Original Research Paper

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ISOLATION AND CHARACTERIZATION OF ENTEROCOCCUS FROM VARIOUS CLINICAL SAMPLES IN TERTIARY CARE INSTUTE WITH SPECIAL REFERENCE TO VRE (VANCOMYCIN RESISTANT ENTEROCOCCUS)AND VISE (VANCOMYCIN INTERMEDIATE SENSITIVE ENTEROCOCCUS)

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ABSTRACT

BACKGROUND: Enterococcus, an indigenous flora of the intestinal tract, is known to be relatively avirulent in healthy individuals, but turns as pathogens in hospitalized patients and are emerging nosocomial pathogen.

OBJECTIVE: To Isolate and characterise Enterococci and to determine their antibiotic resistance pattern with special reference to VRE and VISE.

MATERIAL AND METHODS: This prospective study was conducted in the Department of Microbiology,Gajra Raja Medical College, Gwalior. Isolation and Identification was done by conventional methods and antibiotic susceptibility testing was done by Kirby-Bauer Disc Diffusion method and MIC was determined by an E test strip.

RESULT: In this study enterococci were mostly isolated from urine 65 (59.09%), followed by blood 26 (23.63%) and pus 14 (12.72%). Seven isolate of *E. faecalis* (7.60%) was found resistant to vancomycin by Kirby-Bauer Disc Diffusion test and resistance was of high level (MIC>256 gm/dl). Vancomycin resistance was detected in *E. faecium 9 (50%)*.

CONCLUSION: Only two enterococal species were isolated in this study. They were *E. faecalis* 92 (83.63%) and *E. faecium* 18 (16.36%). In *E. faecalis*, 84 (91.30%) isolates showed resistance to penicillin. In *E. faecalis*, 58 (63.04%) isolates showed resistance to ampicillin . In *E. faecalis*, 50(54.34%) isolates showed Gentamycin Resistance. In *E. faecalis*, 51(55.43%) isolates showed Streptomycin Resistance. Seven vancomycin resistant *E. faecalis* (VRE) was isolated in this study. These isolate was highly resistant to vancomycin showing MIC > 256 gm/dl.All the strain of enterococcus isolates were sensitive to linezolid.

KEYWORDS : VRE, VISE, E-Test

INTRODUCTION:

Enterococcus, an indigenous flora of the intestinal tract, may turn as pathogens in hospitalized patients[1].

Enterococci are normal commensals adapted to the nutrient enriched, oxygen depleted, ecologically complex environment of the intestinal tract of humans and animals[1].However when they colonize sites where they are not normally found these turns as pathogens and cause a wide range of diseases such as urinary tract infections, bloodstream infections, wound infections, and endocarditis[2].

Enterococci have become emerging nosocomial pathogen, in spite of their low levels of virulence.The increasing importance of these bacteria is largely due to their resistance to many antimicrobial agents. In particular multiple drug resistant *Enterococci faecium* strains carrying intrinsic and acquired resistance determinants posses life threatening clinical conditions.

MATERIAL AND METHODS

This prospective study was conducted in the Department of Microbiology, Gajra Raja Medical College,Gwalior, a tertiary care institute. Enterococcus spp. isolated from various clinical samples like urine, pus, pleural fluid, peritoneal fluid, blood, and CSF samples were submitted for pathogen identification and susceptibility testing.

Isolates of Enterococci from throat swab, sputum, vaginal swabs, and stool were excluded from the study, as they formed a part of normal flora[15].

All samples was collected following aseptic procedure in an appropriate sterile container. Blood sample was collected in BacT/ ALERT FN Plus blood culture bottle.

Microscopy: A primary smear is made from the sample and stained with Gram stain, gram positive cocci which appear mainly in pairs slightly ovoid in shape and may appear in short chains, or as single cells were suspected of being enterococcus.[15]

Culture:

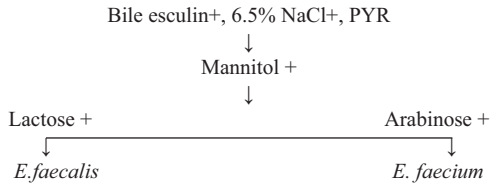
Sample was inoculated on 5% sheep Blood agar and MacConkey and incubated overnight at 37°C.

