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Antimicrobial susceptibility pattern of enterococci isolated from various clinical samples in a tertiary care hospital in India

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Abstract

Background and objectives: Enterococci are significant human pathogens that are capable of causing various nosocomial infections. This study determined the antibiotic susceptibility pattern of enterococcal species isolated from various clinical specimens with special reference to vancomycin-resistant enterococci.

Material and methods: The study was carried out for 6 months on enterococci isolated from various clinical specimens at a tertiary care hospital. Organisms were identified by standard procedures, and subjected to antimicrobial testing as per the standard guidelines.

Results: Total 116 enterococci were isolated from various clinical samples. Of the total isolates, 56.9%, 30.2% and 12.9% were isolated from indoor, intensive care unit and non-hosptalized (outdoor) patients respectively.The most common *Enterococcus* species from blood was *E. faecium* (72%) followed by *E. faecalis* (12%) and *E. galinarrium* (9.4%). Out of 116 enterococci isolates, 31 (26.7%) were resistant to vancomycin and only 1 (0.9%) was resistant to linezolid.

Conclusion:The study demonstrated high prevalence of multidrug-resistant enterococci in our hospital setting, thus posing a serious therapeutic challenge. The result would be useful in monitoring the future trends of antimicrobial susceptibility of enterococci in this region.

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Introduction

Enterococci are inhabitants of normal human intestinal flora which were thought to be harmless. But over the past few decades, it has emerged as an agent of serious nosocomial infection with a dramatic increase in patient morbidity and mortality, thus causing increasing associated costs of healthcare in such patients [1]. Enterococci species cause variety of infections and the most common species that account for 90% of clinical isolates are *Enterococcus faecalis* and *Enterococcus faecium* [2, 3]. Traditionally, enterococcal infections are treated with cell wall active agents namely penicillin or ampicillin. However, *Enterococcus* species are intrinsically resistant to many antimicrobial agents, including cephalosporins, clindamycin, cotrimoxazole and aminoglycosides, with the capacity to acquire resistance genes and mutations [4]. The rapid increase in resistance to vancomycin as well as high-level aminoglycosides resistance is of particular concern as the treatment options for vancomycin-resistant enterococci (VRE) is limited. Nosocomial VRE infections can develop either endogenously, where colonization in critically ill patients is followed by invasive infection, or exogenously, in which the bacteria are transmitted via healthcare workers or contact with contaminated instruments and inanimate surfaces [5]. Prompt and accurate identification of antibiotic-susceptible and resistant enterococci is essential to establish diagnosis, selecting effective therapy, and instituting infection control measures [5,6].

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The main aim of the study was to find out the prevalence of enterococci in various clinical samples and their antimicrobial susceptibility patterns with special reference to vancomycin resistance in a tertiary care Hospital.

Materials and method

The study was carried out over six month period from May 2021 to October 2021 and approved by the institutional ethical committee (Ethical approval letter No. Micro/DPU/2021/148).

All enterococcal species isolated from various clinical specimens were included in the study. Specimens were cultured on blood agar, MacConkey Agar, Cystine-Lactose-Electrolytes-Deficient (CLED) agar by streaking methods and incubate at 37°C for 18-24 hours. Blood specimens were collected in BacTec bottle and loaded in BacT/ Alert automation system for incubation at 37°C for 5 days. After receiving positive signal flag, bottles were removed and subcultured on blood agar and MacConkey agar plates and incubated at 37°C for 18-24 hours.Next day growth was observed and suspected enterococcus colonies were further identified to the species level with the help of conventional phenotypic methods which included Gram stain, colony morphology, catalase test, bile - esculin test, growth in 6.5% NaCl, mannitol fermentation, and pyruvate fermentation tests [7].

All Enterococcal isolates were tested for their susceptibility to various antibiotics by the Kirby-Bauer disc diffusion method. The antibiotics tested were penicillin (10 units), ampicillin (10 mcg), ciprofloxacin (5 mcg), erythromycin (15 mcg), and linezolid (30 mcg) and vancomycin (30 mcg). Vancomycin susceptibility was checked by the disk diffusion as well as by automated Vitek-2C system (Bio-Merieux, France). Enterococcus isolates from urine were tested for their susceptibility to nitrofurantoin and nalidixic acid additionally. The test was performed on Mueller-Hilton agar and interpreted as per the current CLSI guidelines after 18-24 h of incubation at 37°C [8]. Enterococcus isolated from blood were tested for speciation and antibiotic susceptibility by Vitek 2C automation system as per institutional policy. *Enterococcus faecalis* ATCC 29212 and *Enterococcus casseliflavus* ATCC 700327 were used as control strains.

