Enterococcal Infections and Antimicrobial Resistance in a Tertiary Care Hospital, Eastern India

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Background and study aim: During last two decades, there has been a world-wide trend in increasing occurrence of entero-coccal infections in the hospitals. The aim of present study was to determine the spectrum of enterococcal infections, species prevalence, antimicrobial resistance and characteristics of vancomycin resistant enterococci (VRE) in a tertiary care hospital, Eastern India.

Patients and methods: Between January 2013 and July 2014, 152 *Enterococcus* species were obtained from clinical samples. Enterococci were identified using standard biochemical tests. Antimicrobial susceptibility was tested by Kirby-Bauer disk diffusion according to Clinical & Laboratory Standards Institute (CLSI) guidelines.VRE agar base was used to screen VRE isolates. Minimum inhibitory concentration (MIC) values of VRE isolates were determined using Epsilometer-test. VRE isolates were also examined by PCR to detect *vanA* gene.

Results: From 1602 clinical samples, 961 (60%) were culture positive and 152 (15.8%) enterococcal isolates were obtained. Most common species isolated was *E. faecalis* (63.8%) followed by *E. faecium* (35.5%). Majority of enterococcal infections were detected from ICUs and surgical wards and clinically presented as UTIs. Disk diffusion method showed 67.1% were resistant to penicillin, 61.2% ampicillin, 58.5% ciprofloxacin, 46.7% high-level gentamicin, 42. 8% high-level streptomycin, 7.9% teicoplanin and none to linezolid. Twenty (13.2%) enterococcal isolates were vancomycin resistant in VRE screen and disk diffusion method. Epsilometer-test of VRE isolates showed 8 (40%) isolates were resistant and 9 (45%) were intermediately resistant. From 20 VRE isolates, six showed VanA and two VanB phenotypes and all six VanA phenotypes had *vanA* gene cluster.

Conclusion: More accurate and reliable MIC determination tests should be performed in all suspected VRE isolates. Confirmatory PCR is required for identifying resistant gene cluster.

INTRODUCTION

Gram-positive enterococci are normal commensals of the gastrointestinal tract, oral cavity, genitourinary tract and skin especially perineal area in both humans and animals **[1]**. *Enterococci* are traditionally regarded as low grade pathogens but have emerged as an important cause of nosocomial infections in recent years. Although 19 species within the genus have been recognized, *E. faecalis* is the most predominantly isolated pathogen, followed by *E. faecium* **[2]**. The most frequent infections caused by enterococci are urinary tract infections, followed by intra-abdominal and intra-pelvic abscesses, post operative wound infections and blood stream infections **[3,4]**. The rise in prevalence of entero-coccal infections in humans is influenced by the ability of enterococci to escape the action of most commonly used antibiotics.

Acquired high-level aminoglycoside resistance (HLAR), ß-lactamases production, glycopeptide resistance including vancomycin resistance enterococci (VRE) have emerged, thus posing therapeutic challenge to the health care professionals **[5]**. In recent years, VRE are the main sources of infections in humans and carry the transferable vancomycin resistance markers **[6]**. Despite the increasing reports of VRE in different countries, there is little known about its prevalence and characteristics in Eastern India. Therefore, this study was undertaken to identify the different species of enterococci isolated from clinical specimens, their antimicrobial resistance patterns, prevalence and characteristics of VRE in a tertiary care hospital, Eastern India.

PATIENTS AND METHODS

Study design and data collection:
A prospective was conducted from July 2013 to June 2014 in the department of clinical microbiology at a tertiary care hospital, Odisha state, Eastern India. A total of 1602 clinical specimens i.e., urine, pus/wound swabs, blood and body fluids were collected from patients and transferred to the laboratory without delay for further processing. Specimens such as throat swabs, sputum and faeces were excluded as enterococci are usually remain as commensals. A detailed evaluation of patient's age, sex, address, associated co-morbidities, admission to the hospital, duration of stay in the hospital and antibiotic treatment history was carried out. An infection is considered as nosocomial if all the elements of a site specific infection criterion of Center of Disease Control and Prevention (CDC) were first present together on or after the 3 rd hospital day (day of hospital admission is day 1) **[7]**.

