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ORIGINAL ARTICLE

Species Prevalence And Antimicrobial Resistance Pattern Of Enterococcal Isolates In A Tertiary Health Care Centre

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ABSTRACT:

Context: Enterococci are one of leading causes of nosocomial and community acquired infections and in recent years, they have become increasingly resistant to a wide range of antimicrobial agents. **Aim:** The present study was done to determine the species distribution and antimicrobial resistance pattern of enterococcal isolates. **Material and Methods:** 120 enterococcal isolates from different clinical samples were included in the study. They were identified by the standard microbiological methods and their antimicrobial susceptility was done by the Kirby-Bauer disc diffusion method. Vancomycin resistance was detected by the disc diffusion method and the agar dilution method and MIC testing was done by the macrobroth dilution method. High level aminoglycoside resistance (HLAR) was detected as per the CLSI guidelines. **Results:** *E. faecium* was the predominant species (47.50%) which was detected, followed by *E.faecalis* (44.16%) and others. *E. faecium* strains displayed a higher degree of drug resistance. The *E.gallinarum* species expressed low level vancomycin resistance, which was not detected by the disc diffusion method. More than 70% resistance was seen for ampicillin, erythromycin and tetracycline. 9(7.5%) isolates were found to be resistant to vancomycin. 5(4.16%) isolates were resistant to teicoplanin. All the isolates were susceptible to linezolid. HLAR was seen in 73(47.18%) isolates. **Conclusion:** *E.faecium* is now emerging as the predominant enterococcal species which causes infections and most of the enterococcal isolates (>77%) are multidrug resistant. Vancomycin resistance and HLAR in enterococci are rising rapidly. This study emphasizes the need for routinely carrying out a detailed speciation and antibiotic susceptibility testing of the enterococcal isolates in the bacteriology laboratory.

Key words – Enterococcus, VRE, High level aminoglycoside resistance, *Enterococcus faecium*.

Key Messages:

- *E.faecium* is now emerging as the predominant enterococcal isolate from human infections.

- The disc diffusion method fails to detect low level vancomycin resistance of motile enterococci.
- Speciation and antibiotic susceptibility testing should be done on all enterococcal isolates.

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INTRODUCTION

Once regarded as a bacterial genus of little consequence, entrococci in the past several years, have rapidly emerged as important nosocomial and community acquired pathogens. These organisms can cause serious invasive infections including endocarditis, bacteraemia, meningitis and urinary tract infections, with high mortality.[1] Traditionally, of the 19 species of enterococcus which have been recognized so for, *E.faecalis* has accounted for approximately 80-90% of the clinical isolates, while *E.faecium* was isolated in the remaining 5-15% of the cases.[2] Other species are also being isolated. *E. faecium* strains display a higher degree of drug resistance[3],[4]. *E.casseliflavus*, *E.flavascence* and *E.gallinarum* are intrinsically resistant to vancomycin. So, the speciation of the enterococcal isolates has now become important. Enterococci are resistant to a wide range of antimicrobial agents including B-lactams and aminoglycosides, which are frequently used to treat infections caused by gram-positive cocci. Enterococci have the ability to acquire resistance through the transfer of plasmids or transposons, or by mutations.[5] Further, the acquisition of vancomycin resistance leaves limited options for therapeutic management.[6] The present prospective study was carried out to know the species prevalence and antimicrobial resistance pattern of the enterococcal isolates in our hospital.

MATERIALS AND METHODS:

The present study was conducted on 120 enterococcal isolates which were retrieved from clinical samples. The ethical standards laid down by the institutional committee on human experimentation were followed during the study.

Of the 120 enterococcal isolates, 50 were from urine, 35 were from blood, 20 were from pus and 15 were from body fluids. The isolates were identified up to the species level by gram staining, by studying their cultural characteristics and by biochemical tests and motility testing by using the standard microbiological techniques.[6]

Antimicrobial susceptibility testing was done according to the CLSI guidelines[7] by the disc diffusion method of Kirby-Bauer by using MH (Mueller-Hinton) agar. The various antibiotics which were tested were Ampicillin (10µg), Vancomycin (30µg), Teicoplanin (30µg), Erythromycin (15µg), Tetracycline (30 µg), Ciprofloxacin (5µg), Nitrofurantoin (300µg) and Linezolid (30µg). Vancomycin resistance was tested by the disc diffusion method and the agar screen method and MIC testing was done by the macrobroth dilution method.

