A profile of vancomycin-resistant enterococcal infections and a comparison of resistance detection methods

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Abstract

Scope: Vancomycin-resistant enterococci (VRE) are being increasingly reported from hospitals across the world. This study provides a profile of enterococcal infections and compares various methods of detecting vancomycin resistance.

Materials and Methods: All clinically significant isolates of enterococci over a 2-year period were included. Antibiotic susceptibility was carried out as per CLSI guidelines. Vancomycin resistance was detected by 3 methods: disk diffusion, agar screen, E-test. The 3 methods were compared.

Results: 156 clinical samples yielded *Enterococcus* spp. over the study period. Maximum resistance was noted to penicillin, erythromycin, and ciprofloxacin. *E. faecium* strains showed a higher percentage of resistance to the antibiotics tested. 15 (9.6%) enterococcal strains were resistant to vancomycin; 10 (6.4%) strains were intermediate. Compared to E-test, disk diffusion and agar screen had sensitivities of 100%. Disk diffusion had 97.2% specificity and agar screen demonstrated 92.9% specificity.

Conclusion: Prevalence of VRE in Indian hospitals is increasing. Though disk diffusion had a higher specificity than the agar screen at identifying resistant isolates, intermediate strains were identified as sensitive. BHI agar containing 6 µg/ml of vancomycin can be used to screen for VRE, and E-test can be used to confirm resistance.

Keywords: Bacterial infections, Enterococcus, Drug resistance, Vancomycin, Vancomycin resistance.

Introduction

Resistance is a paradoxical, yet natural outcome of antibiotic use. Alexander Fleming, in 1945, warned that misuse of penicillin would lead to resistant organisms.[1] The use of broad-spectrum antibiotic agents as a substitute for precise diagnostics increases the rate of selection of resistant bacteria.

Arguably, the most impressive accomplishment of bacteria in this arena has been the development of vancomycin resistance enterococci (VRE).[2] Vancomycin had been in clinical use for more than thirty years when, in England in 1988, Uttley et al. first reported the isolation of VRE.[3,4]

Enterococci are Gram-positive cocci that are part of the normal gut flora. Among them, *Enterococcus faecalis* and *Enterococcus faecium* are predominantly isolated from clinical samples. Less commonly isolated species include *E. gallinarum, E. casseliflavus, E. durans, E. avium* and *E. raffinosus*.[5] Infections caused by enterococci include urinary tract infections (UTIs), bacteremia, intra-abdominal infections, endocarditis, central nervous system (CNS) infections, skin and soft tissue infections.[5-8]

Enterococci exhibit intrinsic resistance to cephalosporins, low levels of aminoglycosides and clindamycin; acquired resistance is seen against high-level aminoglycosides (HLAR), vancomycin, chloramphenicol, erythromycin, high levels of clindamycin, tetracycline, and fluoroquinolones.[9]

Glycopeptide-resistant enterococci have become a major threat to hospitalized patients, causing outbreaks that increase morbidity, mortality, and healthcare-associated costs.[10]

Currently, there are nine types of operon structure conferring glycopeptide resistance, designated according to the characteristics of a key ligase gene encoding either a D-alanyl-D-lactate or a D-alanyl-D-serine ligase. These include *vanA, vanB, vanC1, canC2, vanC3, vanD, vanE, vanG, vanL, vanM* and *vanN* genes. Except for *vanC*-type resistance, which is intrinsic to *E. gallinarum* and *E. casseliflavus*, all resistant types are acquired.[11] VanA and VanB are the types most frequently detected in enterococci and have also been detected in coryneform bacteria and streptococci.[12]

VanA-resistant strains possess inducible, high-level resistance to vancomycin (MICs, ≥64 µg/ml) and teicoplanin (MICs, ≥16 µg/ml).[13] Levels of vancomycin resistance among VanB isolates may range from 4 to ≥1000 µg/ml whereas susceptibility to teicoplanin is retained.[14]

The risk factors that have emerged for VRE infection are longer duration of hospitalization, longer stay in ICU, history of solid organ transplantation, and use of various antibiotics such as vancomycin, third-generation cephalosporins, aminoglycosides, aztreonam, ciprofloxacin, imipenem, clindamycin and metronidazole.[5,15,16]

