

# Community Acquired Enterococcal Urinary Tract Infections and Antibiotic Resistance Profile in North India

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## ABSTRACT

**Background:** Urinary tract infections (UTIs) remain a major problem both in hospitalized and outdoor patients. Multidrug-resistant enterococci are emerging as a major nosocomial pathogen with increasing frequency. However, the incidence of community-acquired enterococcal infections and species prevalent in India is not thoroughly investigated.

**Objectives:** This study aims to estimate the burden of community-acquired UTIs seen at a tertiary care hospital and to identify the *Enterococcus* species isolated from these patients. The study also aims to determine the antibiotic susceptibility pattern with reference to high-level aminoglycosides and vancomycin.

**Materials and Methods:** Semi-quantitative cultures from a total of 22,810 urine samples obtained from patients seen at various Outpatient Departments were analyzed. From them 115 nonduplicate isolates of enterococci were obtained as significant pure growth ( $>10^5$  cfu/ml) and speciated. Antibiotic susceptibility was performed by Kirby–Bauer disc diffusion method. Vancomycin resistance screening was performed by the vancomycin screen agar method recommended by Clinical and Laboratory Standards Institute and confirmed by determination of minimum inhibitory concentration by agar dilution method.

**Results:** Of 115 enterococcal isolates, 61 were identified as *Enterococcus faecalis*, 42 as *Enterococcus faecium*, 3 each as *Enterococcus dispar*, and *Enterococcus pseudoavium*. High-level gentamicin resistance (HLGR) was higher in *E. faecium* (47.6%) than *E. faecalis* (32.7%) and HLSR also showed the same pattern with 47.6% and 27.9% resistance, respectively. Vancomycin resistant enterococci accounted for 11.3% of the isolates, and out of them 53.8% were *E. faecium* by agar dilution method.

**Conclusion:** High rate of resistance to antibiotics of penicillin group and aminoglycosides was observed in our tertiary care hospital even in community acquired UTIs. Hence, there is an urgent need for more rational and restricted use of antimicrobials.

**Key words:** *Enterococcus faecalis*, *Enterococcus faecium*, urinary tract infection, vancomycin resistant enterococci

## INTRODUCTION

Enterococci are part of the normal resident flora of the gut, oral cavity, and female genital tract. They are well-known as nosocomial opportunistic pathogens.<sup>[1]</sup> *E. faecalis* (80–90%) and *E. faecium* (5–10%) are the two most commonly isolated enterococcal

species from clinical samples.<sup>[2,3]</sup> The most frequent infections caused by these organisms include urinary tract infections (UTIs) followed by intra-abdominal or intrapelvic abscesses and blood stream infections.<sup>[4]</sup> Community-acquired infections due to enterococci are

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on the rise due to intensive use of broad spectrum antibiotics. Moreover, since vancomycin-resistant enterococci (VRE) from animal sources such as poultry and human foods of animal origin play an important role in human colonization and infection, a significant level of VRE colonization may be found among persons not associated with the health care setting.<sup>[5]</sup> High-level aminoglycoside resistance (HLAR),  $\beta$ -lactamase production, and glycopeptides resistance including VRE have been reported among enterococcal species.<sup>[6,7]</sup> Isolation of *E. faecium* and other species from clinical samples is on the rise and poses a significant health concern, as they show intrinsic resistance to many commonly used antibiotics and may lead to a treatment failure.<sup>[8]</sup>

Although a few studies from India have been published which report prevalence of enterococci in hospital settings as well as nosocomial infections with their antimicrobial sensitivity patterns, there is a paucity of information on the status of community-acquired enterococcal infections. This study was undertaken to know the prevalence and antimicrobial susceptibility patterns of enterococci in community-acquired UTI.

