ORIGINAL RESEARCH

Isolation, identification, and antibiotic-resistant pattern of *Enterococcus* species in a tertiary care centre in central India

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Revised date: 17 December, 2023 Acceptance date: 22 January, 2024

ABSTRACT

Background *Enterococci* are common bacteria found in the human mouth, stomach, and female vaginal system that are known to cause nosocomial infections. Urinary tract infections, followed by intra-abdominal abscesses and bloodstream infections, are the most common illnesses caused by Enterococcus spp.**Material method**This prospective observational study was conducted in department of microbiology. Clinical samples such as urine, pus, cerebrospinal fluid and other body fluid were received in department of microbiology, mahatma Gandhi memorial medical college, Indore Madhya Pradesh from February 2019 to January 2020. In this duration 200 *Enterococcus* species isolates were isolated. The antimicrobial susceptibility testing was done by Kirby Bauer's discdiffusion method as per 2018 CLSI guideline.**Result** In this study, 31453 clinical samples received in the bacteriology laboratory in which 2832 morphological suspected colonies were subjected to biochemical testing, 200 isolated strains were found Catalase Negative, Bile Esculin positive, Growth in 6.5% NaCl, Growth at 10°C and 45°C and sugar fermentation. Species of *Enterococci*, *E. faecalis* constituted 155/200 (77.5%), and E. faecium 45/200 (22.5%).**Conclusion** The finding of this study indicated the presence of *Enterococcus* species have shown an increased rate of resistance to most the of tested drug, particularly to Vancomycin. This finding demands an attention from health policy makers for intensified actions to promote rational use of antibiotics in health care settings and surveillance studies in order to monitor changes in Enterococcal resistance patterns.

Key words: *Enterococcus species*, VRE, antibiotic resistance

INTRODUCTION

Streptococci within Group D comprise the genus *Enterococcus*. These organisms are normal inhabitants of the male urethra, vagina, and biliary and digestive tracts. Due in large part to their resistance to antimicrobial treatments, they are becoming an increasingly significant agent of human disease. [1,2] Hospitalised patients' long-term treatment costs, morbidity, and mortality are primarily caused by nosocomial infections. [3,4] *E. faecalis accounts for 80–90% and E. faecium for* 5–10% of human *enterococcal* infections, respectively [5,6].The most frequent infections caused by Enterococcus species are urinary tract infections, which are followed by bloodstream infections and intra-abdominal abscesses [7].The organism's growing resistance to beta-lactam, aminoglycoside, and glycopeptide antibiotics, as well as an inadequate response to treatment, contribute to a high percentage of enterococcal infection death [5,8].The spread of vancomycin-resistant *Enterococci* (VRE) during pandemics and its resistance to current antibiotics need ongoing monitoring and early VRE diagnosis. [9]*Enterococci* are intrinsically resistant to many antibiotics and can also acquire resistance to other antibiotics, including glycopeptides. Resistance is more common with *E. faecium* compared to *E. faecalis*. [10]VRE has emerged as an important cause of HAIs since it was first discovered in the 1980s; it has been associated with increased morbidity, mortality, and healthcare expenditure [11, 12, 13].Risk factors for the development of VRE infection include increased exposure to antibiotics, diabetes mellitus, hemodialysis, neutropenia, and abdominal transplantation [14, 15, 16].Individuals colonised with VRE are asymptomatic and may serve as a reservoir for transmission. Previous studies have shown that

patients colonised with VRE have a high likelihood of developing VRE bacteremia [17, 18, 19].

MATERIAL METHOD

The prospective observational research was carried out in the microbiology department. Clinical samples such as urine, pus, cerebrospinal fluid, and other body fluids were received in the department of microbiology at the Mahatma Gandhi Memorial Medical College, Indore, Madhya Pradesh, from February 2019 to January 2020. In this period, 200 *enterococcus* species isolates were isolated from all clinical samples except blood. A pure, isolated culture of *Enterococcus species* from all clinical samples except blood was included in this study. The exclusion criteria were as follows: 1. All unsatisfactory samples for bacteriological culture 2. Culture showing: mixed culture in urine (more than two microorganisms or normal flora in urine) 3. Duplicate samples from the same patient.

After receiving the sample, direct gram staining was performed to look for the presence of pus cells and the arrangement of gram-positive cocci. Samples were inoculated on blood agar, MacConkey agar, and cysteine lactose electrolyte deficient agar (CLED). After that, plates were incubated at 37 °C for 18 to 24 hours. On Mac Conkey agar, colonies were small, 0.5–1 mm, magenta-coloured, while on CLED agar, small, orange-yellow colonies were seen. In urine culture, a standard protocol was followed for significant bacteriuria.