VRE infections tend to occur in more debilitated or seriously ill hospitalized patients. Mortality rates in patients with VRE bacteremia may reach 60 to 70%; half these deaths may be attributable directly to the infection. However, there is no evidence that VRE are more

virulent than vancomycin-susceptible strains of the same enterococcal species.[5]

Environmental surfaces and medical equipment items in the patient's room frequently become contaminated with VRE and may also serve as a reservoir for the organism in the hospital.[17,18] VRE may remain viable on such surfaces for days or weeks.[19]

Mathematical models argue that VRE endemicity requires a constant introduction of colonized patients. Susceptibility tests that detect vancomycin resistance accurately must be used, or the prevalence of VRE may be underestimated.[16,20] Several vancomycin resistance detection systems, including automated systems may misclassify isolates as intermediately susceptible to vancomycin.[5,21]

Despite increasing reports of VRE from different countries, there is a paucity of information on this issue from our country.[22]

Aims and Objectives

This study aims to identify enterococci isolated from various clinical samples up to the species level. This study also aims to determine the prevalence of vancomycin resistance among the isolates and to compare various methods of detection: disk diffusion; agar screen test – using brain heart infusion (BHI) agar containing 6 micrograms µg of vancomycin per ml; E-test (epsilometer test).

Materials and Methods

This descriptive cross-sectional study was carried out in the Department of Microbiology between June 2011 and July 2013. Permission was obtained from the Institutional Ethics Committee prior to the commencement of the study.

The data collected was entered into Microsoft Excel sheets.

All the enterococcal isolates from clinical samples such as blood, urine, pus, wound swab, cerebrospinal fluid (CSF) and other body fluids were included. All commensal enterococci from the gastrointestinal tract, female genital tract and oral cavity, and repeat isolates from the same patient were excluded.

Samples were processed according to standard procedures.[23] Species-level identification was carried out as per the Facklam and Collins test scheme.[24,25]

All the enterococcal isolates were tested for antibiotic sensitivity against penicillin (10 U), ampicillin (10 µg), chloramphenicol (30 µg), erythromycin (1p5 µg), ciprofloxacin (5 µg), linezolid (30 µg) and vancomycin (30 µg) by the Kirby Bauer disk diffusion method using commercially available disks (Himedia, Mumbai, India) on Mueller-Hinton agar as per CLSI guidelines.[26] HLAR was tested against gentamicin (120 µg) and streptomycin (300 µg). Readings of disk diffusion tests were taken at 24 h for vancomycin, and at 18 h for other antibiotics.[26] Enterococci were screened for resistance using brain heart infusion (BHI) agar supplemented with 6 µg/ml vancomycin.[20] E-tests for vancomycin and teicoplanin were performed as per manufacturer's instructions (Himedia, Mumbai, India).

Quality control of the antibiotic disks was carried out using *Staphylococcus aureus* ATCC 25923 and *Enterococcus faecalis* ATCC 29212.

Results

During the study period, 156 samples yielded isolates of enterococci. Thirty-five percent of the isolates were from the 0-20 year age group. Of the 39 patients in the 0-10 year age group, 31 were neonates, 3 of whom grew VRE. The mean age of patients with enterococcal infections was 32.1 years (Table 1).

Table 1: Age-wise distribution of the isolates

	All enterococci	VRE
Age groups (yr)	**Number (%age)**	**Number (%age)**
0-10	39 (25.0%)	4 (26.7%)
11-20	16 (10.3%)	0 (0%)
21-30	26 (16.7%)	1 (6.7%)
31-40	19 (12.2%)	4 (26.7%)
41-50	15 (9.6%)	0 (0%)
51-60	17 (10.9%)	2 (13.3%)
>61	24 (15.4%)	4 (26.7%)
Total	156	15

Out of the 156 enterococcal isolates, 88 (56.4%) were from female patients, and 68 (43.6%) were from male patients. Of the 15 VRE isolates, 8 (53%) were from female patients.

A majority of the isolates were from urine samples (61.5%), followed by blood cultures (18.6%) and pus samples (16%) (Table 2). Of the 25 pus samples, 7 (4.48%) were from burn patients (4, *E. faecium;* 3, *E. faecalis).* VRE were isolated from urine (66.7%), followed by blood (20%) and pus (13.3%).

36% of the enterococci were isolated from patients admitted in the medicine ward, followed by the paediatric (24.3%) and gynaecology (14.7%) wards. Nine percent of the isolates were from patients admitted in the ICU.

