual, Lab 4.

Susan LF (2018). Enterococcal infection: An overview. Available at http://emedicine.medscape.com/article/216993. [Accessed 08/10/2018]

Ibrahim GM, Sundaramoorthy S, Madhavan R. (2015). Enterococcus: an emerging global superbug, *International*

Journal of Science and Research, 4(2):1539 – 1543. https://www.ijsr.net/search_index_results_paperid.php?id=SUB151518

Devriese LA, Van de Kerckhove A, Kilpper-Bälz R, Schleifer KH (1987). Characterization and identification of Enterococcus species isolated from the intestines of animals. *International Journal of Systematic and Evolutionary Microbiology*. ;37(3):257-9.

Gorbach SL, Falagas M (2001). Enterococcus species. 5-Minute Infectious Disease Consult. 1st Edition. Lippincott Williams and Wilkins. USA.

Guzman Prieto, A. M., van Schaik, W., Rogers, M. R., Coque, T. M., Baquero, F., Corander, J., & Willems, R. J. (2016). Global emergence and dissemination of enterococci as nosocomial pathogens: attack of the clones?. *Frontiers in microbiology*, *7*, 788. https://doi.org/10.3389/fmicb.2016.00788

Collee JG, Fraser AG, Marmion BP & Simmons A [editors] (2012). Mackie and McCartney Practical Medical Microbiology. 14th Ed. Churchill Livingstone, Elsevier Indian reprint. Ch 12 & Ch 20.

Cheesbrough M (2009). District Laboratory Practice in the Tropics, Part II; Microbiology. 2nd Ed. Cambridge University Press, UK.

Facklam RR, Moody MD (1970). Presumptive identification of group D streptococci: the bile-esculin test. *Applied Microbiology*. 1;20(2):245-50.

Facklam RR, Collins MD (1989). Identification of Enterococcus species isolated from human infections by a conventional test scheme. *Journal of clinical microbiology*.1;27(4):731-4.

Clinical Laboratory Standards Institute (2014)."Performance standards for antimicrobial susceptibility testing, twenty-fourth informational supplement. M02-A11, M07-A9 and M11-A8," CLSI Document M100-S24, Clinical and Laboratory Standards Institute, Wayne, PA, USA 2014.

Praveen KD, Srikanth Nandini, Rathai R (2012). Prevalence and antibiogram of Enterococcus species in a Tertiary care centre. *World J Pharm & Pharm Sci.*

Fernandes SC, Dhanashree B (2013). Drug resistance & virulence determinants in clinical isolates of Enterococcus species. *The Indian Journal of Medical Research*. ;137(5):981.

Sharma R, Pai C (2013). Prevalence of Various Enterococcal Infections and Its Antibiotic Susceptibility with Special Reference to Vancomycin and High-Level Gentamicin Resistance in a Tertiary Care Centre in Navi Mumbai. *Int J Pharm Sci Rev Res*.23:122-5.

Tamanna S, Barai L, Ahmed AA, Haq JA (2013). High-level gentamicin resistance and susceptibility to Vancomycin in enterococci in a tertiary care hospital of Dhaka City. *Ibrahim Medical College Journal*. 7(2):28-31.

Golia S, Nirmala AR (2014). Isolation and speciation of enterococci from various clinical samples and their antimicrobial susceptibility pattern with special reference to high-level aminoglycoside resistance. *International Journal of Medical Research and Health Sciences*.1;3(3):526-9.

Padmasini E, Padmaraj R, Ramesh SS (2014). High-level aminoglycoside resistance and distribution of aminoglycoside-resistant genes among clinical isolates of Enterococcus species in Chennai, India. *The Scientific World Journal.* http://dx.doi.org/10.1099/2014/329157

Acharya A, Khanal A, Kanungo R, Mohapatra T (2007). Characterization and susceptibility patterns of clinically important Enterococcus species in eastern Nepal. *Nepal Med Coll J*.;9(4):250-4.