Identification of the *Enterococcus* genus was done by different biochemical tests such as catalase, salt tolerance, bile esculin, and PYR tests. Identification of enterococcus species was done by pyruvate utilisation tests, potassium tellurite reduction tests, arginine dihydrolase tests, motility testing, and sugar fermentation tests including glucose, arabinose, raffinose, mannitol, sorbitol, sucrose, and lactose.

The antimicrobial susceptibility testing was done by Kirby Bauer's disc diffusion method as per the 2018 CLSI guidelines. The antibiotics used were Ampicillin (10 ug), Ciprofloxacin (5 ug), Levofloxacin (5 ug), Doxycycline (30 ug), Erythromycin (15 ug), Norfloxacin (10 ug for urine), Nitrofurantoin (300 ug for urine), Vancomycin (30 ug), Linezolid (30 ug), Teicoplanin (30 ug), and High-Level Gentamicin (120 ug).

RESULT AND OBSERVATION

In this study, 31453 clinical samples were received in the bacteriology laboratory, in which 2832 morphologically suspected colonies were subjected to biochemical testing. 200 isolated strains were found to be catalase negative, bile esculin positive, growth in 6.5% NaCl, growth at 10°C and 45°C, and sugar fermentation (Mannitol, Sorbitol, Pyruvate, and Arabinose).

The age-wise distribution of isolates ranged from 9 months to 78 years. The majority of isolates were from patients between 41 and 50 years of age. The maximum number of isolates were from urine samples. The rest of the isolates were from pus, sputum, body fluids, and CSF. *Enterococci* were isolated more from male patients 112/200 (56%) than female patients 88/200 (44%).

Species of *Enterococci: E. faecalis* constituted 155/200 (77.5%), and *E. faecium constituted* 45/200 (22.5%). In this table age wise distribution of *Enterococcus* spp. shows that, maximum isolation was in age group 41-50years, then 51-60 years, then 21-30 years, 31-40 years, 11-20 years, 70 and above, than 61-70 years, and minimum isolation was in 0-10 years.

The maximum number of isolates of *Enterococcus faecalis* were from urine samples (32%), body fluids (22.5%), pus (11.5%), sputum (8%), C.S.F. (1.5%), catheter tips (1.5%), and *Enterococcus faecium* from urine (7.5%), pus (4%), body fluids (7%), and sputum (3.5%).

Table 1: Age wise distribution of *Enterococcus species.*

Age in Years	No. of Cases	Percentage
0-10	5	2.5
11-20	14	7
21-30	37	18.5
31-40	32	16
41-50	48	24
51-60	39	19.5
61-70	12	6
70 and above	13	6.5
Total	200	100

Table 2: Gender wise distribution

Gender	Number of cases	Percentage
Male	112	56%
Female	88	44%
Total	200	100%

Table 3: Species wise distribution

Species	Frequency	Percent
E. faecalis	154	77.0
E. faecium	46	23.0
Total 200	200	100.0

Table 4: Sample wise Distribution of Isolates

S.N.	Specimen	Frequency	Percent
1.	CSF	4	2.0
2.	Catheter Tip	4	2.0
3.	Sputum	23	11.5
4.	Pus	31	15.5
5.	Body fluids	59	29.5
6.	Urine	79	39.5
	Total	200	100

Table 5: Association between Sample & species

Samples	Species				Total	
	E. faecalis		*E. faecium*			
	No.	%	No.	%	No.	%
Body fluids	45	22.5	14	7.5	58	29.5
CSF	3	1.1	1	0.5	4	2.0
Catheter tip	3	1.1	1	0.5	4	2.0
Pus	23	11.5	8	4.0	31	15.5
Sputum	16	8.0	7	3.5	23	11.5
Urine	64	32.0	15	7.5	79	39.5
	154	77.5	46	22.5	200	100.0