Results

During the study period, a total of 116 enterococci were isolated from various clinical specimens. Out of them, 53.5% and 46.6% were from male and female patients respectively. Of the total enterococci, 33.6%, 28.7% and 27.6% were isolated from samples from 41-60, 18-40 and > 60 years age group cases respectively (Table-1). Of the total isolates, 56.9%, 30.2% and 12.9% were isolated from indoor, intensive care unit and non-hosptalized (out door) patients respectively. Most of the enterococcal isolates were from urine (53.5%) followed by blood (27.6%). Ten (10) enterococci were isolated from body fluids which include: ascetic/peritoneal fluid - 6, pleural fluid- 2 and bile -2. Antimicrobial resistance pattern of isolated enterococci from different clinical specimens is shown in Table-2. Overall, 31 (26.7%) enterococcal isolates were resistant to vancomycin. Vancomycin resistance rate of isolated enterococci was 16.7% to 40.6% in different clinical samples. All vancomycin resistant enterococci (VRE) were resistant to penicillin, ciprofloxacin, and erythromycin too. Except 1 (1.2%), all the enterococci were sensitive to linezolid. Out of 116 isolates, 65.5% and 81% were resistant to ampicillin and erythromycin respectively. Enterococci isolated from the urine specimen showed 96.8% and 38.7% resistance to nalidixic acid and nitrofurantoin respectively by disk diffusion method. Susceptibility

Table-1: *Source of the isolated enterococci (N=116)*

Parameter		Number (%)
Gender	Male	62 (53.5)
	Female	54 (46.6)
Patients' age (years)	< 18	12 (10.3)
	18-40	33 (28.5)
	41-60	39 (33.6)
	Above 60	32 (27.6)
Location	Outdoor	15 (12.9)
	Indoor	66 (56.9)
	ICU	35 (30.2)
Types of sample	Urine	62 (53.5)
	Blood	32 (27.6)
	Pus	12 (10.3)
	Fluids*	10 (8.6)

*Note: ICU: intensive care unit; *Fluids: ascetic/ peritoneal fluid - 6, pleural fluid- 2 and bile -2.*

to tigecycline and levofloxacin was tested only in blood isolates by automation. All the 32 (100%) blood isolates were sensitive to tigecycline and 29 (90.6%) were resistant to levofloxacin.

Resistance pattern of enterococci isolated from samples from different locations is shown in Table-3. Overall, the resistance rate of isolated enterococci from outdoor cases were low compared to indoor and ICU cases. Speciation was done only for enetrococci isolated from blood. The most common enterococcus species isolated was *E. faecium* (72%) followed by *E. faecalis* (12%) and *E. galinarrium* (9.4%). Other species were *E. avium and E. rafinosus* (Table-4). Resistance rates to different antimicrobials were higher among the *E. faecium* compared to other species.

Table-2: *Antimicrobial susceptibility pattern of entrococci isolated from different clinical speciemens*

Antibiotics	Urine (N=62)	Blood (N=32)	Pus (N=12)	Fluid (N=10)	Total (N=116)
	Resistant n (%)	Resistant n (%)	Resistant n (%)	Resistant n (%)	Resistant n (%)
Ampicillin	31 (50)	25 (78.1)	10 (83.3)	10 (100)	76 (65.5)
Penicillin	31 (50)	25 (78.1)	10 (83.3)	10 (100)	76 (65.5)
Erythromycin	57 (91.5)	27 (84.4)	10 (83.3)	10 (100)	94 (81)
Ciprofloxacin	59 (95.2)	29 (90.6)	11(91.7)	10 (100)	99 (85.3)
Vancomycin	12 (19.4)	13 (40.6)	2 (16.7)	4 (40)	31 (26.7)
Linezolid	1(1.6)	0	0	0	1 (0.9)
Tigecycline*	NT	0	NT	NT	0
Levofloxacin*	NT	29 (90.6)	NT	NT	29 (25)
Nalidixic Acid*	60 (96.8)	NT	NT	NT	60 (51.7)
Nitrofurantoin*	24 (38.7)	NT	NT	NT	24 (20.7)

*Note: * Levofloxacin and tigecyclin tested only for blood isolates while nalidixic acid and nitrofurantoin tested only in urine isolates. NT: not tested.*

Table-3: *Antimicrobial susceptibility pattern of entrococci isolated from speciemens from different locations.*

Antibiotics	Indoor (N=66)	ICU (N=35)	Outdoor (N=15)
	Resistant, n (%)	Resistant, n (%)	Resistant, n (%)
Penicillin	41 (62.2)	29 (82.9)	6 (40)
Ampicillin	41 (62.2)	29 (82.9)	6 (40)
Erythromycin	61 (92.4)	30 (85.7)	3 (20)
Ciprofloxacin	65 (98.5)	32 (91.4)	2(13.3)
Vancomycin	17 (25.8)	12 (34.3)	2 (13.3)
Linezolid	0	0	1 (6.7)
Tigecycline[#]	0	0	0
Levofloxacin[#]	7(100)	21 (84)	1 (100)
Nalidixic Acid[#]	41 (96.8)	9 (100)	10 (90.9)
Nitrofurantoin[#]	16 (67.2)	6 (66.7)	2(18.2)