Catalate test- *Enterococci* are catalase negative.The three tests taken as most reliable are : Growth in bile esculin agar, growth in 6.5% Sodium chloride and positive PYR test. (Hydrolysis of α-pyrrolidonyl β-naphthylamide test)[24]

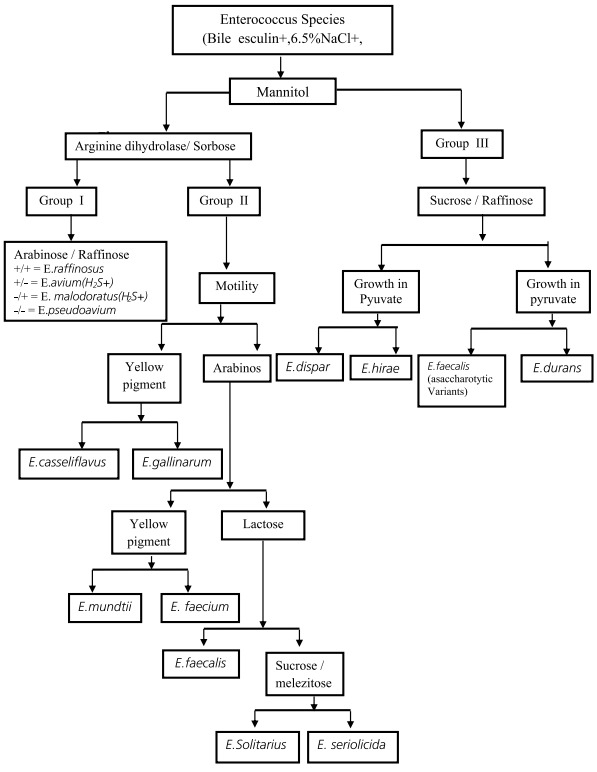
Enterococci were selected by colony morphology from the primary isolation plates. Suspected colonies of the genus Enterococcus on blood agar were small (0.5-1 mm) size, semitransparent, smooth, low convex discs.[24,25] It showed no hemolysis, sometimes showed α and β hemolysis. On MacConkey agar *Enterococci* are identified as small (0.5-1mm), usually magenta coloured colonies. On gram stain (secondary smear)enterococci appear as pairs of oval cocci, the cells in a pair arranged at an angle to each other. These colonies of enterococci are catalase negative.

Enterococci were identified on the basis of their ability to hydrolyse of L-pyrolidonyl- β-naphthylamide (PYR), salt-resistant growth (6.5% NaCl), and growth resistant to 40% bile with esculin hydrolysis.

Identification of enterococcus spp. All above feature suggested genus Enterococcus. Identification of species was done on the basis of biochemicals.[24]



Flowchart used for identification of Enterococcus species.



ANTIBIOTIC SUSCEPTIBILITY TESTING

Each enterococcal isolates was tested by Kirby Bauer disc diffusion testing on Muller Hinton Agar.[141] Determination of MIC of Vancomycin by E test strip method[55]

Table 1 Antimicrobial agents for Enterococci

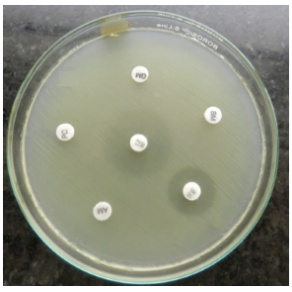
Antimicrobial (For all sample)	Symbol	Disc content in gm	Zone diameter, breakpoints		
			S	I	R
β-lactams					
Penicillin	P	10 U	≥ 15	-	≤ 14
Ampicillin	AMP	10	≥ 17	-	≤ 16
Aminoglycosides					
Gentamicin	GEN	120	≥ 10	7-9	≤ 6
Streptomycin	S	300	≥ 10	7-9	≤ 6
Glycopeptides					
Vancomycin	V	30	≥ 17	15-16	≤ 14
Oxazolidinones					
Linezolid	LZ	30	≥ 23	21-22	≤ 20
Antimicrobial (Only for urine)					
Nitrofurantoin	NIT	300	≥ 17	15-16	≤ 14
Ciprofloxacin	CIP	5	≥ 21	16-20	≤ 15
Levofloxacin	LE	5	≥ 17	14-16	≤ 13
Norfloxacin	NX	10	≥ 17	13-16	≤ 12
Tetracycline	T	30	≥ 19	15-18	≤ 14