Twenty eight (18%) patients presented with UTI, followed by febrile illness (17%, n=27) and septicaemia (13%, n=20). Of the patients who presented with a febrile illness, 70% (n=19) had a urine culture positive for enterococcus.

Table 2: Specimen-wise distribution of the isolates

	All enterococci	VRE
Specimen	**Number (%age)**	**Number (%age)**
Urine	96 (61.5%)	10 (66.7%)
Blood	29 (18.6%)	3 (20.0%)
Pus	25 (16.0%)	2 (13.3%)
Ascitic fluid	3 (1.9%)	0 (0%)
CSF	2 (1.3%)	0 (0%)
ET aspirate	1 (0.64)	0 (0%)
Total	156	15

More than half the isolates came from the medicine and paediatric wards (36.5%, n=57 and 24.3%, n=38, respectively); 4 enterococcal isolates from each of these areas were VRE, accounting for 53% of the VRE strains. The intensive care unit (ICU) yielded 14 strains of enterococci, of which 3 were VRE.

135 (86.5%) of the isolates were identified as *E. faecalis*, and 18 (11.5%) were *E. faecium*. Of the remaining, two were identified as *E. avium*, and one as *E. durans* (Table 3). Thirteen (86.7%) VRE isolates were identified as *E. faecalis*, and 2 (13.3%) were *E. faecium*. The 10 remaining enterococcal isolates exhibited intermediate sensitivity to vancomycin.

Table 3: Species-wise distribution of the isolates

	All enterococci	VSE	VRE
Species	**Number (%age)**	**Number (%age)**	**Number (% age)**
E. faecalis	135 (86.5%)	115 (87.8%)	13 (86.7%)
E. faecium	18 (11.5%)	14 (10.7%)	2 (13.3%)
E. avium	2 (1.3%)	1 (0.76%)	0 (0%)
E. durans	1 (0.6%)	1 (0.76%)	0 (0%)
Total	156	131	15

Maximum resistance was noted to penicillin (86.5%), erythromycin (83.3%), and ciprofloxacin (79.5%). 37.2% of the isolates were resistant to high level gentamicin (HL-G) (120 µg), and 32.7% were resistant to high level streptomycin (HL-S) (300 µg). The enterococci were least resistant to teicoplanin (8.3%) and vancomycin (9.6%). None of the isolates were resistant to linezolid (Table 4).

A higher proportion of resistance was noted among *E. faecium* strains than *E. faecalis* against all but two antibiotics: penicillin and ampicillin.

Table 4: Antibiotic resistance pattern of *Enterococcus* spp

	All enterococci (n=156)	*E. faecalis* (n=135)	*E. faecium* (n=18)
Antibiotic	**No. of R strains (%age)**	**No. of R strains (%age)**	**No. of R strains (%age)**
Penicillin (10 U)	135 (86.5%)	117 (86.7%)	15 (83.3%)
Ampicillin (10 µg)	83 (53.2%)	72 (53.3%)	9 (50.0%)
Ciprofloxacin (5 µg)	124 (79.5%)	106 (78.5%)	16 (88.9%)
Gentamicin (HL) (120 µg)	58 (37.2%)	48 (35.6%)	9 (50.0%)
Streptomycin (HL) (300 µg)	51 (32.7%)	43 (31.8%)	6 (33.3%)
Chloramphenicol (30 µg)	92 (59.0%)	78 (57.8%)	12 (66.7%)
Erythromycin (15 µg)	130 (83.3%)	110 (81.5%)	17 (94.4%)
Linezolid (30 µg)	0 (0%)	0 (0%)	0 (0%)
Teicoplanin (E-test)	13 (8.3%)	11 (8.1%)	2 (11.1%)
Vancomycin (disk) (30 µg)	19 (12.2%)	15 (11.1%)	4 (22.2%)
Vancomycin (agar screen)	25 (16.0%)	20 (14.8%)	4 (22.2%)
Vancomycin (E-test)	15 (9.6%)	13 (9.6%)	2 (11.1%)
	No. of I strains (% age)	No. of I strains (%age)	No. of I strains (%age)
Vancomycin (E-test)	10 (6.4%)	7 (5.2%)	2 (11.1%)

