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Screening of VRE with special emphasis on the determination of MIC of Vancomycin and Teicoplanin for *Enterococci*

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Abstract

Introduction:
Vancomycin resistant Enterococci (VRE) have recently emerged as nosocomial pathogen with intrinsic resistance to many antimicrobial agents making them difficult to treat. We investigated the prevalence of vancomycin resistance in *Enterococci* isolated in a tertiary health care set up.

Materials and Methods:
128 Enterococcal isolates form patients specimens were screened for vancomycin resistance. Screening for vancomycin resistance was done by utilizing vancomycin screen agar. Vancomycin resistance was also confirmed phenoypically by determining the Minimum inhibitory concentration of both Vancomycin and teicoplanin by broth microdilution method.

Results:
Vancomycin screen agar detected four resistant isolates of Enterococcus faecium (12.90%) which is inclusive of an additional isolate which Kibybaeur disk difusion method failed to identify .

Interpretation and conclusion:
Being an emerging pathogen, VRE acts as a sensitive marker for measuring the effectiveness of
Infection control programme and the appropriate application of preventive measures. The study resulted in an increased awareness about VRE and implementation of control measures in the hospital to restrict spread of VRE

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Introduction

Enterococci are gut commensals which inhabit the gut of humans and animals and in the recent decades have emerged as one of the major causes of nosocomial pathogens. Vancomycin has been used extensively for the treatment of serious infections due to Enterococci. However, after the first isolate of Vancomycin Resistant Enterococci (VRE) was reported in Europe in1986, there has been a steady increase in the number of VRE infections across the globe (1)

In the last two decades, the emergence of Vancomycin Resistant *Enterococci* (VRE) and their increasing prevalence worldwide has made it difficult to treat serious Enterococcal infections. VRE can remain viable in the environment for an extended period of time and therefore pose a problem for infection control in hospitals.Enterococcus; particularly *E.faecium* is intrinsically resistant to many antibiotics such as cephalosporins, clindamycin and penicillinase resistant penicillins. Later on the organism emerged as having acquired resistance to Ampicillin, Amino glycosides and Vancomycin (2-4)

The pathogen thus became untreatable with most available antibiotics. The organism was also noted to transfer the resistance horizontally to other Gram positive cocci. Control of VRE by preventing its colonization and spread at centers where VRE is endemic was observed to be unsuccessful (5-6)

The present study was aimed to know the prevalence of vancomycin resistance in Enterococci isolated as pathogen.

Material and Methods

This was a cross-sectional study carried out over a period of 12 months from April 2013 to March 2014 after obtaining approval from the institute scientific advisory and ethics committees. The study was conducted in the Department of Microbiology, SRM Medical college Hospital and research centre, kattan kulathur . A total of 128 strains of Enterococci were isolated from various clinical samples (Urine, pus, blood, wound swab, catheter tips, tissues and body fluids).The samples were processed within two hours of collection. The strains isolated were identified and speciated according to standard laboratory procedures as per the scheme of Facklam &Collins(7).

Enterococci isolated from clinical samples were screened for vancomycin resistance by using brain heart infusion agar (Hi media Lab Pvt. Ltd, India) with 6µg/ml vancomycin (Hi media Lab Pvt.Ltd, India) – **Vancomycin Agar screen**. Inoculation was done via spotting of 10µl of Enterococcal suspension matching 0.5 McFarland standard. Alternatively,spoting an area of 10-15 mm using a swab or streaking was also desired. Plates were incubated at 37°C in ambient air for 24 hours. Presence of more than one colony indicates presumptive vancomycin resistance.Antimicrobial susceptibility testing was done by Kirby- Bauer disc diffusion method as per there commendations of CLSI .Various antibiotics tesed were Ampicillin (10 µg), Penicillin (10 units /disc), Gentamicin-high content (120 µg), Streptomycin-high content (300µg), ciprofloxacin (5µg), tetracycline (30 µg), (nitrofurantoin (300µg), vancomycin (30 µg), teicoplanin (30 µg) and linezolid (30 µg). (8)

Broth microdilution method was performed with powders of vancomycin and Teicoplanin (source-Himedia) for the determination of Minimal inhibitory concentration as per the recommended standards. *E.faecalis* ATCC 29212 was used as Quality control .