S= sensitive, I= intermediate, R=resistant

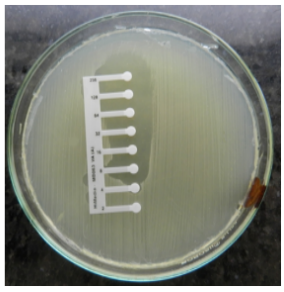
RESULTS:

(A) ANTIMICROBIAL RESISTANCE PROFILE OF CLINICAL ENTEROCOCCAL ISOLATES BY DISC DIFFUSION TEST

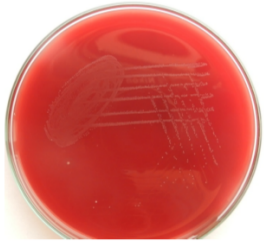
Based upon Kirby Bauer disc diffusion testing, isolates with resistance and decreased susceptibility (intermediate category in DDT) to various antimicrobial was identified . In *E. faecalis* 84 (91.30%) isolates showed resistance to penicillin, 58(63.40%) to ampicillin. Seven *E. faecalis* showed no zone in Disc Diffusion Test to vancomycin and labeled as vancomycin resistant enterococci (VRE). All *E. faecalis* were sensitive to linezolid. *E. faecalis* isolated from urine showed low resistance to nitrofurantoin 22 (23.91%) as compared to ciprofloxacin 38 (41.30%), tetracycline 40 (43.47%), levofloxacin 42 (45.66%), norfloxacin 48 (52.17%),. In *E. faecium* 15(88.88%) isolates showed resistance to penicillin, 10(55.55%) to ampicillin. *E. faecium* were resistant to vancomycin 11(61.11%) and all *E. faecium* were sensitive to linezolid *E. faecium* isolated from urine showed low resistance to nitrofurantoin 04 (22.22%), compare to ciprofloxacin 08 (44.44%), levofloxacin 10 (55.55%), norfloxacin 12 (66.66%), tetracycline 12 (66.66%).



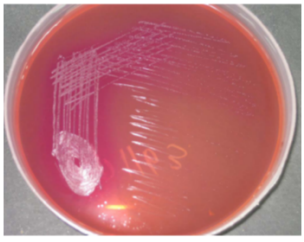
AST by Kirby Bauer Disc Diffusion method



E-test for Vancomycin MIC-2-256µg/ml



Showing colony of Enterococcus faecalis on Blood Agar

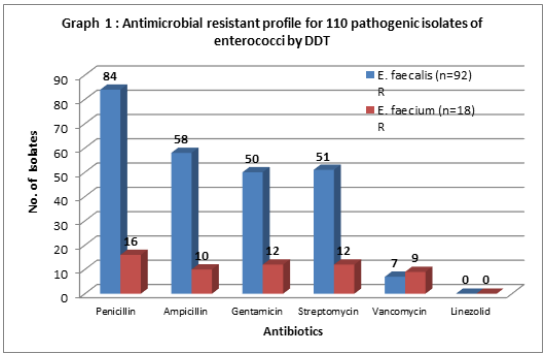


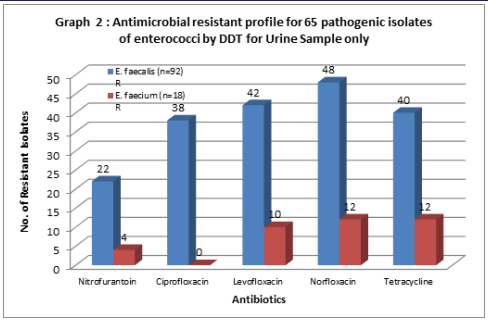
Showing colony of Enterococcus faecalis on Mac Conkey Agar