Note: #Levofloxacin and Tigecyclin tested only for blood isolates while Nalidixic acid and Nitrofurantoin tested only in Urine isolates

Table-4: *Distribution of enterococcal species from blood sample and their antimicrobial susceptibility pattern (N=32)*

Antibiotics	*E. faecium* (N=23)	*E. faecalis* (N=4)	*E. galinarrium* (N=3)	Other enterococcus species** (N=2)
	Resistant, n (%)	Resistant, n (%)	Resistant n (%)	Resistant, n (%)
Penicillin	20 (86.9)	2 (50)	3 (100)	2 (100)
Ampicillin	20 (86.9)	2 (50)	3 (100)	0
Erythromycin	22 (95.7)	3 (75)	1 (33.3)	1 (50)
Ciprofloxacin	23 (100)	3 (75)	2 (66.7)	1 (50)
Vancomycin	11 (47.9)	0	NT *	0
Linezolid	0	0	0	0
Tigecycline	0	0	0	0
Levofloxacin	23 (100)	3 (75)	2 (66.7)	1 (50)

*Note: * Enterococcus galinarium is intrinsically resistant to vancomycin, ** E. avium-1 and E. rafinosus-1; NT: not tested*

Discussion

Enterococci contribute significantly to hospital-associated infections. In our study, isolation of the enterococcal species was found to be more in males (53.5%) as compared to females. Similar results have been shown in studies by Yielma *et al* (54.3%) and Jada S *et al* (55.6%) [9,10]. In the current study, we found that the majority of the enterococcal species were isolated from adults and geriatric age groups (33.6% and 27.6%).This is in accordance with the study done by Jada *et al.* [10] who isolated most of the enterococcus from the adult age group (35.8%) and geriatric pateints (39.9%). This is contrary to the findings of Yielma *et al* [9] who reported 54.2% isolates from the pediatric age group in Ethiopia.This difference could be due to variation in the clinical specimens as their study was on urine specimens while our study included various clinical specimens.

Isolation of the enterococcus in hospitalized patients is common. In the present study, 87.1% of enterococci were isolated from hospitalized patients which included indoor and ICU patients. Similar rate (83.3%) of isolation of eneterococci was reported from hospitalized patients by Yielma *et al*. [9].

Several studiess have reported high isolation of enterococci (40.3%,-46.6%) from urine samples [9,10,12]. Our study also showed similar results (53.4%). However, Sreeja S *et al* [11] reported that majority of their isolates were from pus specimens (55.4%).

The prevalence of the enterococcal species can vary depending on the region of the study. Studies from India [11,12] reported *E. faecalis* as the most common species (58 - 76%) whereas, in the current study, we found only 12% *E. faecalis*. On the contrary, we found 72% of the enterococci as *E. faecium* while the other studies reported 24% - 42% [12-14]. This variation could be due to types of clinical samples and patients.Uroisolate enterococcal species were tested for nitrofurantoin susceptibility. In the present study, we found 38.7% enterococci resistant to nitrofurantoin which was higher (11.7% - 14.2%) than other reported studies [12,13]. Beta lactam antibiotics were effective in infection by enterococcus. But recently, resistance against penicillin is emerging among enterococci. In the present study, 65.5% enterococci were resistant to either penicillin or ampicillin. Similar high prevalence of penicillin resistance was reported by others [9,10]. Similarly, most of our enterococci were resistant to macrolides. VRE is one of the major issues in-hospital care setups all over the world. In the current study, we found 26.7% VRE among all isolated enetrococci. However, the rate was lower than those reported from Ethiopian (41.7%), Nigerian (42.9%), Serbian (54.1%), and Iraq (71.4%) [9,15,16,17]. This variations in the prevalence of VRE could be due to variation of the specimen, study duration, use of

antimicrobial agents and types of patients. Ethiopian and Iraq study evaluated only urine specimens while the Serbian study evaluated enetrococci isolated only from blood. Only, Rudy et al [13] reported 100% susceptibility of their enterococci to vancomycin. All species of enetrococci in our study were found sensitive to linezolid except one isolate which was isolated from urine.

This study was conducted only for a short period (6 months) of time and the sample size was small. Hence, a multicentric study with large number of samples and of longer duration would give a better perspective of the prevalence of enterococcal species and their antimicrobial susceptibility pattern in our region. Also, we did not test the enterococcal isolates for high-level resistance to aminoglycosides as per institutional policy. Also, minimum inhibitory concentration (MIC) of vancomycin was not determined.

Enterococcus sp is one of the major organisms responsible for hospital acquired infection. The current study has demonstrated high prevalence of vancomycin resistant enterococci in our hospital setting. Therefore, regular monitoring of the antimicrobial susceptibility pattern of enterococci would be useful to control its spread within the hospital and in the community.

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Conflict of interest

None of the authors have declared any conflict of interest

Fund

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Ethical Approval

Ethical clearance was taken from the institutional sub-ethical committee before the study was conducted. Ethical approval letter No. Micro/DPU/2021/148.

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