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ORIGINAL ARTICLE

Speciation and Antimicrobial Susceptibility pattern of *Enterococci* from a Tertiary Health Care Center of North India.

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ABSTRACT

Introduction: In recent years, *Enterococci* have become important nosocomial pathogens. Therefore, it is important for a hospital setting to continuously monitor such infections and to determine their species and antimicrobial susceptibility pattern from time to time. Keeping these objectives in mind, the present study was conducted in our tertiary health care center of North India.
Methods: A total of 100 enterococcal strains isolated from urine and blood samples were speciated as per the scheme of Facklam and Collins. Antibiotic susceptibility was determined for Amoxycillin, Penicillin, Cephalexin, Erythromycin, Cotrimoxazole, Gentamicin, Vancomycin, Teicoplanin, Linezolid, Imipenem, Piperacillin, Ampicillin- sulbactam and Nitrofurantoin by Kirby Bauer disc diffusion method. MIC detection was done by Agar dilution method for penicillin and vancomycin. HLAR detection was done by agar dilution method for gentamicin and streptomycin by supplementing the Mueller Hinton agar with 500 µg/ml and 2000 µg/ml of the antibiotics respectively.
Results: 96 of the strains were *Enterococcus faecalis* and 4 were *Enterococcus faecium*. Antibiotic susceptibility tests showed high level resistance to cephalexin (100%), gentamicin(96.42%), cotrimoxazole (87.03%), erythromycin (77.19%) and penicillin (61.17%). However, only two strains were found to be resistant to vancomycin and teicoplanin. All the strains were sensitive to linezolid. HLAR was seen in 75% of the strains for gentamicin and in 69% strains for streptomycin. In case of penicillin, MIC values were found to be >16 µg/ml for 14 strains (14%). 6 strains had MIC values upto 4 µg/ml for vancomycin. Out of these, one *E. faecalis* strain came out to be Vancomycin resistant *enterococci* (VRE) showing MIC value as high as 512 µg/ml.
Conclusion: We conclude that enterococcal strains with high rate of resistance to penicillin and aminoglycosides are prevalent in our nosocomial setting and emergence of VRE strain has further worsened this situation. There is urgent need for more rational and restricted use of antimicrobials, in order to minimize the selection and spread of such strains.
Key Words: *Enterococci*, VRE, HLAR.

Introduction:

Since the advent of Vancomycin resistant *Enterococci* (VRE) by Uttley et al [1] in 1988, Enterococcal infections have been a cause of great concern among the clinicians especially in nosocomial settings. In western countries, especially USA, they have been reported as third most common pathogen associated with blood stream infections and second most common isolated pathogen over all [2].

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Though primarily, they are opportunistic pathogens, their inherently low virulence is well compensated for by their intrinsic resistance to

antibiotics and their ability to acquire resistance to several broad spectrum antibiotics[3]. Vancomycin resistance in *Enterococci* not only leaves fewer options for disease management, but also is important due to potential risk of vancomycin resistant gene transfer from *Enterococci* to *Staphylococcus aureus*[4]. VRE has been frequently reported from USA and Europe, but there are not much reports on their isolation from many Asian countries including IndiA[5]. In addition to it, *Enterococci* are also showing acquired High level resistance to Aminoglycosides (HLAR). Traditionally, a combination of penicillin/ampicillin with an aminoglycoside remains the treatment of choice for *Enterococci* with vancomycin as last resort. Therefore, VRE along with HLAR is making the treatment of these infections extremely difficult and they pose a great challenge to the health professionals.

Although 12 species in the Genus *Enterococcus* have been recognized, most common species implicated in human infection is *E. faecalis* (causing 90% of the infections) followed by *E. faecium*. *E. faecium* predominantly is more resistant species than the *E. faecalis* and emergence of vancomycin resistance in it has caused an increase in the frequency of its isolation[6]. Considering all these facts, the present study was conducted in the tertiary health care centre of North India, to speciate as well as to study the antibiogram of enterococcal strains isolated from the clinical samples –urine and blood. Minimum inhibitory concentration (MIC) values were also determined for penicillin and vancomycin along with HLAR detection in these isolates.