For vancomycin susceptibility testing by the disc diffusion method, a zone diameter of less than or equal to 14mm was taken as resistant, 15-16mm was taken as intermediate and more than or equal to 17mm was taken as sensitive, after 24hrs of incubation.

For agar screening, BHI agar with 6µg/ml of vancomycin was used. 10 µl of 0.5 Mc Farland's suspension organism was spot inoculated and incubated at 35^0C for 24hrs.

The growth of more than one colony was taken as presumptive resistance.

MIC was done by the macrobroth dilution method in cat ion- adjusted Mueller-Hinton broth. An MIC of less than or equal to 4µg /ml was taken as susceptible, 8-16µg /ml was taken as intermediate and that which was more than or equal to 32µg /ml was taken as resistant, after 24hrs of incubation.

The detection of high-level aminoglycoside resistance (HLAR) was performed by the agar dilution method for gentamycin and streptomycin by supplementing the BHI agar with 500µg/ml and 2000 µg /ml of antibiotics respectively. 10µg of 0.5Mc Farland's suspension was spot inoculated on the agar surface and incubated at 35^{o}C for 24hrs for gentamycin and 48hrs for streptomycin. The growth of more than one colony was taken as resistant.

The source of antimicrobials was Hi-Media Ltd (Mumbai) India. The standard strains, *E. faecalis* ATCC 29212 and *E. faecalis* ATCC 51299 were used as the susceptible and resistant quality control strains.

RESULTS:

A total of 120 Enterococcal stains were isolated from various clinical samples. These included 57(47.50%) *E.faecium*, 53(44.16%) *E.faecalis*, 5(4.16%) *E.mundti,* 2(1.66%) *E.durans*, 2(1.66%) *E. dispar* 1(0.83%) and

The antimicrobial resistance profile of the isolates is shown in [Table/Fig 1]. Most of the isolates were resistant to the tested antibiotics. More then 70% resistance was seen for ampicillin, erythromycin and tetracycline. 5(4.16%) isolates were resistant to teicoplanin and all isolates were susceptible to linezolid.

[Table/Fig 1]: Antimicrobial resistance profile among enterococcal isolates.

Antimicrobial agent	*E.faecium.* n=57(%)	*E.faecalis.* n=53(%)	*E.mundti.* n=5(%)	*E.durans.* n=2(%)	*E.dispar.* n=2(%)	*E.gallinarum.* n=1(%)	Total. n=120(%)
Ampicillin	40(70.17)	37(69.98)	3(60)	2(100)	1(50)	1(100)	84(70)
Vancomycin	4(7.01)	2(3.77)	0(0)	2(100)	0(0)	0(0)	8(6.66)
Teicoplanin	1(1.75)	2(3.77)	0(0)	1(50)	1(50)	0(0)	5(4.16)
Erythromycin	42(73.68)	40(75.47)	5(100)	2(100)	2(100)	1(100)	92(76.66)
Tetracycline	45(78.94)	34(64.15)	4(80)	2(100)	2(100)	1(100)	88(73.33)
Ciprofloxacin	39(68.42)	26(49.05)	5(100)	2(100)	1(50)	0(0)	73(60.83)
Nitrofurantion	20(35.08)	22(41.50)	4(80)	2(100)	2(100)	1(100)	51(42.50)
Linezolid	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)

8 (6.66%) isolates were vancomycin resistant enterococci (VRE) which were detected by the disc diffusion method, but 9(7.50%) isolates were found to be vancomycin resistant by the agar screening method and the MIC method. Disc diffusion showed one *E.gallinarum* isolate which was susceptible (18mm) to vancomycin, but it was found to be intermediately resistant by the MIC method (8µg/ml). This was a major error of the disc diffusion method [Table/Fig 2].

[Table/Fig 2]: Comparison of disc diffusion and agar dilution test for detection of vancomycin resistant enterococcus (VRE).

Enterococcal Species	No. of VRE by Disc Diffusion	No.of VRE by Agar dilution	No. of VRE by MIC
E.faecium	4	4	4
E.faecalis	2	2	2
E.durans	2	2	2
E.gallinarum	0	1	1
Total	8	9	9

[Table/Fig 3] shows High level aminoglycoside resistance. High level gentamycin resistance (HLGR) was seen in 93 (77.69%) isolates and High level streptomycin resistance (HLSR) was seen in 94(61.63%) isolates . Overall, HLAR (HLGR+HLSR) was seen in 73(47.18%) isolates.