Table 2 Antimicrobial resistant profile for 110 isolates of enterococci by DDT

Antimicrobial (For all sample)	Number of resistant pathogenic isolates (n=110)					
	E. faecalis (n=92)			*E. faecium* (n=18)		
	R	I	Total (%)	R	I	Total (%)
β-lactam						
Penicillin	84	0	84(91.30%)	16	0	16(88.88)
Ampicillin	58	0	58(63.04%)	10	0	10(55.55%)
Aminoglycosides						
Gentamicin	50	O	50(54.34%)	12	0	12(66.66%)
Streptomycin	51	0	51(55.43)	12	0	12(66.66)
Glycopeptides						
Vancomycin	07	05	12(13.04%)	9	2	11(61.11%)
Oxazolidinones						
Linezolid	0	0	0	0	0	0
Antimicrobial (only for urine) (n=65)						
Nitrofurantoin	22	0	22(23.91%)	04	0	4(22.22%)
Ciprofloxacin	38	0	38(41.30%)	O8	0	08(44.44%)
Levofloxacin	42	0	42(45.65%)	10	0	10(55.55%)
Norfloxacin	48	0	48(52.17%)	12	0	12(66.66%)
Tetracycline	40	0	40(43.47%)	12	0	12(66.66%)

R=Resistance, I=Intermediate





(B) CORRELATION OF ANTIMICROBIAL RESISTANCE IN ENTEROCOCCI WITH SITE OF ISOLATION

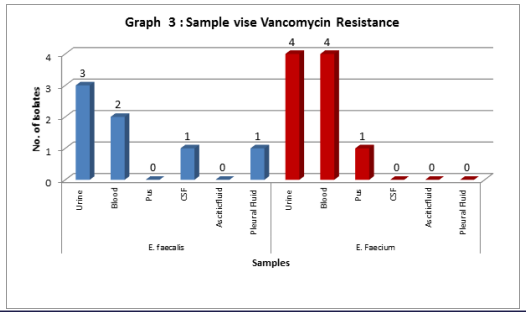
Table 3 Correlation of antimicrobial resistance in enterococci with the site of isolation

Antimicrobial	Urine	Pus	Blood	Ascitic fluid	Pleural fluid	CSF	Total
E. faecalis							
β-lactam							
Penicillin	50	09	20	2	2	1	84
Ampicillin	35	8	10	2	2	1	58
Aminoglycosides							
Gentamicin	32	7	6	2	2	1	50
Streptomycin	28	6	12	2	2	1	51
Glycopeptides							
Vancomycin	3	0	2	0	1	1	7
Oxazolidinones							
Linezolid	0	0	0	0	0	0	0
E. faecium							
β-lactam							
Penicillin	10	2	3	0	0	0	15
Ampicillin	7	1	2	0	0	0	10
Aminoglycosides							
Gentamicin	8	2	2	0	0	0	12
Streptomycin	8	2	2	0	0	0	12
Glycopeptides							
Vancomycin	5	1	3	0	0	0	9
Oxazolidinones							
Linezolid	0	0	0	0	0	0	0

Above table highlights that drug resistant *E. faecalis* and drug resistant *E. faecium* were isolated from all clinical specimen and hence they are capable of pathogenesis any site in the body. More frequent isolation of drug resistant *E. faecalis* and drug resistant *E. faecium* from urine is in proportion to the higher frequency isolation of enterococci from urine.

Table 4 Sample vise Vancomycin Resistance :

Species of Enterococci	Sample	VR	VISE	Total
E. faecalis	Urine	3	3	6
	Blood	2	2	4
	Pus	-	-	-
	CSF	1	-	1
	Asciticfluid	-	-	-
	Pleural Fluid	1	-	1
E. Faecium	Urine	4	1	5
	Blood	4	1	5
	Pus	1	-	1
	CSF	-	-	-
	Asciticfluid	-	-	-
	Pleural Fluid	-	-	-



DISCUSSION

Recent years have witnessed increased interest in enterococci because of their ability to cause serious infections because of their increasing resistance to many antimicrobial agents.[49,54,142]

In a CDC survey of nosocomial infection, enterococci accounted for 13.9% of urinary tract infections (UTIs), second only to *Escherichia coli* as a sole agent of nosocomial UTIs.[41] Enterococci are responsible for causing UTIs (1st most frequent) intra-abdominal and intra-pelvic abscesses or postsurgery wound infections (2nd most frequent), blood stream infections (3rd most frequent).[40,41]

