

Prevalence and antimicrobial susceptibility of high-level gentamicin resistant enterococci isolated from urine at a hospital in Pune, India

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Abstract

Introduction: Enterococci are one of the common organisms isolated from hospitalized patients with urinary tract infections. Guidelines recommend testing enterococci for susceptibility to high-level gentamicin (HLG) and streptomycin. The present study was planned to determine the susceptibility of uropathogenic enterococci to high-level gentamicin in a tertiary care hospital.

Materials and Methods: Prospective observational research was carried out at a tertiary care hospital for two years on all isolated enterococci from urine specimens. Identification and antibiotic susceptibility were performed as per standard methods. All the isolated enterococci were tested for high level gentamicin ((120µg) resistance and susceptibility to other recommended antimicrobial agents by standard methods.

Results: A total of 320 uropathogenic enterococci were isolated and tested for antibiotic susceptibility. The majority of enterococci were isolated from elderly (34.06%) and admitted patients (69.06%). A total of 68.4% isolated enterococci were HLG resistant. HLG resistant enterococci were highly resistant to erythromycin (96.3%), ciprofloxacin (96.8%) and nalidixic acid (97.7%). Enterococci sensitive to HLG were significantly ($p < 0.05$) less resistant to the other antimicrobial agents except nalidixic acid. Only 20.5% isolated Enterococci were resistant to vancomycin. All isolated enterococci were susceptible to linezolid.

Conclusion: The study demonstrated high prevalence of HLG resistant enterococci causing UTI in our hospital setting. Compared to HLG sensitive enterococci, HLG resistant enterococci were more resistant to other antimicrobial agents tested. The findings highlight the need for mandatory testing of enterococci for HLG resistance to determine effective antimicrobials for treatment.

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Introduction

Enterococci, a Gram-positive facultative anaerobic catalase-negative cocci, are one of the common organisms responsible for hospital-associated infections (HAI) in healthcare settings [1,2]. The majority of enterococci are isolated from individuals with bacteremia, urinary tract infection

(UTI), infective endocarditis, and occasionally meningitis [3]. Enterococci are capable of producing biofilm, enhancing adhesion in urinary catheters, artificial heart valves, and dental prostheses [4]. Among all uropathogens identified from urine specimens, enterococci are the second most common [5].

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Isolation of organisms from clinical specimens is pointless unless antibiotic susceptibility is performed. Clinical Laboratory Standard Institute (CLSI) guideline is useful for assessing antibiotic susceptibility in enterococcus [6]. Beta-lactam, macrolides, fluoroquinolones, glycolipopeptides, and linezolid are commonly used antibiotics for enterococci. Few drugs namely aminoglycosides, cephalosporins, cotrimoxazole, and clindamycin though effective on gram-positive organisms are not effective on enterococcus because enterococci are intrinsically resistant to them [6]. However, aminoglycosides can be used in combination with bacterial cell wall biosynthesis inhibitor drugs such as penicillin, ampicillin or vancomycin [7]. Only gentamicin and streptomycin are recommended for combination use in enterococcal infection to have the synergistic effects while other aminoglycosides are not used in this application [8,9].

CLSI guidelines recommend testing enterococcal species for susceptibility to high-level gentamicin (HLG) and streptomycin from the aminoglycoside group because their mechanisms of action differ from other aminoglycosides and are effective drugs in combination with other drugs or alone at higher doses than the standard therapeutic dose [4]. Several studies reported high prevalence of enterococci in urinary tract infections [9-12].

The present study investigated the susceptibility of uropathogenic enterococci to high-level gentamicin at a tertiary care hospital. Also, the study evaluated susceptibility patterns of various antibiotics in the context of high-level gentamicin resistance in enterococci.

Materials and method

Prospective observational research was carried out at a tertiary care medical college hospital for two years, from December 2020 to November 2022. The study included all urine specimens with significant (10^5 CFU/ml) growth of enterococci from patients with features of urinary tract infection. Other specimens and uropathogens were not included in the investigation. The study was approved by the institutional Ethical sub-

committee before the study was initiated. Approval was granted by letter no: I.E.S.C./154/2022 dated 12 November 2022. Written informed consent was taken from patients or their attendants regarding sample testing, their results, and further use of results for research purposes.