Salem-Bekhit MM, Moussa IM, Muharram MM, Alanazy FK, Hefni HM (2012). Prevalence and antimicrobial resistance pattern of multidrug-resistant enterococci isolated from clinical specimens. *Indian journal of medical microbiology*. 1;30(1):44.

Jain S, Kumar A, Kashyap B, Kaur IR (2011). Clinico-epidemiological profile and high-level aminoglycoside resistance in enterococcal septicemia from a tertiary care hospital in east Delhi. *International Journal of Applied and Basic Medical Research*. 1(2):80.

Miskeen PA, Deodhar L (2002). Antimicrobial susceptibility pattern of Enterococcus species from urinary tract infections. *The Journal of the Association of Physicians of India*. 50:378-81.

Rahangdale VA, Agrawal G, Jalgaonkar SV (2008). Study of antimicrobial resistance in enterococci. *Indian journal of medical microbiology*. 1;26(3):285.

Deshpande VR, Karmarkar MG, Mehta PR (2013). Prevalence of multidrug-resistant enterococci in a tertiary care hospital in Mumbai, India. *The Journal of Infection in Developing Countries*. 15;7(02):155-8.

Palanisamy S, Sankari Karunakaran SN (2013). Antimicrobial resistance profile and characterisation of Enterococcus species from various clinical samples in a tertiary care hospital. *International Journal of Medical Research and Health Sciences*. 1;2(3):328-33.

Desai PJ, Pandit D, Mathur M, Gogate A (2001). Prevalence, identification and distribution of various species of enterococci isolated from clinical specimens with special reference to urinary tract infection in catheterized patients. *Indian journal of medical microbiology*. 1;19(3):132.

Fisher K, Phillips C (2009). The ecology, epidemiology, and virulence of Enterococcus. *Microbiology.*155(Pt 6): 1749–1757. http://dx.doi.org/10.1099/mic.0.026385

Madani TA, Kabani A, Orr P, Nicolle L (1999). Enterococcal bacteremia in a tertiary care centre in Winnipeg. *Canadian Journal of Infectious Diseases and Medical Microbiology*. 10(1):57-63.

Montravers P, Lepape A, Dubreuil L, Gauzit R, Pean Y, Benchimol D, Dupont H (2009). Clinical and microbiological profiles of community-acquired and nosocomial intra-abdominal infections: results of the French prospective, observational EBIIA study. *Journal of Antimicrobial Chemotherapy*. 5;63(4):785-94.

Olawale KO, Fadiora SO, Taiwo SS (2011). Prevalence of hospital-acquired enterococci infections in two primary-care hospitals in Osogbo, Southwestern Nigeria. *African Journal of infectious diseases*. 5(2).

Jandhyala SM, Talukdar R, Subramanyam C, Vuyyuru H, Sasikala M, Reddy DN (2015). Role of the normal gut microbiota. *World J Gastroenterol* 21(29): 8787-8803 DOI: http://dx.doi.org/10.3748/wjg.v21.i29.8787.

Randhawa VS, Kapoor L, Singh V, Mehta G (2014). Aminoglycoside resistance in enterococci isolated from paediatric septicaemia in a tertiary care hospital in north India. *Indian Journal of Medical Research*. 1;119:77-9.

Lall N, Basak S (2014). High-level aminoglycoside resistant Enterococcus species: A study. *International Journal of Current Research and Review*. 6(3):16-21.

Robert CM (1998). Enterococcal Resistance. Clinical updates in infectious disease. National Foundation for Infectious Diseases NFID Publication Vol(4):3 Englewood USA; Vol(4):3

Maradia, M. R., Mehta, K., Prajapati, K., Vadsmiya, M., Shah, P., & Vegad, M. (2017). Prevalence of multidrug-resistant Enterococcus species isolated from urine samples in a tertiary care hospital, Western India. *International Journal of Medical Science and Public Health*, *6*(4), 715-720. DOI:10.5455/ijmsph.2017.0850530112016