In the present study, enterococci were mostly isolated from urine (59.09%), followed by blood (23.63%) and pus (12.72%) and Recently, Bose et al from Maharashtra published the similar finding. According to them enterococci were isolated most commonly from urine (62.13%), blood (27.02%), pus (7.9%).[9] Similar finding were also reported from Manipal (Sikkim) and by Agarwal et al from Lucknow.[14,16]

It has been documented that *E. faecalis* and *E. faecium* are the most common species accounted for approximately 80-90% and 10-15% respectively of enterococcal isolates.[20] Based upon the scheme of Facklam and Collins (1989), out of 110 enterococci two enterococcal species were identified: *E. faecalis* (83.63%) and *E. faecium* (16.36%). Other enterococcal species were not isolated in present study. A study done by Bose et al reported similar finding.[9] Study from Sevagram and Nagpur also isolated two species of Enterococci namely *E. faecalis* (most common) and *E. faecium*.[8,17]

Seven isolate of *E. faecalis* (7.60%) was found resistant to vancomycin by DDT and resistance was high level (MIC>256 gm/dl). Vancomycin resistance was detected in *E. faecium(50%)*. Resistance to vancomycin is widely variable. Agrawal et al, Titze-de-Almeida et al, Rahangdale et al, did not get any VRE in their study.[17,19,143]

But in a study from Mumbai 10% vancomycin resistance in *E. faecalis* and 28.57% in *E. faecium* was noted.[20] Similarly, SalenBekhit et al found 1.8% vancomycin resistance in *E. faecalis* and 18.5% in E. faecium.[144]Agarwal et al from Lucknow found 9.52% vancomycin resistance in *E. faecalis* but did not find any vancomycin resistance in *E. faecium*.[16]

Table 5 : Comparison of VRE Isolation with other studies

S. No.	Study	Total Sample	VRE	MIC value (µgm/ml)
1	Present study (2017)	110	16(14.54%)	64-256
2	FawziaE.Alotaibi et al (2017)	231	40(17.3%)	
3	V. Dillirali et al (2016)	120	4(3.3%)	
4	Priyanka Paul et al (2016)	250	36(14.4%)	8-64
5	Seemamittal et al (2016)	100	5(5%)	>64
6	Kheya Mukherjee et al (2016)	395	15(3.8%)	
7	Ayan K. Das et al. (2015)	146	8(5.4%)	32-128
8	Nita Gangurde et al (2014)	180	15(8.33%)	
9	PreetiShrivastava et al (2013)	100	14(14%)	
10	Latika Shah et al (2012)	92	8(8.6%)	8-32
11	Ghoshal et al (2006)	685	10(1.4%)	62-256
12	Karmarker et al (2004)	52	12(23%)	
13	Mathur et al (2003)	444	5(1%)	26-512

In our study we also found that enterococci were resistance to three-four drugs, common for all isolates. In *E. faecalis* only 5 isolates (6.95%) were sensitive to all drugs, common for all isolates. They are resistant to minimum one and maximum five number of drug. In *E. faecium* all isolates were resistant to minimum one and maximum five number of drug. 28(32.94%) isolates of enterococci were resistance to four drugs (penicillin G, ampicillin, Gentamycin, Streptomycin). 63(57.27%) isolates were resistant to three drugs out of which maximum isolates were resistance to penicillin G, ampicillin, Gentamicin.

MDR in enterococci is very high in our study place. Resistance in aminoglycoside as well as in ampicillin is very high that may lead to failure to synergism. Because of intrinsic resistance to much antibiotic and high level resistance to effective antibiotic very few antibiotics are

left for treatment of enterococcal infection. Although prevalence of VRE is low in our study place at present but this may rise.

CONCLUSION

Increased incidence of vancomycin resistant enterococci (VRE) and vancomycin intermediate sensitive enterococci(VISE) from this study emphasize the immediate need for intervention in current antimicrobial management and monitoring. Our study concludes that the rational and appropriate usage of antimicrobials in the community and health care centers will minimize the emerging multidrug and vancomycin resistant strains of enterococci.

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