Preparation of stock solution

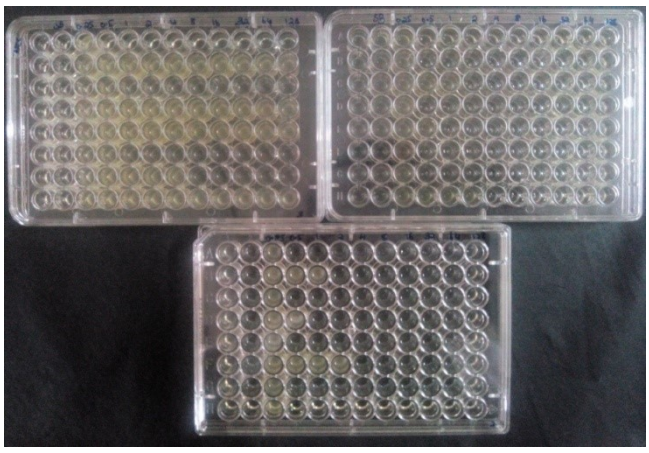
Stock solution were prepared using the formula **(1000/P) * V * C=W**, where P = potency of the antibiotic base, V=volume in ml required, C=final concentration of solution and W=weight of the antimicrobial to be dissolved in V.

Preparation of Dilutions of Antibacterial Agents

Step	Concentration (µgm/ml)	Volume (ml)	CAMHB Volume (ml)	Final concentration (µg/ml)
1	512	1	1	256
2	512	1	3	128
3	512	1	3	64
4	64	1	1	32
5	64	1	3	16
6	64	1	7	8
7	8	1	1	4
8	8	1	3	2
9	8	1	7	1
10	1	1	1	0.5
11	1	1	3	0.25

Procedure:

Suspensions of organisms were prepared by inoculation of BHI broth. Final inoculum of 10^5CFU/ml is required and therefore suspensions were diluted 1:100 in broth medium.100µl of each antibiotic dilution was added to all the rows of wells.100 µl of control strain *E.faecalis* ATCC 29212 was dispensed in the first row followed by the isolates in the subsequent rows. Inoculated and uninoculated wells of antibiotic free broth were also included. The former controls the broth adequacy to support the growth of the organism , while the latter is for sterility check. Plates were covered with lid and incubated at 37° C in ambient air. The endpoint of MIC is expressed as the lowest concentration of drug that inhibits the growth of the strain. (8-9).



Results

Table 1: VANCOMYCIN MIC RANGE OF *E.faecalis* by BROTH MICRODILUTION METHOD

Drug concentration (µg/ml)	0.25	0.5	1	2	4	8	16	32	64	128	256
Control ATCC (29212)	-	1	-	-	-	-	-	-	-	-	-
MIC Value (No of isolates)	-	48	23	14	10	1	1	-	-	-	-
Percentage (%)	-	49.48	23.71	14.43	10.3	1.03	1.03	-	-	-	-

Table 2: VANCOMYCIN MIC RANGE OF *E.faecium* by BROTH MICRODILUTION METHOD

Drug concentration (µg/ml)	**0.3**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**
Control ATCC (29212)	-	1	-	-	-	-	-	-	-	-	-
MIC Value (No of isolates)	-	-	-	9	17	1	-	-	-	-	4
Percentage (%)	-	-	-	29	54.8	3.2	-	-	-	-	12.9

Table 3: COMPARISON OF MIC AND DISK DIFFUSION METHOD AND VANCOMYCIN SCRREN AGAR FOR DETECTION OF VANCOMYCIN RESISTANCE

MIC (µg/ml)	**No of Isolates**	**Percentage**
≤ 4 µg/ml (Susceptible)	121	94.53
8-16µg/ml (Intermediate)	3	2.34
32µg/ml (Resistant)	4	3.12
Vancomycin Screen agar	4	3.12
By disk diffusion	3	2.34

