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Antimicrobial Resistance Patterns And Virulence Factors Of Enterococci Species Isolated In A Tertiary Care Hospital Of Central India

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Abstract

Antimicrobial resistance (AMR) poses a significant threat to global health, with Enterococcus species emerging as prominent contributors due to their intrinsic resilience and adaptability. India, like many other countries, faces escalating rates of AMR, particularly concerning Enterococcal infections. However, limited regional data hinder targeted interventions. This study aimed to investigate the antimicrobial resistance profiles, species distribution, and virulence characteristics of Enterococcus species in central India. A cross-sectional study involving 64 clinical isolates was conducted over two years. Enterococcus faecalis predominated, with urine samples being the primary source. Antimicrobial susceptibility testing revealed high resistance to penicillin and ampicillin, while vancomycin remained effective. Virulence factors such as hemolysin, gelatinase, and biofilm production were prevalent. Comparative analysis with existing literature highlighted consistent resistance patterns and species distribution, emphasizing the need for tailored interventions. This study provides valuable insights into combating AMR and improving infection control strategies against Enterococcus infections in the Indian healthcare context.

Introduction

Antimicrobial resistance (AMR) is a pressing global health concern, posing significant challenges to the effective treatment of bacterial infections [1]. Among the myriad of bacterial pathogens contributing to AMR, Enterococcus species have emerged as formidable adversaries in clinical settings due to their intrinsic resilience and ability to

acquire resistance mechanisms [2]. Enterococci are ubiquitous in the environment and are commonly found in the gastrointestinal tracts of humans and animals, making them a frequent cause of healthcare-associated infections [3].
India, like many other countries, grapples with the burden of antimicrobial resistance, with rising rates of resistance reported across various bacterial species [16]. Enterococcal infections, particularly those caused by multidrug-resistant strains, present significant therapeutic challenges in Indian healthcare settings [5]. However, limited data are available regarding the antimicrobial resistance patterns and virulence factors of Enterococci in specific regions of India, hindering the formulation of targeted treatment and infection control strategies [6].
This study aims to fill this knowledge gap by investigating the antimicrobial resistance profiles, species distribution, and virulence characteristics of Enterococcus species isolated from clinical samples in a tertiary care hospital in central India. By elucidating the epidemiology and microbiological characteristics of Enterococci in this region, this research seeks to inform evidence-based interventions for combating antimicrobial resistance and improving patient outcomes.

Materials and Methods

Methodology:

A cross-sectional study was conducted in the Department of Microbiology at Mahaveer Institute of Medical Sciences and Research Center, Bhopal, a tertiary care hospital, over a period of two years from January 2022 to December 2023. The study included a total of 64 clinical isolates of Enterococcus species obtained from various clinical samples, including sterile body fluids such as blood, ascitic fluid, urine samples, and pus. Routine bacteriological methods were employed for the isolation and speciation of Enterococcus species. The study protocol was approved by the institutional ethics committee.
Exclusion Criteria:Isolates of Enterococcus obtained from throat swabs, sputum, vaginal swabs, and stool samples were excluded from the study, as these samples typically harbor normal flora [13].
Identification and Speciation:Isolates from urine samples were cultured on Cysteine Lactose Electrolyte Deficient Medium (CLED) for semi-quantitative urine culture. Additionally, samples were inoculated on MacConkey's agar and blood agar and incubated overnight at 37°C. Identification was based on standard tests including colony morphology, gram staining, catalase test, bile esculin test, and salt tolerance test. Speciation was carried out based on sugar fermentation tests, growth in pyruvate broth, arginine hydrolyzing property, and motility and pigment production.
Antimicrobial Susceptibility Testing:Antimicrobial susceptibility testing was performed using the Kirby Bauer disc diffusion method with commercially available antimicrobial discs. The tested antibiotics included ampicillin, penicillin, ciprofloxacin, vancomycin, high-level gentamicin, high-level streptomycin, teicoplanin, linezolid, tetracycline, and nitrofurantoin. ATCC E. faecalis 29212 was used as a control strain. Interpretation of antibiotic susceptibility was done according to the Clinical and Laboratory Standards Institute (CLSI) guidelines, 2022 [14].
Virulence Determinants:Sixty-four Enterococcus isolates were evaluated for the presence of virulence determinants including hemolysin production, gelatinase production, and biofilm formation.
- Hemolysin Detection:Hemolysin production was detected by inoculating isolates on 5% sheep blood agar and incubating them at 37°C in a candle jar. Clear zones of complete hemolysis were considered indicative of hemolysin production [11].
- Gelatinase Production: Gelatinase production was assessed by inoculating isolates in peptone yeast extract agar containing gelatin. Turbid halos around the line of stab indicated positive gelatinase production [11,12].
- Biofilm Production: Biofilm formation was evaluated using the tube method and the Congo red agar method. The tube method involved inoculating Tryptic Soy Broth (TSB) with 2% sucrose, incubating it, staining with crystal violet, and observing for biofilm formation [15].