[Table/Fig 3]: HLAR among Enterococcal isolates

Enterococcal species (No. of isolates)	No.(%) of isolates showing resistance to		
	HLGR	HLSR	HLGR + HLSR
E.faecium (57)	46 (80.70)	47 (82.45)	39 (68.42)
E. faecalis (53)	40 (75.47)	41 (77.35)	29 (54.71)
E. mundti (5)	3 (60)	3 (60)	3 (60)
E.durans (2)	1 (50)	2 (100)	1 (50)
E. dispar (2)	2 (100)	1 (50)	1 (50)
E.gallinarum (1)	1 (100)	0 (0)	0 (0)
Total(120)	**93 (77.69)**	**94 (61.63)**	**73 (47.18)**

HLGR – High level gentamycin resistance
HLSR - High level streptomycin resistance

DISCUSSION:

In the present study, we determined the species prevalence and the antimicrobial resistance pattern of enterococcal isolates from different clinical samples in our tertiary care teaching hospital.

Earlier studies from various parts of India [8],[9],[10],[11] have shown *E.faecalis* as the predominant species isolated from humans. In our study, we isolated *E.faecium*(47.50%) as the predominant isolate, followed by *E. faecalis* (44.16%), *E.mundti* (4.16%), *E.durans* (1.66%), *E. dispar* (1.66%) and *E.gallinarum* (0.83%). Changes in the hospital's patient population and the antimicrobial use pattern, coupled with the greater antibiotic resistant nature of *E.faecium*

probably conferred a greater selective survival advantage to *E.faecium* as compared to *E.faecalis.* This explains the emergence of *E. faecium* as the predominant isolate.[2]

Multidrug resistant enterococci are being increasingly reported from all over the world. Our study also revealed multidrug resistance in most of the enterococcal isolates. Drug resistance is rapidly acquired by enterococci by plasmids[12], conjugative transposition[13] or by mutations.[5]

Our study revealed *E.faecium* to be more resistant to antimicrobials than *E.faecalis.* Similar findings have been reported by other studies also.[10],[14]

Till recently, many Indian studies have shown vancomycin resistance of 0-5% in the enterococcal isolates. [8],[9],[10],[11] In the present study, vancomycin resistance was seen in 9 (7.50%) isolates. Our study showed increasing vancomycin resistance in the enterococcal isolates. The agar screen method and MIC by the macrobroth dilution method detected vancomycin resistance in all the 9 isolates, but the disc diffusion method failed to detect vancomycin resistance in one *E.gallinarum* isolate. Studies done at CDC, Atlanta [15] and Minnesota USA [16] have also shown this error. This type of an error occurs because motile enterococci have the VAN C genes (*E.casseliflavus, E.flavascence, E.gallinarum*) which encode low level vancomycin resistance (MIC less than or equal to 8 µg/ml) and they are intrinsically resistant to vancomycin. Modification of the CLSI break points, especially for the motile enterococcal species, may resolve the problem, or otherwise, MIC should be done for all motile enterococci. The agar screening method which contains 6µg/ml of vancomycin, should be further evaluated for the detection of such low level resistance, as in our study, we encountered only one motile enterococcus.

HLAR was seen in >75% isolates for gentamycin and in >60% isolates for streptomycin. Overall, HLAR (HLGR+HLSR) was seen in 47.18% isolates. Such high level resistance has been shown by other studies also. [2],[17] This is of great concern, since it eliminates the synergy of the aminoglycosides with the B-lactam antibiotics which is the therapy of choice for enterococcal infections, thus limiting the therapeutic options.

Linezolid has demonstrated good antienterococcal activity and may be kept as a second line drug for VRE.

It can be concluded that this study illustrated the changing epidemiology of enterococcal infections and the high rate of resistance to most of the antibiotics, with an increasing rate of vancomycin and high level aminoglycoside resistance. Measures should be taken to routinely identify the enterococcal species, test their antimicrobial susceptibilities properly and to implement a sound antibiotic policy in every hospital to prevent further increases in resistance and the spread of enterococcal infections.

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