Pearson Chi-Square = 1.610, df = 5, P value = .900, Not Significant

Table 6: Antibiotic Susceptibility Pattern of the *Enterococcus* species

Antibiotic	Sensitive		Resistant	
	No.	%	No.	%
Ampicillin (n = 200)	74	37.0	126	63.0
Doxycycline (n = 200)	110	55.0	90	45.0
Erythromycin (n = 200)	48	24.0	152	76.0
Gentamycin (n = 200)	116	58.0	84	42.0
Vancomycin (n = 200)	170	85.0	30	15.0
Teicoplanin (n = 200)	170	85.0	30	15.0
Linezolid (n = 200)	200	100.0	0	0.0
Norfloxacin (n = 79)	22	27.8	57	72.1
Nitrofurantoin (n = 79)	19	24.05	60	75.9
Ciprofloxacin (n = 200)	42	21.0	158	79.0
Levofloxacin (n = 200)	64	32.0	136	68.0

Table 7: Antibiotic Resistance Pattern of *E. faecalis* and *E. faecium* in the total *Enterococccal i*solates

Antibiotic	*Enteroccus faecalis*		*Enteroccus faecium*		Total resistant	
	No.	%	No.	%	No.	%
Ampicillin (n = 200)	108	54.0%	18	9.0%	126	63.0%
Doxycycline (n = 200)	76	38.0%	14	7.0%	90	45.0%
Erythromycin (n = 200)	130	65.0%	22	11.0%	152	76.0%
Gentamycin (n = 200)	71	35.5%	13	6.5%	84	42.0%
Vancomycin (n = 200)	19	9.5%	11	5.5%	30	15.0%
Teicoplanin (n = 200)	21	10.5%	9	4.5%	30	15.0%
Linezolid (n = 200)	0.0	0.0%	0.0	0.0%	0.0	0.0%
Norfloxacin (n = 79)	47	37.6%	11	8.8%	58	46.4%
Nitrofurantoin (n = 79)	49	39.2%	12	9.6%	61	48.8%
Ciprofloxacin (n = 200)	131	65.5%	27	13.5%	158	79.0%
Levofloxacin (n = 200)	117	58.5%	19	9.5%	136	68.0%

DISCUSSION

Enterococcus spp. are important nosocomial pathogens because of their innate resistance to several classes of antibiotics (Cephalosporin) and ability to acquire additional resistance, such as glycopepetide resistance.

In the present study, out of 200 *Enterococci* isolates, *E. faecalis* constituted 155/200 (77.5%), and *E. faecium constituted* 45/200 (22.5%). *E. faecalis* isolates fermented most of the sugar and also showed positive reactions for arginine hydrolysis and pyruvate utilisation, while *E. faecium* isolates were negative for sorbitol fermentation and did not utilise pyruvate. Arginine hydrolysis was positive.

In the present study, 200 *Enterococcus* isolates were identified.Themajority of clinical isolates were *E. faecalis* (77.5%) and *Enterococcus faecium* (22.5%) (table 3.). similarly shown by S. Sreeja *et al.*, *Enterococcus faecalis* (76%), and *Enterococcus faecium* (24%),respectively [20].

The maximum number of patients were in the age group of 41–50 years, i.e., 48 (24%), followed by 51–60 years, i.e., 39 (19.5%), 21–30 years, i.e., 37 (18.5%), 31–40 years, i.e., 32 (16%), 11–20 years, i.e., 14 (7%), 61–70 years, i.e., 12 (6%), and the least in the age group of 0–10 years, i.e., 5 (2.5%).

The majority of patients were male (56% in the study) compared to females (44% in Table 2). Most of the male patients belong to the age group of 41–50 years (14.5%) and females are in the age group of 21–30 years (11.5%), which is comparable to the studies of Telkar Anjana et al. (2012) and Golia et al. (2014) [21, 22].

In the present study, most of the strains of *Enterococcus sp. were isolated from a* urine sample, followed by body fluids, pus, sputum, catheter tip, and CSF (Table 4). Similarly, Nautiyal et al. (2016) and Mokherjee et al. (2016) reported the maximum number of isolates from urine [23, 24].

As demonstrated in the table above, the isolates were resistant to a variety of antibiotics. Vancomycin was also used in the susceptibility test. *E. faecalis* shows the highest resistance to Vancomycin (9.5%), followed by *E. faecium* (5.5%). The total percentage of vancomycin-resistant isolates was 15%. Nitrofurantoin was used in only urine isolates and showed 48% resistance. However, no isolate showed resistance against Linezolid.

CONCLUSION

The findings of this study indicated that *Enterococcus species* have shown an increased rate of resistance to most of the tested drugs, particularly vancomycin. This finding demands attention from health policymakers for intensified actions to promote rational use of antibiotics in health care settings and surveillance studies in order to monitor changes in antibiotic resistance patterns.

Source of funding: none

Conflicts of interest: none declared

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