Research Article

Prevalence of multidrug-resistant *Enterococcus* species isolated from urine samples in a tertiary care hospital, Western India

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ABSTRACT

Background: *Enterococci* have emerged as an important cause of nosocomial infections, and antibiotic resistance *Enterococcus* is a major obstacle for treatment. **Objective:** The present study was carried out to determine the species of *Enterococci* isolated from urine samples and to determine its multidrug-resistant pattern. **Materials and Methods:** *Enterococcus* spp. were isolated and identified from urine samples between February 2014 and June 2015 by the standard biochemical tests. Antimicrobial susceptibility testing was performed by modified Kirby-Bauer disc diffusion method as per the Clinical and Laboratory Standards Institute guidelines. **Result:** Among the 156 isolates, *Enterococcus faecium* constituted the predominant isolate. They were found to be susceptible to linezolid and vancomycin with least sensitive to ampicillin and ciprofloxacin. **Conclusion:** Routine speciation and *in vitro* antimicrobial susceptibility testing of *Enterococcus* in urine samples are emphasized due to the prevalence of a wide variety of *Enterococcus* species and also appearance of high-resistant strains.

KEY WORDS: *Enterococcus* spp.; Antimicrobial Susceptibility Testing; Multidrug Resistance; High-level Gentamicin Resistance; Vancomycin-resistant *Enterococci*

INTRODUCTION

Enterococci contain a C-carbohydrate that reacts with Lancefield Group D antisera. Therefore, in the past, they were considered Group D *Streptococci*.[1] Today, DNA analysis and other properties have placed them in their own genus. *Enterococci* are regular inhabitants of the bowel. They are found in the intestine of nearly all animals, from cockroaches to humans. *Enterococci* are readily recovered outdoors from vegetation and surface water probably because of contamination by animal excrement or untreated sewage. In humans, typical concentrations of *Enterococci* in stool are up to 10^8 CFU per gram. Although the oral cavity and vaginal tract can become colonized, *Enterococci* are recovered from these sites in fewer than 20% of cases.[2]

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Enterococci, leading cause urinary tract infection (UTI), are becoming resistant to many and sometimes all standard therapies. *Enterococci* are not very virulent, but they have become prominent as a cause of nosocomial infections as a result of their multiple antibiotic resistance.[3]

Genus *Enterococcus* includes more than 17 species, but only a few cause clinical infections in humans. *Enterococcus faecalis* is the most prevalent species cultured from humans, accounting for more than 90% of clinical isolates. Other *Enterococcal* species known to cause human infections include *Enterococcus avium, Enterococcus gallinarum, Enterococcus casseliflavus, Enterococcus durans, Enterococcus raffinosus,* and *Enterococcus mundtii*. *E. faecalis* is isolated from approximately 80% of human infections, and *Enterococcus faecium* represents most vancomycin-resistant *Enterococci*

(VRE). Infections to other *Enterococcal* species are rare.[2] Most *Enterococcal* infections are caused by *E. faecalis*, which are more likely to express traits related to retain sensitivity to at least one effective antibiotic.[2] The remaining infections are mostly caused by *E. faecium*, a species virtually devoid of known overt pathogenic traits but more likely to be resistant to even antibiotics of the last resort.

Two types of *Enterococci* cause infection:

1. Those originating from patients' native flora, which are unlikely to possess resistance beyond that intrinsic to the genus and are unlikely to be spread from bed to bed.
2. Isolates that possess multiple antibiotic resistance traits and are capable of nosocomial transmission.

The therapeutic challenge of multiple drug-resistant (MDR) *Enterococci* has brought their role as important nosocomial pathogens into sharper focus.[4]

Enterococci are intrinsically resistant to many antibiotics. Unlike acquired resistance and virulence traits, which are usually transposon or plasmid encoded, intrinsic resistance is based on chromosomal genes, which typically are nontransferable. Penicillin, ampicillin, piperacillin, imipenem, and vancomycin are among the few antibiotics that show consistent inhibitory, but not bactericidal, activity against *E. faecalis*. *E. faecium* are less susceptible to beta-lactam antibiotics than *E. faecalis* because the penicillin-binding proteins of the former have markedly lower affinities for the antibiotics.[5] *Enterococci* often acquire antibiotic resistance through exchange of resistance encoding genes carried on conjugative transposons, pheromone-responsive plasmids, and other broad host range plasmids.[6]

The past two decades have witnessed the rapid emergence of MDR *Enterococci*. High-level gentamicin resistance (HLGR) occurred, and simultaneously, sporadic outbreaks of nosocomial *E. faecalis* and *E. faecium* infection appeared with penicillin resistance due to beta-lactamase production; however, such isolates remain rare. Finally, MDR *Enterococci* that had lost susceptibility to vancomycin were reported.[4] Among several phenotypes for vancomycin resistance *Enterococci*, Van A (resistance to vancomycin and teicoplanin) and Van B (resistance to vancomycin alone) are most common. Inducible genes encoding these phenotypes alter cell wall synthesis and render strains resistant to glycopeptides.[5] Van A and Van B types of resistance are primarily found among *Enterococci* isolated from clinical, veterinary, and food specimens but no other common intestinal or environmental bacteria.[4,5]

MATERIALS AND METHODS

Study Area

From February 1, 2014, to June 30, 2015, urine samples collected in a tertiary care hospital from patients clinically diagnosed to be suffering from UTI were processed for bacteriology culture and sensitivity. Clean catch mid-stream urine samples received in sterile containers and processed as per the Clinical and Laboratory Standards Institute (CLSI) guidelines.