Table 4: TEICOPLANIN MIC RANGE OF *E.faecalis* by BROTH MICRODILUTION METHOD

Drug concentration (µg/ml)	**0.3**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**
Control ATCC (29212)	-	1	-	-	-	-	-	-	-	-	-
MIC Value (No of isolates)	-	19	38	16	22	1	1	-	-	-	-
Percentage (%)		19.6	39.2	16.5	22.7	1	1	-	-	-	-

Table 5: TEICOPLANIN MIC RANGE OF *E.faecium* BROTH MICRODILUTION METHOD

Drug concentration (µg/ml)	**0.3**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**256**
Control ATCC (29212)	-	1	-	-	-	-	-	-	-	-	-
MIC Value (No of isolates)	-	4	5	7	6	3	2	-	-	1	3
Percentage (%)	-	12.9	16.1	22.6	29	9.7	6.5	-	-	3.2	9.7

Table 6: COMPARISON OF MIC AND DISK DIFFUSION METHOD FOR DETECTION OF TEICOPLANIN SENSITIVITY

MIC (µg/ml)	**No of Isolates**	**Percentage**
≤ 8 µg/ml (Susceptible)	117	91.4
16µg/ml (Intermediate)	7	5.46
≥32µg/ml (Resistant)	4	3.12
By disk diffusion	4	3.12

Discussion

The emergence of vancomycin resistant *Enterococci* poses a serious threat to the hospitalized patients with impaired host defense. Mathur *et al* from New Delhi were the first to report VRE from India in 1999[10]. Athough the prevalence of VRE infections in India is much lower than the western world, it has been increasing since a decade [11].

The prevalence of VRE infections in India range from 0-30% [12-15]

In this study, three isolates of *E.faecium*(9.67%) were identified as vancomycin resistant by disk diffusion method. Vancomycin screen agar detected four resistant isolates of *E.faecium*(12.90%) including an isolate which Disk diffusion method failed to identify. Similarly in a study by Oberoi *et al* ,14 (18.42%) isolates were resistant to vancomycin by KBDDM ,while by vancomycin screen agar, resistance was observed in 16 (21.05%) [17].Thus vancomycin screen agar proves as a useful medium for the screening of vancomycin resistant Enterococci.

Minimum inhibitory concentration for vancomycin determined by Broth microdilution method showed that 83.86% and 3.22% of *E.faecium* isolates were in the susceptible and the intermediate ranges whereas 12.90% of the isolates screened as vancomycin resistant had MICs higher than 256µg/ml. Majority of the *E.faecalis* isolates (97.92%) were susceptible and 2.06% of isolates were in intermediate range. The commonest phenotype seen among VRE strains is the Van A phenotype in which high inducible resistance to both vancomycin and teicoplanin is seen (MIC ≥ 64 µg/ml) [18].Van A phenotype was seen in 100% off all VRE isolates in our study.

Of the four Vancomycin resistant *Enterococcus faecium* isolated, urine and pus yielded 1 each isolate , whereas the other 2 isolates were from blood .

In our study, no vancomycin resistance has been detected in isolates of *E.faecalis*. Vancomycin resistance in Enterococci not only leaves fewer options for the disease management but it is also carries the potential risk of VRE gene transfer from Enterococci to *Staphylococcus aureus*.[15]

In the present study, resistance to Teicoplanin (12.90%) was seen in four isolates of *Enterococcus faecium* . The MICs of Teicoplanin by broth microdilution showed that 87.08% of *E.faecium*were susceptible, 5 isolates (16.12%) were within the intermediate range. Among resistant isolates one had the MIC of 128µ/ml whereas the other three had MIC higher than 256µg/ml. Majority of *E.faecalis* strains (97.93%) had MICs within the susceptible range and 2.06% of isolates were in the intermediate range. The table provided below provides information about the rates of Teicoplanin resistance in india.

Teicoplanin resistance in our study falls within the range mentioned by several authors.