## MATERIALS AND METHODS

A total of 22,810 urine samples screened for UTI from the patients attending various Outpatient Departments (OPDs) of the All India Institute of Medical Sciences, New Delhi over a period of 14 months from April 2013 to May 2014 were included in the study. Mid-stream urine samples were cultured by a semi-quantitative method on cysteine lactose electrolyte deficient medium (Hi-Media Laboratories, Mumbai, India). All the samples were screened for *Enterococcus* and nonduplicate isolates obtained in significant colony count ( $>10^5$  CFU/ml) were included in the study. Community-acquired VRE included VRE cultured from patient  $<48$  h after hospital admission, or as an outpatient. Patients who were previously hospitalized or residing in a nursing home for  $>24$  h during the last 31 days, previous history of positive VRE culture or infection, received vancomycin in recent past, or those patients who had outpatient surgery were excluded. Coexisting medical or other factors possibly associated with healthcare exposure (diabetes mellitus, prior antimicrobial drug therapy, renal failure, and immunosuppression) were assessed for all VRE cases.

The strains were identified and speciated according to standard laboratory procedures as per the scheme of Facklam and Collins.<sup>[9]</sup> Antimicrobial susceptibility was

determined by Kirby–Bauer disc diffusion method. Various antibiotics tested were ampicillin (10  $\mu$ g), amoxycyclav (30  $\mu$ g), norfloxacin (10  $\mu$ g), tetracycline (30  $\mu$ g), ciprofloxacin (5  $\mu$ g), nitrofurantoin (300  $\mu$ g), vancomycin (30  $\mu$ g), teicoplanin (30  $\mu$ g), fosfomycin (200  $\mu$ g), and linezolid (15  $\mu$ g) (Hi-Media Laboratories, Mumbai, India).

HLAR was determined by disc diffusion method using high-level gentamicin (120  $\mu$ g) and streptomycin (300  $\mu$ g) discs (Hi-Media Laboratories Pvt., Ltd., Mumbai, India). Vancomycin screen agar test was performed using brain heart infusion agar with 6  $\mu$ g/ml vancomycin (Hi-Media Laboratories, Mumbai, India) to look for resistance to vancomycin. One or more colonies or a film of growth on this screen agar was considered to be resistant to vancomycin. Control strains included were *Staphylococcus aureus* ATCC 25923, *E. faecalis* ATCC 51299, and *E. faecalis* ATCC 29212.

## Minimum inhibitory concentration testing

Determination of minimum inhibitory concentration (MIC) of vancomycin for enterococcal isolates which grew on vancomycin agar screen was done by agar dilution method.<sup>[10]</sup> Muller-Hinton agar was supplemented with different concentrations of vancomycin. Ten microliter of bacterial culture was spot inoculated after adjusting the turbidity with McFarland 0.5 standard. The plates were incubated at 37°C for 24 h and examined for growth. The minimum concentration of vancomycin which inhibited bacterial growth was considered MIC. Enterococci which had MIC  $>32$   $\mu$ g/ml were considered resistant; 8–16  $\mu$ g/ml as intermediately resistant; and MIC of 4  $\mu$ g/ml as susceptible to vancomycin as per Clinical and Laboratory Standards Institute guidelines.<sup>[10]</sup> Data were analyzed, and descriptive percentages were mentioned using SPSS statistical software (version 20) [IBM Corp. NY, USA].

## RESULTS

Of the 22,810 urine samples screened for UTI, 115 (0.5%) *Enterococcus* isolates were obtained during the study period. The ages of the study population ranged between 1 and 75 years with a mean age of  $36.8 \pm 20.0$  years. Of 115 patients, 62 (53.9%) were males, and 53 (46.1%) were females. Most cases were from Urology OPD (24.3%) followed by Medicine OPD (18.3%) in our hospital. The most common enterococcal isolate was *E. faecalis* (61/115 [53%]), followed by *E. faecium* (42/115 [36.5%]), *E. pseudoavium* (3/115 [2.6%]),

*E. dispar* (3/115 [2.6%]), *E. casseliflavus* (2/115 [1.7%]), *E. avium* (2/115 [1.7%]), and *E. gallinarum* (2/115 [1.7%]).