The patients who satisfied the following criteria were included in the study.

All urine specimens isolates of *Enterococci* spp. isolated in a microbiology laboratory.

I. All male and female patients suspected from UTI.
II. All patients including from all intensive care units.

Examination of samples was done by direct microscopy, followed by bacterial culture.

Urine was examined microscopically as a wet preparation to detect significant pyuria, i.e., WBCs in excess of 10^7 WBC/l of urine, red cells, casts, yeast cells, bacteria. A gram stain smear of the urine was examined when bacteria and/or white cells were seen in the wet preparation. All urine samples were cultured on nutrient agar, blood agar, MacConkey agar and incubated at 37°C for 18-24 h. Any significant growth obtained was identified using general appearance of the colonies and characters such as pigment production, hemolysis, and negative catalase. On nutrient agar, colonies were 1 mm diameter, convex with regular margin. On blood agar, it gave non-hemolytic colonies. On MacConkey agar, small, tiny, deep (0.5-1 mm), usually magenta-colored colonies were seen.

Bacterial Colony Count of Bacteria in UTI

A measured amount of urine, using calibrated loop method, was inoculated into blood agar medium for colony count. Equal or more than 10^5 CFU/ml of a single potential pathogen interpreted as positive UTI and a result of 10^2-10^4 CFU/ml was repeated. A $<10^2$ CFU/ml was interpreted as negative UTI.

Gram stain was done from nutrient agar and it showed that Gram-positive cocci characteristically larger, oval arranged in pair, and short chain in pair were arranged at an angle to each other. Motility was carried out by hanging drop method to detect *E. casseliflavus* and *E. gallinarum* which like *E. faecium* ferment arabinose but are motile. All isolates were non-motile. Entero-set consisting of growth on esculin agar in the presence of 40% bile, 6.5% NaCl, and arabinose test was used to identify *Enterococci*.[7] Antimicrobial susceptibility testing of the isolates was carried out using modified Kirby-Bauer disc diffusion method on Mueller-Hinton agar as recommended by the CLSI.[8-10] Thirteen isolates were interpreted as susceptible or resistant according to the sensitivity zones of the particular antimicrobial as recommended by the CLSI. Age in years and gender were

demographic while isolation of *Enterococci* and their sensitivity to different antibiotics were research variables.

RESULTS

A total of 156 *Enterococcal* isolates obtained from urine samples from February 1, 2014, to June 30, 2015. During this period, 13,971 urine samples were received and processed for bacteriological culture at our hospital. Table 1 shows that most common isolate among *Enterococcal* spp. was *E. faecium* (67.95%), followed by *E. faecalis* (32.05%).

Table 2 shows that 97.44% isolates were resistant to penicillin-G, 91.67% resistant to ampicillin, 76.28% resistant to ciprofloxacin, and 95.51% resistant to erythromycin. It also shows that 68.59% isolates were sensitive to levofloxacin and 43.59% sensitive to tetracycline. It shows that 70.51% were sensitive to HLG. All isolates of *Enterococci* were resistant to low-level gentamicin and 29.49% were resistant to HLG. Out of 156, 151 isolates were sensitive to vancomycin and 5 were resistant to vancomycin. Hence, the prevalence of VRE was 3.20% in urine samples. Among 5 isolates of VRE, 4 were *E. faecium* and 1 was *E. faecalis*. The vancomycin MIC for one of these isolate was more than 256 μg/ml by Ezy MIC™ (E-test), so they were high-level resistance to vancomycin according to the CLSI guidelines. Out of 5 VRE, 1 was resistant and 4 were sensitive to teicoplanin, so they were of Van A and Van B phenotype, respectively. All isolates were sensitive to linezolid. The main concern in *Enterococci* is the high amount of drug resistance that has been reported in present study, and according to our study, vancomycin remains the drug of choice.

Table 1: Distribution of *Enterococcus* spp. in urine specimens

Clinical specimens	*E. faecalis*	*E. faecium*	Number of *Enterococci* (%)
Urine (%)	50 (32.05)	106 (67.95)	156 (100)

E. faecalis: *Enterococcus faecalis*, *E. faecium*: *Enterococcus faecium*

Table 2: Antimicrobial susceptibility pattern of various antibiotics against *Enterococcus* by disc diffusion method

Name of antibiotic	Isolates (%)	
	Susceptible (S)	Resistant (R)
Penicillin-G (Pe)	4 (2.56)	152 (97.44)
Ampicillin (Am)	13 (8.33)	143 (91.67)
Ciprofloxacin (Ci)	37 (23.72)	119 (76.28)
Levofloxacin (Le)	107 (68.59)	49 (31.41)
Erythromycin (Er)	7 (4.49)	149 (95.51)
Tetracycline (Te)	68 (43.59)	88 (56.41)
HLG	110 (70.51)	46 (29.49)
Vancomycin (Va)	151 (96.79)	5 (3.20)
Teicoplanin (Tei)	153 (98.08)	3 (1.92)
Linezolid (Li)	156 (100)	0 (0)