Sample processing and identification of Enterococcus species:
In the laboratory, all the collected samples were cultured aerobically on solid media i.e., blood agar and MacConkey agar. Blood specimen was cultured in trypticase soy broth (TSB) and subcultured on blood agar, chocolate agar and MacConkey agar. All cultured plates were incubated at 37°C for 48 hours and examined for growth. Identification of genus Enterococcus was done by using colony morphology, Gram stain, catalase test, bile esculin agar hydrolysis test, growth in 6.5% NaCl (salt tolerance test), growth at 10°C and 45°C (heat tolerance test) **[8]**. Speciation was done by detecting fermentation of arabinose, mannitol, raffinose and sorbitol as well as motility and pigment production, if any **[9]**.

Antimicrobial susceptibility testing and phenotypic detection of vancomycin resistance enterococci (VRE):
All enterococcal isolates were subjected to antimicrobial susceptibility testing by the standard Kirby-Bauer disk diffusion method (KBDDM) **[10]**. The following standard antibiotic disks were placed on Mueller-Hinton agar plate: penicillin (10U/disk), ampicillin (10µg), ciprofloxacin (5µg), high-level gentamicin (120µg), high-level streptomycin (300µg), vancomycin (30µg), teicoplanin (30µg), and linezolid (30µg). Zone diameter was measured and interpreted according to standards of the clinical and laboratory standards institute (CLSI) **[11]**. Quality control strain *E. faecalis* (ATCC 51299) were used to ensure the potency of each antimicrobial agent used.

Vancomycin agar screen test was performed by using VRE agar base supplemented with vancomycin and meropenem. Cultural characteristics of luxuriant growth along with blackening of the medium were seen after an incubation of 35° C for 24 to 48 hours in VRE isolates. Vancomycin sensitive strain *E. faecalis* (ATCC 29212) was used as negative control and vancomycin resistant strain *E. faecalis* (ATCC 51299) was used as positive control **[11]**.

Minimum inhibitory concentration (MIC) of VRE isolates were determined by Epsilometer test (E-test) method. The MIC determination paper strip was coated with vancomycin in a concentration gradient manner capable of showing MICs in the range of 0.016 to 256 µg/ml, when testing against *Enterococcus* species. MIC values of ≤4 µg/ml, 8-16 µg/ml, and ≥32 µg/ml were considered as sensitive, intermediate resistant and resistant isolates respectively. Similarly, the MIC values of VRE isolates were detected for teicoplanin and linezolid respectively.

All culture media, biochemical reagents, antibiotic discs, vancomycin agar screen test, MIC test strips and standard reference strains used in the study were purchased from Hi Media Laboratories Pvt. Ltd., Mumbai, India.

Genotypic detection of Enterococci and VRE isolates
16S rRNA genes expression for enterococcal isolates:
16S rRNA sequence was amplified from genomic DNA obtained for PCR with the upstream primer:

27F Primer–5'-GAGTTTGATCMTGGCTCAG-3' and the downstream primer: 1492R Primer–5'-GGYTACCTTGTTACGACTT-3',which generated a DNA fragment of approximately 1500 bp. Both of forward and reverse primers were ordered from IDT genomics, Japan.

PCR amplification of 16S rRNA genes:
Total 31DNA isolate mixtures were used in 16S rRNA amplification. PCR was performed in a reaction volume of 25 µL containing 1X Taq polymerase buffer with 1.5 mmol/L –1 of MgCl2 (New England Biolab, USA), 100 µL/mol/L–1 of each dNTP (3B black bio, JAPAN), 0.5 pmol of forward and reverse primers, 50-100 ng of genomic DNA and1.0 U of Taq DNA polymerase (New England biolab, USA). PCR amplifications were performed in a thermal cycler (Biorad, USA). Primers specific to bacterial 16SrRNA were used for PCR amplification. The thermal conditions for 16SrRNA primer pair were [94˚C for pre-denaturation, 5 min (one cycle); 94˚C for denaturation, 1 min, annealing 55˚C, 1 min, extension 72˚C, 2 min (30cycles);final extension 72˚C, 10 min (one cycle)]. The amplified products were resolved in 1.5% agarose gel, stained with ethidium bromide (10 mg/ml) and photographed under UVP version gel documentation system. Negative and positive controls were included in PCR reactions.