Table 5: Antibiotic susceptibility pattern of vancomycin-resistant enterococci

Antibiotic	Resistant	%age
Penicillin (10 U)	15	100.0%
Ampicillin (10 µg)	12	80.0%
Chloramphenicol (30 µg)	13	86.7%
Erythromycin (15 µg)	13	86.7%
Ciprofloxacin (5 µg)	14	93.3%
Gentamicin (HL) (120 µg)	14	93.3%
Streptomycin (HL) (300µg)	12	80.0%
Linezolid (30 µg)	0	0%
Teicoplanin (E-test)	13	86.7%
Vancomycin (E-test)	15	100.0%

The strains of vancomycin-resistant enterococci showed significant resistance to all the other antibiotics tested, except to linezolid. All 15 VRE strains were resistant to penicillin and 93.3% were resistant ciprofloxacin, as well as to high-level gentamicin (Table 5). A vast majority of the HLAR strains also exhibited resistance to penicillin (98%), ampicillin (76%), ciprofloxacin (98%) and erythromycin (100%).

Eleven *E. faecalis* strains and 2 of *E. faecium* exhibited VanA phenotype (vancomycin MIC ≥ 64 µg/ml, teicoplanin MIC ≥ 16 µg/ml) and 2 isolates of *E. faecium* had VanB phenotype (vancomycin MIC ≥ 64 µg/ml, teicoplanin MIC ≤ 8 µg/ml).

Resistance to vancomycin was tested by 3 methods, viz. Kirby Bauer disc diffusion with vancomycin disk (30 µg), BHI screen agar containing vancomycin (6 µg/ml), and vancomycin E-test (Table 6). The E-test showed resistance in 9.6% and intermediate sensitivity in 6.4% of the isolates.

Table 6: Vancomycin resistance detected by various methods

	Number	%age of enterococci
Vancomycin disk diffusion	19	12.2%
Vancomycin screen agar	25	16.0%
Vancomycin E-test	15	9.62%

While 79% of the strains indicated to be resistant to vancomycin by disk diffusion had a vancomycin MIC ≥ 32 µg/ml (resistant) by the E-test, only 60% of the strains that grew on vancomycin screen agar had vancomycin MIC ≥ 32 µg/ml. Vancomycin disk diffusion had 100% sensitivity, 97.2% specificity and vancomycin agar screen showed 100% sensitivity, 92.9% specificity.

The *E. durans* strain and one of the *E. avium* strains were sensitive by vancomycin disk diffusion, did not grow on the vancomycin screen agar and had vancomycin MICs ≤ 4 µg/ml in the E-test. The remaining *E. avium* strain exhibited intermediate sensitivity (MIC, 8-16 µg/ml) to vancomycin and grew on the vancomycin screen agar, but tested sensitive to the vancomycin disk.

Discussion

Enterococci are the second most common cause of nosocomial infections in the United States, and are responsible for approximately 8% of all nosocomial bloodstream infections.[27]

The purpose of this study was to generate data on enterococcal infections in a rural tertiary care hospital, to identify the isolates to the species level, to assess the antibiotic resistance pattern to widely prescribed antibiotics with a focus on vancomycin resistance, and to compare the detection of vancomycin resistance by various methods.

In this study, 45 (35.3%) of the enterococci were isolated from patients in the 0-20 year age group, out of which 31 (19.9%) were <1 month old. The mean age of incidence of enterococcal infections was 32.13 years. This is in contrast to a studies where the average age was 62 years, and another where 96% of the patients were >18 years of age.[28,29]

The prevalence of enterococcal infections was found to be higher in female patients (56.4%). Other studies have shown the prevalence to be 46% to 50% in females.[28,29]

Maximum isolates in our study were obtained from urine samples (61.5%). Studies have reported similar observations.[29,30] In other studies, pus samples contributed the most to the number of enterococcal isolates.[31,32]

Urinary tract infections (UTIs) are the most common bacterial infection, and almost half of all women will experience one episode of UTI during their lifetime.[33] Eighteen percent of our patients (n=28) presented with UTI and additionally, 19 of patients who presented with a febrile illness grew enterococci in their urine.