HLG: High-level gentamicin

Table 3 shows that *E. faecium* was more resistant to antimicrobial agents than *E. faecalis*. The highest number of *Enterococci* was isolated from 2 to 12 year of age (21.79%), followed by 41-55 years age group (19.87%) and 26-40 years (17.31%). *Enterococcal* infection was more common in male (60.25%) than female (39.74%). Highest isolation rate of *Enterococcus* spp. was from medical ward (25.6%) and pediatric ward (21%).

DISCUSSION

Isolation rate of *Enterococci* from urine was 3.21%. *E. faecium* (67.95%) was the most common species isolated followed by *E. faecalis* (32.05%). In the present study, the prevalence of HLG was 46 (29.49%) and VRE was 3.20%. Overall, resistance to penicillin, ampicillin, ciprofloxacin, and erythromycin among strains of *E. faecium* was higher than among strains of *E. faecalis*. For all other antibiotics, there was no significant difference between resistance pattern of *E. faecalis* and *E. faecium*.

Isolation rate was comparable to study done at Krishna Institute of Medical Sciences, Karad, Mumbai (4.2%). It was not comparable with study at Shri B. M. Patil Medical College, Bijapur (12.1%) and study at M. G. Karmarkar, G.S. Medical College, Mumbai (10.28%). Reasons for these higher urinary isolates than present study include active surveillance for *Enterococcal* infection and differentiation between colonization and infection might not be properly carried out.[11] The resistant to HLG in this study was comparable to University Teaching Hospital located in Northwest, Iran (47.3%), incomparable to B. M. Patil Medical College, Bijapur (64.67%). Prevalence of VRE in the present study was comparable to study at Krishna Institute of Medical Sciences, Karad, Mumbai, and Armed Forces Institute of Pathology, Rawalpindi (1.4% and 3%, respectively). VRE in the present study was incomparable to study at B. M. Patil Medical College, Bijapur (36%), Medical science Tehran University, Tehran (16.93%), and University Teaching Hospital located in Northwest, Iran (18.6%).[12-16] This difference may be related to settings under which the studies were carried out. Gordon et al.[17] reported that *E. faecium* was found more resistant to commonly used antibiotics as compared with *E. faecalis*. Reasons for these incomparability in antibiotic susceptibility pattern were surveillance for colonization, identification of colonized and infected patients, isolation of colonized patients, the use of gowns and gloves by health-care worker (barrier method), handwashing with an antiseptic after gloves removal, and avoid contact with environmental surfaces after gloves removal.[18] Medical equipment (stethoscopes, blood pressure cuffs, etc.,) must be dedicated to HLAR patients.[19]

Table 3: Antimicrobial susceptibility pattern of various antibiotics against *Enterococcus* species by disc diffusion method

Species	Antibiotics																					
	Pe		Am		Ci		Le		Er		Te		Va		Tei		Lz		HLG			
	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R		
E. faecalis n=50 (%)	2 (4)	48 (96)	6 (12)	44 (88)	19 (38)	31 (62)	36 (72)	14 (28)	5 (10)	45 (90)	23 (46)	27 (54)	49 (98)	1 (2)	50 (100)	0 (0)	156 (100)	0 (0)	32 (64)	18 (36)		
E. faecium n=106 (%)	2 (2)	104 (98)	7 (7)	99 (93)	18 (17)	88 (83)	71 (67)	35 (33)	2 (2)	104 (98)	45 (43)	61 (57)	102 (96)	4 (4)	103 (97)	3 (3)	156 (100)	0 (0)	78 (74)	28 (26)		
Total	4	152	13	143	37	119	107	49	7	149	68	88	151	5	153	3	156	0	110	46		

E. faecalis: *Enterococcus faecalis*, *E. faecium*: *Enterococcus faecium*

Our study was conducted in a medical college hospital in which children as well as adults were treated both as inpatients and outpatients.

CONCLUSION

E. faecium (67.95%) was the most common species isolated followed by *E. faecalis* (32.05%). The species most commonly implicated in human infections is *E. faecalis*, the increasing occurrence of *E. faecium* is of particular concern due to high resistance to antibiotics especially in nosocomial settings, *E. faecium* was more resistant to antimicrobial agents than *E. faecalis*.[20-23] *Enterococci* have emerged from being harmless commensals to versatile, lethal pathogens. The rising multidrug resistance is worrisome as the commonly used antibiotics for the treatment of nosocomial UTI are less effective. Thus, prevention and control of spread of MDR *Enterococci* require coordination effort from various departments and can only be achieved by,

- Education of hospital staff regarding problem of drug resistance.
- Injudicious usage of antibiotics must be curtailed, and local antibiotic policies must be formulated.
- Early detection and reporting, screening of health-care workers, and immediate implementation of appropriate infection control measure.
- Improved surveillance mainly in intensive care units.

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