Reproducibility of PCR reactions was checked by performing duplicate reactions for each template DNA isolated. DNA templates used in the study were able to give reproducible PCR amplification results in duplicated experiment. All amplified products were separated by electrophoresis in 1.5% agarose gels with 10 mg/mL–1 of ethidium bromide at 50 V constant voltages for 2 h. The gels were scanned in Gel documentation system (UVP Laboratories, USA) [Figure 1].

Primer designing for vancomycin resistance gene:
One candidate *vanA* vancomycin resistance gene cluster was evaluated in this study. Resistance genes sequences were collected from the previously submitted NCBI data base (http://www.ncbi.nlm.nih.gov/). All the collected sequences were multiple aligned for online EMBL clastalW data base (http://www.ebi.ac.uk/Tools/msa/clustalw2/). Primers were designed using Primer3 online software (http://primer3.ut.ee/). Forward primer: 5'-GGATAGCTACTCCCGCCTTT-3' and reverse primer: 5'-CCGTTTCCTGTATCCGTCCT-3' were used which generated an amplicon size of approximately 350 bp for *vanA* gene cluster. The specificity of the primer sets were confirmed by the presence of a single band of appropriate size obtained after PCR amplification. The amplicons were sequenced and submitted to gene bank data base by BLASTN analysis against the vancomycin resistance bacterial genome; the uniqueness of the primers was examined.

PCR amplification for vancomycin resistance gene:
PCR was performed in a total volume of 25 µl containing 10 pmol each of forward and reverse primers, 2.5 mM of MgCl2, 200 µM each of the four deoxyribonucleotide triphosphates (dNTPs), 0.5 U of Taq DNA polymerase, 1x concentration of PCR buffer (Invitrogen, Life Technologies, Brazil) and 50 to 100 ng of isolated bacterial genomic DNA. The template was denatured by heating at pre-denaturation of 95°C for 5 min. This was followed by 39 cycles of denaturation 30 sec at 95°C, 45 sec annealing and 1 min elongation at 72°C, with a final extension of 7 min at 72°C. The Bio-Rad PCR system was used for PCR amplification. The amplicons were resolved in 1.5%agarose gel using 0.5x tris-acetate-EDTA (TAE) buffer.

RESULTS

Enterococcal isolates:
Out of 1602 clinical specimens, 961 (60%) were culture positive and 152 (15.8%) non-repeated enterococcal isolates were obtained. *E. faecalis* was the commonest species 97 (63.8%) isolated, followed by *E. faecium* 54 (35.5%) and *E. casseliflavus* 1 (0.7%). Maximum number of *Enterococcus* species were detected from urine 89 (58.6%), followed by wound and soft tissue discharge 48 (31.6%), blood 9(5.9%) and ascitic fluid 6 (3.9%) [Table-1]. Majority of them 116 (76.3%) were detected from in-patients i.e, intensive care units 49 (32.3%), surgical wards 37 (24.3) and medicine wards (19.7%) [Table-2]. The mean age of *Enterococcus* isolated study participants was 55.8 ± 13.6 years.

Antimicrobial resistance pattern:
From 152 enterococcal isolates, 20 (13.2 %) were detected as VRE in VRE agar based screening method. In KBDDM, out of 20 VRE isolates, 12 were VR *E. faecalis* and 8 were VR *E. faecium*. The distribution of antimicrobial

susceptibility patterns of isolated enterococci were summarized in Table (3). The results showed that majority of isolates were resistant to penicillin (67.1%), followed by ampicillin (61.2%), ciprofloxacin (58.5%), high-level gentamicin (46.7%), high-level streptomycin (42.8%), vancomycin (13.2%), teicoplanin (7.9%). All enterococcal isolates were sensitive to linezolid.
Out of 20 VRE, 8 (40%) were resistant, 9 (45%) were intermediately resistant and 3 (15%) were sensitive in E-test. The distribution pattern of teicoplanin E-test for VRE isolates were 6 (30%) resistant, 6 (30%) were intermediately resistant and 8 (40%) were sensitive [Table-4]. All VRE isolates had MIC values of ≤ 2 µg/ml and were interpreted as linezolid sensitive.