We identified 135 (86.53%) isolates as *E. faecalis*. Eighteen (11.54%) were *E. faecium*, 2 (1.3%) were *E.*

avium and 1 (0.6%) was *E. durans.* This is similar to studies where 87% to 90% of the strains were *E. faecalis* and 8% were *E. faecium.*[29,30] Other species (*E. avium, E. durans,* etc.) accounted for only 2% to 3% of the isolates.[29,30]

The predominance of infections by *E. faecalis* can be related to the fact that it is found in higher numbers than other species in the faeces of most healthy adults.[9] Some studies have reported a higher incidence of non-*faecalis,* non-*faecium* species.[34,35] In a Brazilian ICU, 84% of the VRE species recovered from faecal specimens of critical patients were *E. gallinarum,* a species with intrinsic resistance to vancomycin.[36]

Species identification of enterococci may be useful both as an epidemiological tool during nosocomial outbreaks and for clinical decisions about therapy.[29]

Of the 15 isolates of VRE, 3 (20%) isolates were from patients admitted in the ICU. The infection rate by VRE in ICUs has ranged from 7% to 45%.[37,38] In India, 3.7% of the VRE isolates were from the ICU.[39-41]

The observed rates of penicillin (86.5%) and ampicillin (53.2%) resistance are comparable to other studies The rate of ciprofloxacin resistance (79.5%) is much higher than what has been reported.[29,35,31]

High-level gentamicin resistance has ranged from 12.6% to 100% among *E. faecalis.*[30,42] Thirty-seven percent of the enterococci in this study were resistant to high-level gentamicin (*E. faecalis,* 35.6% and *E. faecium,* 50%). The high proportion of resistance of these HLAR strains to other classes of antibiotics is comparable to other reports.[43]

Vancomycin resistance among enterococci was observed to be 9.6% (*E. faecalis,* 9.6%; *E. faecium,* 11.1% isolates). Vancomycin resistance has been observed to be on the rise: from 0.3% in 1992;[29] 1.5% in 1996;[32] 20% observed in 2002;[40] to 28.6% in 2004.[42]

Eleven *E. faecalis* strains and 2 of *E. faecium* exhibited VanA phenotype, and 2 isolates of *E. faecium* had VanB phenotype. Seventy percent of VRE in the United States exhibited the VanA phenotype, and 25% exhibited the VanB phenotype.[44] All the vancomycin-resistance *E. faecium* isolates in a study from India had VanA phenotype.[39] In another report, out of 5 VRE isolates, 4 had VanA phenotype, and 1 had VanB phenotype.[22]

We used three methods of antibiotic susceptibility testing to detect vancomycin resistance. Fifteen (9.6%) strains were noted to be resistant (MIC $\geq$ 32 µg/ml) by the E-test and 10 exhibited intermediate sensitivity (MIC 8-16 µg/ml). Disc diffusion indicated resistance in 19 isolates. The agar screen indicated resistance in 25 isolates.

Major errors have been reported with disc diffusion for the detection of vancomycin resistance.[45,46] Similar to other reports, more than half the intermediate strains in our study were reported susceptible by disk diffusion.[47]

The agar screen method for detecting VRE has been demonstrated to have 96% to 99% sensitivity and 100% specificity.[20] This, however, requires careful monitoring of the vancomycin concentration in the plates.[48] We observed 100% sensitivity, 92.9% specificity with the agar screen; vancomycin disk diffusion had 100% sensitivity, 97.2% specificity. All the intermediate strains grew on the agar screen.

Once suspected, vancomycin resistance should be confirmed using a different method.[16] Once VRE have been detected in a hospital, all enterococci should be tested for susceptibility to vancomycin.[49]

The E-test is a reliable susceptibility testing technique that combines the convenience of agar disk-diffusion with the precision of broth/agar dilution methods.[47] Results obtained with the E-test had a high level of agreement with broth microdilution and agar dilution.[50,51] This can be used as a confirmatory test for vancomycin resistance in settings that lack automated testing or molecular testing facilities.

Conclusion

Vancomycin resistant enterococci are being increasingly isolated from hospitals across the country. It is necessary to identify enterococci to the species level, as non-*faecalis,* non-*faecium* species are being increasingly isolated; some of these species are inherently resistant to vancomycin. Detection of vancomycin resistance by disk diffusion is error-prone, particularly misidentifying vancomycin-intermediate strains. Resistance may be screened for using vancomycin screen agar and should be confirmed by another method. The E-test is a suitable method for confirming resistance. Judicious use of glycopeptides is stressed on. Active surveillance for vancomycin-resistant strains may be considered in endemic hospitals.

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