Material and Methods:

The present study was conducted in the Department of Microbiology, Government Medical College Hospital, and Chandigarh. A total of 100 strains of *Enterococci* were isolated from clinical samples namely- urine (49) and blood (51). The strains isolated were identified and speciated according to standard laboratory procedures as per the scheme of Facklam and CollinS[7].

Antimicrobial susceptibility testing was done by Kirby- Bauer disc diffusion method as per the recommendations of CLSI [8]. Various antibiotics tested were: Amoxycillin (10 µg), Penicillin (10 units/disc), Cephalexin (30 µg), Erythromycin (15 µg), Cotrimoxazole (25 µg), Gentamicin (30 µg), Vancomycin (30 µg), Teicoplanin (30 µg), Linezolid (30 µg), Imipenem (10µg), Piperacillin (100 µg), Ampicillin- sulbactam (10/10 µg) and Nitrofurantoin (300 µg).

MIC detection was done by Agar dilution method [9] for penicillin and vancomycin for the MIC values of 2 – 512µg/ml. HLAR detection was done by agar dilution method for gentamicin and streptomycin by supplementing the Mueller Hinton agar with 500 µg/ml and 2000 µg/ml of the antibiotics respectively.

The source of media and antibiotic discs was Hi - Media Ltd. (Mumbai) India. Standard strain *E. faecalis* ATCC 29212 was used as control.

Results:

Of the total enterococcal strains isolated, 96 of the strains came out to be *E. faecalis* and 4 were *E. faecium* on final species level identification. We could not isolate any other species of *Enterococci* from our settings.

Antibiotic susceptibility tests showed high level resistance to various antibiotics tested. Only two strains were found to be vancomycin and teicoplanin resistant. All the strains were sensitive to linezolid [Table/Fig 1]

Table/Fig 1

Percentage resistance of *Enterococci* to various antibiotics by Disc Diffusion Method (n=100)

Sr. No.	Antibiotic	Percentage resistance
1.	Penicillin	61.17
2	Ampicillin	38.88
3	Cephalexin	100
4	Gentamicin	96.42
5	Cotrimoxazole	87.03
6	Erythromycin	77.19
7	Piperacillin	25
8	Imipenem	31.18
9	Ampicillin Sulbactam	34.09
10	Nitrofurantoin	15.90
11	Vancomycin	2.1
12	Teicoplanin	2.1
13	Linezolid	0

HLAR was seen in 75% of the strains for gentamicin and 69% strains for streptomycin. [Table/Fig 2] In case of penicillin, MIC values were found to be >16 µg/ml for 14 strains. Out of these 14 strains, 6 had raised MIC values upto

256 µg/ml. For vancomycin, 6 strains had MIC values upto 4 µg/ml. Out of these, one *E. faecalis* strain came out to be Vancomycin resistant *Enterococci* (VRE) showing MIC value as high as 512 µg/ml.

Table/Fig 2
HLAR in *Enterococci*

***Enterococcus* species**	**Total number isolated**	**HLGR strains (%)**	**HLSR strains (%)**
Enterococcus faecalis	96	71 (73.95)	66 (68.75)
Enterococcus faecium	4	4 (100)	3 (75)
Total	100	75	69

HLGR: High Level Gentamicin Resistance
HLSR: High Level Streptomycin Resistance

Discussion

Combination of colonizing abilities and drug resistance both inherent and acquired has made *Enterococci* significant human pathogens. In the present study, *E. faecalis* (96%) was the predominant species isolated followed by *E. faecium* (4%). Most of the studies done on *Enterococci* support the same finding. Reason could be the predominance of *E. faecalis* in the endogenous flora of the body[10].