Detection of vancomycin phenotypes and genotypes:
From 20 VRE isolates, 6 had high-level vancomycin resistance (MIC ≥64 µg/ml) and high-level teicoplanin resistant (MIC ≥16 µg/ml) were detected as VanA phenotype and 2 had high-level vancomycin resistance (MIC ≥32 µg/ml) and teicoplanin sensitive (MIC ≤0.25 µg/ml) were detected as VanB phenotype. All VanA phenotype isolates were positive for *vanA* gene cluster [Figure 2].

Table (1): Distribution of Enterococcus Species in various clinical specimens

Type of clinical specimen	Number (%) of Enterococcal species isolated			Total (%)
	E. faecalis	*E. faecium*	*E. casseliflavus*	
Urine	58	31	0	89 (58.6)
Skin and soft tissue discharge	29	18	1	48 (31.6)
Blood	6	3	0	9 (5.9)
Ascitic Fluid	4	2	0	6 (3.9)
Total (%)	97 (63.8)	54 (35.5)	1 (0.7)	152 (100)

Table (2): Distribution of Enterococcus in various clinical units

Type of clinical units	Number (%) of Enterococcal species isolated (n=152)			Total (%)
	E. faecalis	*E. faecium*	*E. casseliflavus*	
Out-patient units	23	13		36 (23.7)
In-patient units				
ICUs	32	17		49 (32.3)
Surgical units	22	14	1	37 (24.3)
Medicine units	20	10		30 (19.7)
Total (%)	97 (63.8)	54 (35.5)	1 (0.7)	152 (100)

Table (3): Antimicrobial resistance pattern of enterococcal isolates

Antibiotic (disk content)	Resistance		
	E. faecalis n=97 (%)	*E. faecium* n=54 (%)	Total* n=152 (%)
Penicillin (10 Units)	63 (64.9)	39 (72.2)	102 (67.1)
Ampicillin (10 µg)	58 (59.8)	35 (64.8)	93 (61.2)
Ciprofloxacin (5 µg)	54 (55.7)	35 (64.8)	89 (58.6)
High-level gentamicin (120 µg)	46 (47.4)	25 (46.3)	71 (46.7)
High-level streptomycin (300 µg)	42 (43.3)	23 (42.6)	65 (42.8)
Vancomycin (30 µg)	12 (12.4)	08 (14.8)	20 (13.2)
Teicoplanin (30 µg)	06 (6.2)	06 (11.1)	12 (7.9)
Linezolid (30 µg)	0	0	0

*One *E. casseliflavus* isolate was sensitive to vancomycin, teicoplanin and linezolid

Table (4): Distribution of vancomycin resistant enterococci according to its phenotypic and genotypic characteristics

Isolate number ***E. faecalis***	**MIC values of vancomycin (µg/ml)**			**MIC values of teicoplanin (µg/ml)**			**Phenotype of vancomycin resistance**	**Genotype of vancomycin resistance**
	≤ 4 S*	**8-16 I****	**≥ 32 R*****	**≤ 2 S***	**4 I****	**≥ 8 R*****		
1	2			1.5				
2			32	0.25			VanB	
3			96			32	VanA	*vanA*
4		8			4			
5		8		2				
6			256			96	VanA	*vanA*
7			64			64	VanA	*vanA*
8		16			4			
9		8		2				
10	4			0.5				
11			32	0.125			VanB	
12		16		2				
Isolate number *E. faecium*								
1		8			4			
2	4			0.75				
3			256			16	VanA	*vanA*
4			128			32	VanA	*vanA*
5		16			4			
6		8			4			
7			96			32	VanA	*vanA*
8		8			4			