The intrinsic and extrinsic patient risk factors wherein the VRE be considered despite being commensals are(21);

Intrinsic

Intrinsic Immunosuppression Includes;

1. Haematology/oncology conditions, solid organ transplantation, and neutropenia
2. Renal dialysis - May relate to underlying renal disease or regular healthcare contact
3. Recent/current antibiotic use - Third-generation cephalosporin, fluoroquinolones and b-lactam/ b-lactamase inhibitors
4. Chronic underlying disease, previous hospitalization

Extrinsic

1. Intensive care unit
2. Transfer from LTCF
3. Previous patient in single room
4. Prior hospitalization/transfer from another hospital .

Effectiveness/ culture survey of stools or rectal swabs:

In tertiary medical centers and other hospitals that have many critically ill patients (e.g., ICU, oncology, and transplant patients) at high risk for VRE infection or colonization, periodic culture surveys of stools or rectal swabs of such patients can detect the presence of VRE. Because most patients colonized with VRE have intestinal colonization with this organism, fecal screening of patients is recommended even though VRE infections have not been identified clinically.

The frequency and intensity of surveillance should be based on the size of the population at risk and the specific hospital unit(s) involved. If VRE have been detected in other health-care facilities in a hospital's area and/or if a hospital's staff decides to determine whether VRE are present in the hospital despite the absence of recognized clinical cases, stool or rectal-swab culture surveys are useful.

The cost of screening can be reduced by inoculating specimens onto selective media containing vancomycin and restricting screening to those patients who have been in the hospital long enough to have a substantial risk for colonization (e.g., 5-7 days) or who have been admitted from a facility (e.g., a tertiary-care hospital or a chronic-care facility) where VRE have been identified. After colonization with VRE has been detected, all the Enterococcal isolates (including those from urine and wounds) from patients in the hospital should be screened routinely for vancomycin resistance, and efforts to contain the spread of VRE should be intensified (i.e., by strict adherence to hand washing and compliance with isolation precautions.

Intensified fecal screening for VRE might facilitate earlier identification of colonized patients leading to more efficient containment of the microorganism.(22)

Conclusion

Preventive measures need to be taken especially in ICU's to curtail the spread of vancomycin resistance among Enterococci. Although isolation or cohorting of colonized patients may be ideal, they may not be very practical. Instead strict adherence to hand hygiene and education of health care workers may be more achievable methods of infection control.Being an emerging pathogen, VRE acts as a sensitive marker for measuring the effectiveness of infection control programs and the appropriate application of preventive measures.In the present study, all measures recommended in CDC guidelines were discussed with hospital staff and recommendations were put up in the wards. VRE isolation was seen to be reduced following control measures in the wards. The study resulted in increased awareness about VRE and implementation of control measures to prevent the spread of VRE in the hospital. However periodic re-enforcement needs to be done to monitor the spread of VRE.

Table 7: Vancomycin resistance among *Enterococci* in India

Authors	Year of Study	Vancomycin Resistance		Publication
		E.faecalis	*E.faecium*	
Karmarkar*et al*	2004	23%		IndJ Med Res .2004; 119,22-25. [12]
Ghoshal*et al*	2006	10%		Ind J Path Microbiol 2006; 49 (4):620-2 [1]
Lathika*et al*	2011	6%	2%	Nat J Med Res. Jan - March 2012. 2 (1): 25 - 27. [14]
Praharaj*et al*	2013	9.26%		Ind J Med Res. 2013; 138:549-556 [11]
Gangurdhe*et al*	2014	4.6%	13.7%	Open Journal of Medical Microbiology 2014,4,11-15. [16]
Present study	2014		12.96%	-

Table 8: Teicoplanin resistance among Enterococci in India

Authors	Year of Study	Vancomycin Resistance		Publication
		E.faecalis	*E.faecium*	
Karmarkar*et al*	2004	9.52%		Ind J Med Res .2004; 119,22-25. [12]
Jain *et al*	2011	-		Int j App Basic Med Res 2011;1:80-3[19]
Lathika*et al*	2011	-		Nat J Med Res. Jan - March 2012. 2 (1): 25 - 27. [15]
Praharaj*et al*	2013	7.6%		Ind J Med Res. 2013; 138:549-556 [11]
Deshpande *et al*	2013	4.4%	27.6%	J Infect Dev Ctries 2013; 7(2):155-158. [14]
Present study	2014	-	12.96%	-

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