Penicillin along with aminoglycosides is the mainstay of therapy for infections with *Enterococci*. Therefore, resistance of *Enterococci* against these antibiotics has important clinical implications. In the present study, about 61% of the strains were resistant to penicillin by disc diffusion method and 14 (14%) of the strains had raised MIC values (>16µg/ml). Rather 6 of them had MIC values more than 200 µg/ml which is considered as cut off for high level resistance to penicillin.[2] Mechanism of this resistance could be low affinity penicillin binding proteins or production of beta lactamases.

Among aminoglycosides, 96% of the isolates exhibited resistance to gentamicin by disc diffusion method. HLAR was seen in 75% of the strains for gentamicin and 69% for streptomycin. HLAR was more in *E. faecium* than *E. faecalis* [Table/Fig 2] as has been reported previously also [11], [12] . Both HLGR and HLSR was seen in 55 isolates. HLAR in these strains can well nullify the efficacy of combination therapy. Therefore, distinguishing HLAR from simple intrinsic resistance is important and should be adopted as a part of routine microbiology laboratory.

Only 2 strains were found to be resistant to vancomycin and teicoplanin by disc diffusion method. Out of these, one strain did not show any rise in MIC value but the other strain came out to be VRE with highly raised MIC value of range upto 512µg/ml. This strain (VRE) was isolated from the blood sample of a female patient of left Guillain Barre' Syndrome with polyneuritis cranialis. Blood samples taken from the patient on the day 1 and 3 of admission revealed the growth of *Enterococcus* species organisms, which on further confirmation was reported as *E. faecalis*. On antibiogram, the organism was found resistant to ampicillin, penicillin, erythromycin, gentamicin, cotrimoxazole, imipenem, piperacillin, teicoplanin and vancomycin, but sensitive to nitrofurantoin and linezolid. MIC detection for vancomycin showed value upto 512 µg/ml. Strain also showed HLAR for streptomycin, but was negative for HLAR to gentamicin.

Patient was managed conservatively and was administered a combination drug piperacillin-tazobactam along with clarithromycin, to which she responded well. In present case, the organism isolated probably belongs to VanA phenotype, as it showed resistance to vancomycin upto MIC of 512 µg/ml and was resistant to teicoplanin by disc diffusion test. The various risk factors associated in this case were history of previous hospitalization, admisssion to ICU, catheterization and prolonged antibiotic treatment. Previously, from India, there are few reports of emergence of vancomycin resistance in enterococcal strains with increased MIC values [Table /Fig 3][13], [14], [15], [16]. The strain isolated in our case had MIC value as high as 512 µg/ml. Previously also, only a single case of vancomycin resistant

E. faecalis strain with MIC value as high as 512 µg/ml has been reported from the blood sample of an ICU patient from Delhi, India.

We conclude that enterococcal strains with high rate of resistance to penicillin and aminoglycosides are prevalent in our nosocomial setting and emergence of VRE strain has further worsened this situation. The time has come when proper control measures should be taken to prevent the spread of such infections.

Table/Fig 3
VRE isolation: Indian Scenario

Year	Author	Number positive	Sample (no. positive)	Species isolated	Phenotype	MIC values (µg/ml)
2003	Mathur *et al*	5	Blood (3), urine (1), soft tissue (1)	*E. faecalis*	4 Van A, 1 Van B	256-512
2004	Taneja *et al*	8	Urine	*E. faecium* (5), *E. faecalis* (1), E.casseliflavus (1), E.pseudoavium (1)	Van B and Van C	8-32
2004	Karmarkar *et al*	12	Urine, blood, pus	*E. faecalis*, *E. faecium*	Van B	> 4
2005	Kapoor *et al*	4	Blood (in pediatric age group)	*E. faecalis* (2), *E. faecium* (2)	-	8
2006	Ghoshal *et al*	10	Blood,Tissue,Urine, CVP Tip	*E. faecium*	Van A	62-256

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