*S- Sensitive, I**- Intermediately resistant, R***- Resistant

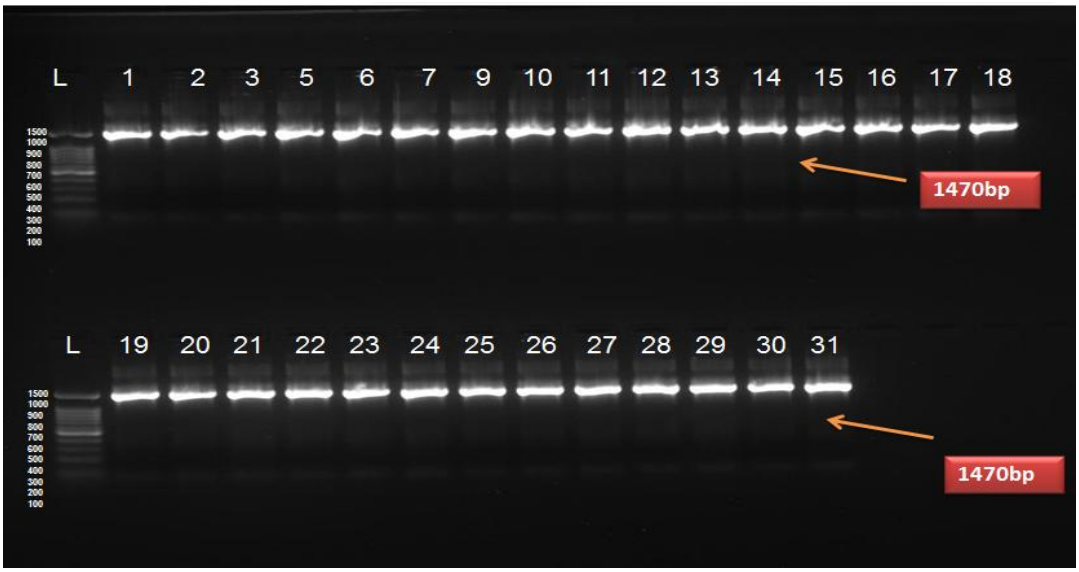

Figure (1): Gel image analysis of 16s rRNA amplification of enterococcal isolates
L: Marker (100 to 1500 bp, DNA ladder) 1 to 31: Enterococcus species (1470 bp)

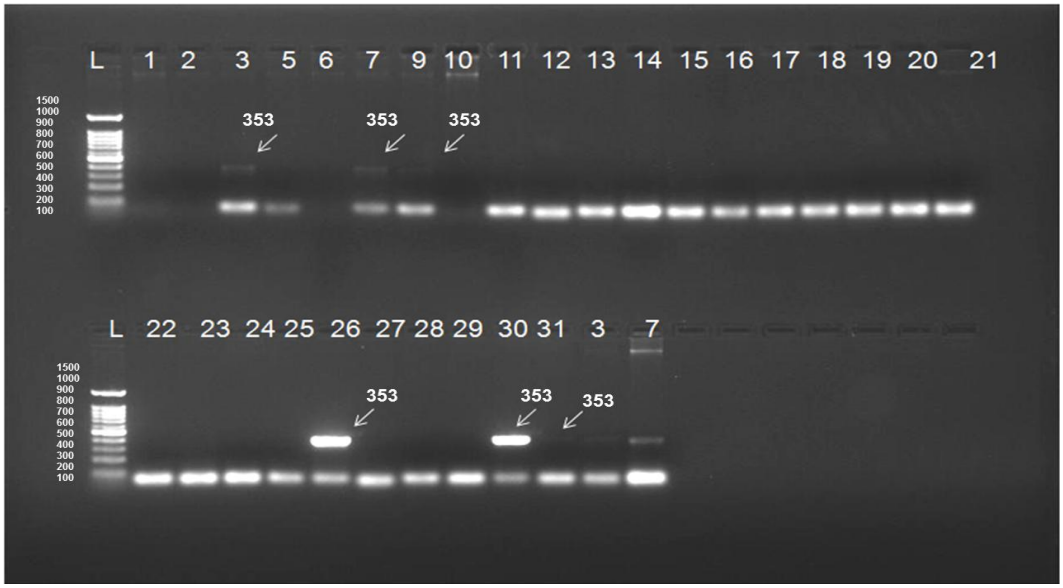

Figure (2): Agarose gel electrophoresis showing positive amplification of 353 base pair fragments specific for *vanA* from VRE isolates. Lane 1: Marker (100 to 1500 bp, DNA ladder), Lane 3, 7 & 9: *E. faecalis vanA* gene cluster, Lane 26, 30 & 31: *E. faecium vanA* gene cluster, Lane 3: Negative control (without template DNA), Lane 7: Positive control (*E. faecium* 15559 containing *vanA* gene cluster)

DISCUSSION

Enterococcus is a part of normal human intestinal flora and traditionally regarded as low grade pathogen. During last two decades, it has emerged as increasingly important nosocomial infections. Vancomycin in combination with an aminoglycoside can provide effective treatment for severe *Enterococcus* infections, while increasingly resistant to both antibiotics have been reported in different parts of the world **[12,13,14]**. In this study the pattern of antibiotics resistance, prevalence of VRE and most commonly associated *vanA* gene cluster were explored. Present study was conducted at a tertiary care hospital, Odisha state, Eastern India consisted of 152 non-repeated strains of *Enterococcus* species that were isolated from hospitalized and out-patients.