Antimicrobial resistance profile of enterococcal isolates showed that resistance was most frequently observed with tetracycline (77.4%), ciprofloxacin (74.8%), and norfloxacin (66.1%). *E. faecium* strains as compared to *E. faecalis* display a higher degree of drug resistance to multiple other antibiotics including ampicillin, amoxycylav, ciprofloxacin, gentamicin, nitrofurantoin, norfloxacin, streptomycin, and teicoplanin. All the enterococcal strains were sensitive to fosfomycin and linezolid by standard disc diffusion method.

Sixteen enterococcal isolates (13.9%) were vancomycin resistant by Kirby–Bauer disc diffusion method (*E. faecalis*-8, *E. faecium*-6, *E. gallinarum*-2) [Table 1]. Risk factors and demographic characteristics for community-acquired VRE patients are summarized in Table 2. By vancomycin agar screen method, fifteen isolates showed growth, giving an overall VRE positivity of 13%. Therefore, the concordance between the two methods was more than 93.7%. Thirteen isolates showed MIC of vancomycin  $\geq 128$   $\mu\text{g/ml}$  and were resistant to vancomycin by agar dilution method. Two enterococcal isolates were intermediately resistant to vancomycin with MIC of 8  $\mu\text{g/ml}$  and 16  $\mu\text{g/ml}$ , respectively. Therefore, 13 isolates were identified as VRE and two isolates showed intermediate resistance.

Of the 13 isolates of *Enterococcus* found to be resistant to vancomycin by MIC testing, 12 were found to be resistant to teicoplanin by disc diffusion method also. These 12 isolates (92.3% of VRE), therefore, showed the *Van A* type of phenotype (resistance to both vancomycin and teicoplanin). On speciation, 6 of these were identified as *E. faecalis*, 4 *E. faecium*, and 2 as *E. gallinarum*. One isolate of

*E. faecium* showed the *Van B* type of phenotype (resistant to vancomycin, sensitive to teicoplanin).

High-level gentamicin resistance (HLGR) was more in *E. faecium* (47.6%) than *E. faecalis* (32.7%) and HLSR was also same with 47.6% and 27.9% resistance, respectively [Table 1]. HLGR was detected in 39.1% and HLSR in 35.6% of the total isolates. Forty-one isolates (35.6%) showed both HLGR and HLSR which included 14.7% ( $n = 17$ ) of the *E. faecalis* and 17.4% ( $n = 20$ ) of the *E. faecium* isolates. Out of thirteen vancomycin-resistant isolates, HLGR and HLSR were found in 92.3% and 84.6% isolates, respectively.

## DISCUSSION

In this study, *E. faecalis* (53%) was found to be the most predominant isolate in community acquired enterococcal UTIs. Most clinical infections were due to either *E. faecalis* or *E. faecium*. Most of the studies done on enterococci support the similar findings which could be attributed to the predominance of *E. faecalis* in the endogenous flora of the body.<sup>[11]</sup>

The sources and reservoirs that play a role in the resistance to antibiotics of enterococci that are community acquired are not known. Reports from developed countries suggest that VRE colonization can frequently occur in the community, suggesting a reservoir in the community.<sup>[12,13]</sup> VRE have also been found in sewage, from stools of healthy farm animals and animal products. In many countries, glycopeptides containing animal feeds are believed to be responsible for VRE intestinal colonization in farm animals and pets which ultimately results in colonization of humans via the food chain.<sup>[14]</sup> Therefore, transmission of VRE from these sources may result in an increased human reservoir of VRE colonization. Control