Samples were inoculated on Cystine Lactose Electrolyte Deficient (CLED) agar plate with a calibrated single-loop wire. Culture plates were incubated at 37°C for 18-24 hours. Following incubation, culture plates were examined for growth of enterococci. Only significant colony-forming units (10^5 /ml or more) were considered pathogenic. Suspected colonies were confirmed using a Gram stain smear and other standard biochemical tests. Catalase and bile esculin tests were performed for identification of enterococci [13]. Antibiotic susceptibility testing was performed on cation-adjusted Muller Hilton agar by Kirby Bauer disc diffusion method. Following discs were used: erythromycin (15µg), ciprofloxacin (5µg), vancomycin (10µg), linezolid (30µg), ampicillin (10µg), and gentamicin (120µg). Lawn culture was performed and the above discs were placed on a lawn-cultured plate. Culture plates were incubated at 37°C for 18-24 hours. Antibiotic susceptibility was interpreted using current CLSI guidelines M-100 (2021 and 2022) [6,14].

Enterococcus faecalis ATCC 29212 and *Enterococcus casseliflavus* ATCC 700327 were used as the quality control strains. From time-to-time QC strain was checked by disc diffusion as well as automation to maintain the quality of the test and study.

Results

During the study period, a total of 320 enterococci were isolated and tested for HLG susceptibility by the Kirby Bauer disc diffusion method. Out of 320 isolates, 68.4% were resistant to HLG. Of the total isolates, 158 (49.37%) were from male and 162 (50.6%) were female patients. The highest numbers of enterococci were isolated from 41-60 years age group (34.1%) followed by the 21-40 years (29.1%) and above 60 years (24.4%) age groups (Table-1).

Table-1: Distribution of high level gentamicin (HLG) resistant and susceptible enterococci according to the gender, age, location and speciality (n=320)

Category	Enterococci		
	Number (%)	HLG Resistant n (%)	HLG Sensitive
Total isolates	320	219 (68.4) [CI: 0.632, 0.733]	101 (31.6) [CI: 0.267, 0.368]
Gender			
Male	158 (49.4)	110 (69.6)	48 (30.4)
Female	162 (50.6)	109 (67.3)	53 (32.7)
Age groups			
< 1 year	9 (2.8)	8 (88.9)	1 (11.1)
1-17 year	31 (9.7)	23 (74.2)	8 (25.8)
18-40 year	93 (29.1)	58 (62.7)	35 (37.6)
41-60 year	109 (34.1)	80 (73.4)	29 (26.6)
>60 years	78 (24.4)	50 (64.1)	28 (35.9)
Location			
In-patient	221 (69.1)	147 (66.5)	65 (29.4)
ICU	65 (20.3)	51 (78.5)	14 (38.5)
Outpatient	34 (10.6)	21 (61.8)	13 (38.2)
Speciality			
Medicine	111 (34.7)	75 (67.6)	36 (32.4)
Urology	73 (22.8)	46 (63)	27 (37)
Pediatric	27 (8.4)	23 (85.2)	4 (14.8)
Nephrology	25 (7.8)	14 (56)	11 (4)
Critical Care	25 (7.8)	19 (76)	6 (2)
Surgery	21 (6.6)	16 (76.2)	5 (23.8)
OBGY	19 (5.9)	13 (68.4)	6 (31.6)
Others	19 (5.9)	13 (68.4)	6 (31.6)

CI: Confidence Interval, ICU: Intensive Care Unit, OBGY: Obstetrics and Gynaecology

During the study period, specimens were received from out and in patients departments and ICU. The highest number of specimens was received from admitted patients (69.1%) but the highest HLG resistant enterococci were found in samples from ICU-admitted patients (78.5%). Among all the patients, the majority of the specimens were received from the medicine department (34.7%) followed by urology department (22.8%). However, the highest HLG resistant enterococci were isolated in samples from patients of pediatric ward (85.2%) followed by patients from surgery (76.2%) and

critical care medicine (76%). Details are shown in Table-1.