In our study from 961 culture positive growth, 152 (15.8%) *Enterococcus* species were isolated. The most common species isolated was *E. faecalis* (63.8%) followed by *E. faecium* (35.5%) and *E. casseliflavus* (0.7%) which was comparable to the distribution of Enterococcus species in different parts of the world **[15-22]**. Predominance of *E. faecalis* in the endogenous flora of the body could be the reason behind its high proportion among hospital isolates. While *E. faecalis* remains the predominant species in clinical infections, *E. faecium* isolates are increasing in proportion.

Present study revealed the most frequent infection was urinary tract infections (58.6%), followed by skin and soft tissue infections (31.6%) and blood stream infections (BSIs). Similar distribution of Enterococcal infections were reported in previous studies **[22,24,25]**. In these settings, enterococci are part of endogenous mixed flora commonly found in the gastrointestinal tract **[26]**. Majority of Enterococcal infections were detected from hospitalized patients and more commonly seen among patients in intensive care units (ICUs) and surgical wards. Similar incidence rate were found by other authors **[25]**. Prolonged ICU hospital stay and increased invasive procedure applications are some possible explanations.

Resistance to ß-lactam antibiotics i.e., penicillin and ampicillin were observed in 67.1% and 61.2% respectively in our study. Similar ß-lactam resistant rates were detected in other studies **[18,21,22,27]**. Recently, a study conducted by Bhatt et. al. had shown 97.5% of all enterococcal isolates were resistant to both penicillin and ampicillin **[28]**. Since ß-lactams remain the drug of choice for most non-severe enterococcal infections, increasing resistance to these antibiotics is of global concern. High-level ß-lactam resistance in enterococci is due to the production of low affinity penicillin binding proteins 5 (PBP 5), or the production of ß-lactamases **[29]**. Our study revealed HLG and HLS resistance were 46.7% and 42.8% of all isolates respectively. Similar resistance rates were detected in other studies **[19,20,27,28,30,31]**. However, in a study performed by Deshpande et al. **[18]** had revealed a high proportion of test isolates exhibited resistance to HLG (73.5%) and HLS (70.8%) respectively. High-level resistance to amino-glycosides is acquired through two mechanisms: modification of ribosomal attachment sites and the production of aminoglycoside-modifying enzymes **[32]**. The presence of high level resistance to aminoglycosides destroys the bactericidal activity obtained with ß-lactam and aminoglycoside synergy in clinical practice **[33]**.

In our study from 152 enterococcal isolates, 20 (13.2%) VRE were obtained by VRE agar screen test and KBDDM. Similarly percentage of VRE isolates were obtained from other studies **[17, 20,21,22,24,28]**. Higher vancomycin resistance rates of 21.05% and 19.6% were reported by Oberoi et. al. and Deshpande et al. respectively **[19,18]**. Some researchers even did not find a single isolate of VRE from their studies **[25,30]**. The prevalence of VRE is reported to be between 0% to 30% in various studies across the world [**16,17,19,24,25]**. Glycopeptides such as vanco-mycin and teicoplanin are cell-wall active agents, exerting their antimicrobial effect by binding with high affinity to D-Ala-D-Ala termini of pentapeptide precursors in order to inhibit the synthesis of peptidoglycans. Glycopeptide resistance arises when low-affinity pentapeptide precursors D-Ala-D-Lac or D-Ala-D-Ser are formed and high affinity precursors D-Ala-D-Ala are eliminated **[34]**. Among VRE isolates, 40% were resistant with high MIC values (64 to 256 µg/ml), 45% were intermediately resistant and 15% isolates were sensitive in E-test. Similarly, Sreeja et al. **[31]** had reported 27% of the enterococci which showed intermediate resistant to vancomycin by KBDDM, were found to be sensitive to vancomycin in E-test. The inaccuracy of disc diffusion and screening method has resulted in inappropriate use of this drug as a part of treatment regimen. Our study revealed 12.4%