**Table 1: Antibiotic resistance (%) among the enterococcal isolates by Kirby-Bauer disc diffusion method**

Antibiotic	<i>Enterococcus faecalis</i> (n=61)	<i>Enterococcus faecium</i> (n=42)	<i>Enterococcus pseudoavium</i> (n=3)	<i>Enterococcus dispar</i> (n=3)	<i>Enterococcus casseliflavus</i> (n=2)	<i>Enterococcus avium</i> (n=2)	<i>Enterococcus gallinarum</i> (n=2)
Tetracycline (30 $\mu\text{g}$ )	78.7	76.2	66.7	66.7	100	50	100
Ciprofloxacin (5 $\mu\text{g}$ )	68.8	83.3	33.3	33.3	100	100	100
Norfloxacin (10 $\mu\text{g}$ )	59	76.2	33.3	66.7	100	100	50
Ampicillin (10 $\mu\text{g}$ )	36.1	45.2	33.3	33.3	100	100	100
Gentamicin (120 $\mu\text{g}$ ) (HLGR)*	32.7	47.6	33.3	33.3	50	50	50
Streptomycin (300 $\mu\text{g}$ ) (HLSR)*	27.9	47.6	33.3	0	50	50	50
Nitrofurantoin (300 $\mu\text{g}$ )	14	23.8	33.3	66.7	50	0	0
Vancomycin (30 $\mu\text{g}$ )	22.9	14.3	0	0	0	0	100
Teicoplanin (30 $\mu\text{g}$ )	11.5	11.9	0	0	0	0	50
Fosfomycin (200 $\mu\text{g}$ )	0	0	0	0	0	0	0
Linezolid (15 $\mu\text{g}$ )	0	0	0	0	0	0	0
Amoxycylav (30 $\mu\text{g}$ )	19.7	28.6	0	0	50	50	50

\*HLGR: High-level gentamicin resistance, HLSR: High-level streptomycin resistance

**Table 2: Risk factors and demographic characteristics for community-acquired vancomycin-resistant enterococci patients**

Characterization	Number
VRE cases without coexisting factors	13
VRE cases with coexisting factors	3
Coexisting factors	
>65 years of age	2
Diabetes mellitus	1
Renal failure	0
Prior antimicrobial therapy	0
Immunosuppression	0
Sex	
Male	10
Female	6

VRE: Vancomycin-resistant enterococci

of these cases, therefore, requires community-based initiative.

*E. faecium* strains as compared to *E. faecalis* display a higher degree of drug resistance to multiple other antibiotics. This emphasizes the need to speciate enterococcal isolates from clinical samples.

The past records of the patients did not indicate prior exposure to vancomycin in any of these patients. In our study, 11.3% were found to be VRE, of which 53.8% were *E. faecalis*. Interestingly our observations were in accordance with two Indian studies, one from Gujarat and one from South India, in which *E. faecalis* was found to be a predominant isolate among VRE.<sup>[15,16]</sup> However, in most of the other studies *E. faecium* was found to be the most common isolate among VRE.<sup>[17]</sup> Emergence of VRE is of concern due to the limited therapeutic options.<sup>[17-19]</sup> This study shows that 92.3% of VRE isolates had *Van A* phenotype. This finding is in accordance with a previous study by Mathur *et al.*, who also reported *Van A* phenotype in 80% of their VRE isolates.<sup>[19]</sup>

In present study, HLAR was seen in 39.1% and 35.6% of the enterococcal isolates for gentamicin (HLGR) and streptomycin (HLSR), respectively. Among *E. faecalis* and *E. faecium* isolates resistance rate was more against streptomycin than gentamicin (HLSR), as reported by others which is similar to previous studies.<sup>[19,20]</sup> This observation is important because the HLAR in enterococci isolates can well nullify the efficacy of combination therapy. The high prevalence of HLSR strains even if it is due to intrinsic low-level resistance for streptomycin, restricts the clinical use of aminoglycosides for enterococci.

## CONCLUSION

The vancomycin resistance rate among the *Enterococcus* isolates was 11.3% in our study which was high as compared to other reports from North India. The most common phenotype of glycopeptide resistance seen in our study was the *Van A* phenotype (resistance to both vancomycin and teicoplanin). Other phenotypes seen were *Van B* (resistant to vancomycin, sensitive to teicoplanin).

The acquisition of HLAR and vancomycin resistance has limited the therapeutic options available for clinicians. Therefore, it is suggested that clinical microbiology laboratories should establish effective detection methods for vancomycin resistance. Sources for community-acquired VRE should be investigated on top priority, to contain the spread of VRE to the hospital environment and other populations.

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## Conflicts of interest

There are no conflicts of interest.

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