Table-2 shows the susceptibility of HLG resistant and sensitive enterococci isolates to several antimicrobial agents tested. Except resistance to nalidixic acid, HLG resistant enterococci were significantly ($p < 0.05$) more resistant to ampicillin, erythromycin, nitrofurantoin and vancomycin compared HLG sensitive isolates. Overall, 15% enterococci were resistant to vancomycin. All the isolated enterococci were sensitive to linezolid.

Table-2: Susceptibility of HLG resistant and sensitive enterococci isolates to antimicrobial agents tested

Antimicrobial agents	Enterococci			p value*
	HLG resistant (N=219)	HLG sensitive (N=101)	Total (N=320)	
	Resistant, n (%)	Resistant, n (%)	Resistant n (%)	
Nalidixic acid	214 (97.7)	96 (95)	310 (96.9)	$p > 0.05$.
Ciprofloxacin	212 (96.8)	81 (80.2)	293 (91.6)	
Ampicillin	136 (62.1)	39 (38.6)	175 (54.7)	$p < 0.05$
Erythromycin	211 (96.3)	88 (87.1)	299 (93.4)	
Nitrofurantoin	112 (51.1)	27 (26.7)	139 (43.4)	
Vancomycin	45 (20.5)	3 (2.9)	48 (15)	
Linezolid	0	0	0	-

Note: p value calculated by Z test

Discussion

Drug-resistant enterococci play a significant role in hospital acquired infections. Detection of HLG resistance in enterococci is important for successful management of infection. With this background, the current study was planned.

In the present study, there was no significant difference of isolation rate of enterococci from urine samples of male and female cases. Several Indian and international studies also reported almost similar rates (52.3% to 59.7%) of enterococcal infection in male and female patients [15-19]. However, these studies were conducted with blood, urine and others clinical specimens while the current study was conducted only on urine specimens.

In the current study, 89.4% of isolates were from the admitted patients which include 69.1% from wards and 20.3% from ICU admitted patients and the findings were comparable to other reported studies [15,20,21]. Age can play a major role in causing urinary infections. Elderly patients are more prone to acquire UTIs. In the current study more than half of the UTI patients (55.8%) with enterococcal was above the age of 40 years. Other studies also reported similar rates [15,19].

Several studies investigated the magnitude of HLG resistance in enterococci isolated from different clinical samples. In our study 68.4% enterococcal isolates from urine was HLG-resistant. Studies from different regions of India and other countries also reported the rates from 41% to 86.2% [6,8,12,13,16,17,20-25]. In our study, resistance

against other commonly used antibiotics was found significantly higher in HLG resistant enterococci compared HLG sensitive isolates. Similar results were also reported by Dadfarma N *et al*, specifically for penicillin, ciprofloxacin, and erythromycin [11]. Overall, the resistance against ampicillin, quinolones, macrolides and nitrofurantoin was high in our isolates. Several other studies also reported almost similar resistance rate in enterococci [12,16-20,22-24,26,27].

Vancomycin is used to treat infections due to methicillin resistant *S. aureus* (MRSA) and enterococci. In our series, overall 15% of the enterococci was resistant to vancomycin, but the rate was significantly higher in HLG resistant than that of sensitive enterococci (20.5% vs. 2.9%). Several earlier studies reported the resistance to vancomycin from 12% to about 37% [12,19,20,23,24,26]. Linezolid is increasingly used to treat infections due to vancomycin resistant and sensitive enterococci. Recently, resistance to linezolid has been reported by many studies. The reported resistance to linezolid varied from 0.5% to 4% [12,19,23,24]. However, in the current study, all the enterococci isolates were susceptible to linezolid.

The present study had some limitations. The organism could not be identified up to the species level and minimum inhibitory concentrations (MIC) for the tested antibiotics were not done due to limited resources. Occurrence of high rates (68%) of HLG resistant enterococci in the present study highlights the need for testing and reporting enterococci for HLG resistance.

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Conflict of interest

None of the author has conflict of interest.

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