were VR *E. faecalis* and 14.8% were VR *E. faecium*. Similar resistance pattern was observed by Deshpande et. al. **[18]** in contradiction to these studies, Vidyalakshmi et. al. **[35]** reported, out of 450 enterococcal isolates, 18(4%) were VRE and all isolates belonged to *E. faecium*. In the early 1990s, *E. faecalis* resistance was low against vancomycin and ampicillin while *E. faecium* were high (60% and 80% respectively) and are raising trend **[36]**. In recent times, the species difference is very unusual, because the gene responsible for this resistance can be transferred easily in the laboratory between the two species carried on pheromone responsive plasmids or conjugative transposons **[23]**. In our study, from 152 enterococcal isolates 12 (7.9%) were resistant to teicoplanin in KBDDM. Out of 20 VRE isolates, 6 were resistant (MIC ≥8 µg/ml), 6 were intermediately resistant (4 µg)/ml and 8 isolates were sensitive in E-test. Similar resistance pattern of enterococci towards teicoplanin were observed by various authors **[18,24,28]**. Fernandes et. al. **[20]** were noticed that from thirteen VRE isolates, five were resistant to teicoplanin. Based on MIC values of vancomycin and teicoplanin, majority of isolates 6 (30%) in our study were VanA phenotype (three *E. faecalis* and 3 *E. faecium*) and 2 (10%) were VanB phenotype (*E. faecalis*).

In our study all enterococcal isolates were sensitive to linezolid in KBDDM. MIC values of all VRE isolates were ≤ 2µg/ml. Other studies have also reported 100% enterococcal isolates were sensitive to linezolid **[22,24,25,30,31]**. Few studies had also detected linezolid resistance cases **[17,18,28]**. Linezolid resistance is rare in enterococci. Variety of sporadic point mutations in rRNA genes that confer linezolid resistance have been identified, the most common of which is G2576T **[37]**.

Genotypic detection for the *vanA* gene using conventional PCR revealed that 6 (30%) VRE isolates were positive for this gene yielding a band size of 390 bp. These isolates also showed a high level resistance to vancomycin and teicoplanin (VanA phenotype). The *vanA* gene was present in three isolates each of *E. faecalis* and *E. faecium*. Recently, Zadeh et al. **[24]** in Iran had reported 59% of vancomycin-resistant strains carried *vanA* gene and 7.95% of VRE strains carried *vanB* gene. Similarly, Yasliani et al. **[17]** had studied 17 VRE for their van genotypes by PCR found 6 (35.2%,), 4 (23.5%) and 1 (5.88%) of *vanA*, *vanB* and *vanC* gene respectively. In another study by Salem-Bekhit et al. **[16]** in Saudi Arabia detected all seven VanA phenotypes were positive for *vanA* gene and a 732-bp PCR product was obtained in all positive isolates. One intermediate vancomycin resistant isolate was detected as *vanB* genotype (635-bp). VanA phenotype associated with *vanA* gene cluster found on the transposon, or "jumping" genetic element, Tn 1546 is responsible for most of the human cases of VRE around the world, and is mostly carried by *E. faecium* **[38]**.

There were few limitations in our study. The E-test for detection of MIC values could be performed only in VRE isolates using vancomycin, teicoplanin and linezolid antibiotic strips. The most common *vanA* gene cluster could be detected in conventional PCR. The availability and the cost factor were major hindrance for this study.

CONCLUSION

The prevalence of VRE in vancomycin screen agar and KBDDM was 13.2 %. Enterococci were most commonly isolated from ICUs and surgical wards and UTI was the most commonly associated infection. VanA and VanB phenotype were obtained in 40% of VRE isolates and 30% of isolates were associated with *vanA* gene. More reliable MIC determination test may be performed in all suspected VRE isolates, so that vancomycin resistance phenotypes can be detected and appropriate therapy may be initiated. When-ever feasible PCR should be used for confirmation of VRE genotypes. Thus, a cooperative effort between health care providers and hospital microbiology laboratory personnel is required that will allow VRE to be promptly and accurately detected. Surveillance cultures in high prevalence areas such as ICUs and surgical wards are immediate requirements in order to keep the spread of vancomycin resistance in control.

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