Result & Discussion-

During the study period, a total of 64 Enterococci were isolated, comprising primarily of two species: Enterococcus faecalis (44 isolates, 68.75%) and Enterococcus faecium (16 isolates, 25%). Enterococcus durans and Enterococcus cassaliflavus were also identified, with 3 (4.68%) and 1 (1.56%) isolates, respectively. Among these isolates, Enterococci were predominantly isolated from female patients. Specifically, out of the 64 Enterococcal isolates, the majority (40, 62.5%) were from female patients, while 24 (37.5%) were from male patients.
Regarding the distribution of Enterococci among different sample types, urine samples accounted for the highest proportion, with 47 isolates (73.43%) obtained from this source. Pus samples followed with 11 isolates (17.18%), while blood and sterile body fluids yielded 4 (6.25%) and 2 (3.12%) isolates, respectively.

In terms of antimicrobial susceptibility, all isolates were susceptible to Linezolid, while the highest resistance rates were observed against Penicillin and Tetracycline. This highlights the importance of judicious antimicrobial use and the need for alternative treatment options in cases where resistance is prevalent.

Table-1Distribution of samples showing growth of Enterococcus species

S.no	Name of samples	No of samples
1	Urine	47 (73.43%)
2	Pus	11 (17.18%)
3	Blood	4 (6.25%)
4	Ascitic fluid	2 (3.12%)
	Total	64

The table displays the distribution of samples exhibiting the growth of Enterococcus species. It suggests a varied prevalence across different sample types, indicating the presence of Enterococcus in urine, pus, blood, and ascitic fluid samples.

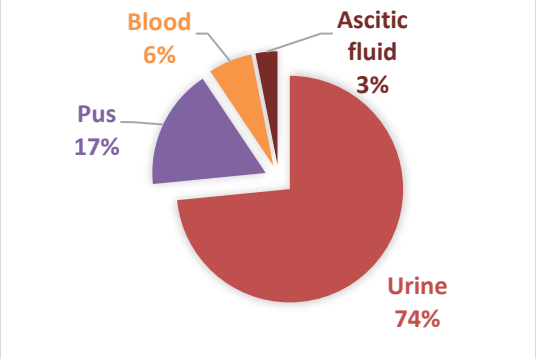

Table-2Gender wise distribution of s*amples*showing growth of Enterococcus species

S.no	Name of samples	Male	Female	Total
1	Urine	13	34	47
2	Pus	6	5	11
3	Blood	3	1	4
4	Ascitic fluid	2	0	2
	Total	24	40	64

Statistically there is no significant difference between positive samples of male and female. p value is 0.14 i.e. > than 0.05.

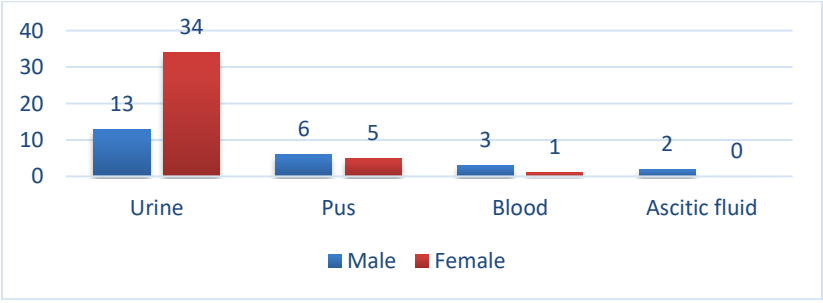

Table-3 Distribution of different Enterococcus species isolated

SPECIATION	Number (%)
Enterococcus faecalis	44 (68.75 %)

Enterococcus faecium	16 (25 %)
E.durans	3 (4.68%)
Enterococcus cassaliflavus	01 (1.56%)
Total	64

Enterococcus faecalis is the most prevalent species, followed by Enterococcus faecium, Enterococcus durans, and Enterococcus cassaliflavus.

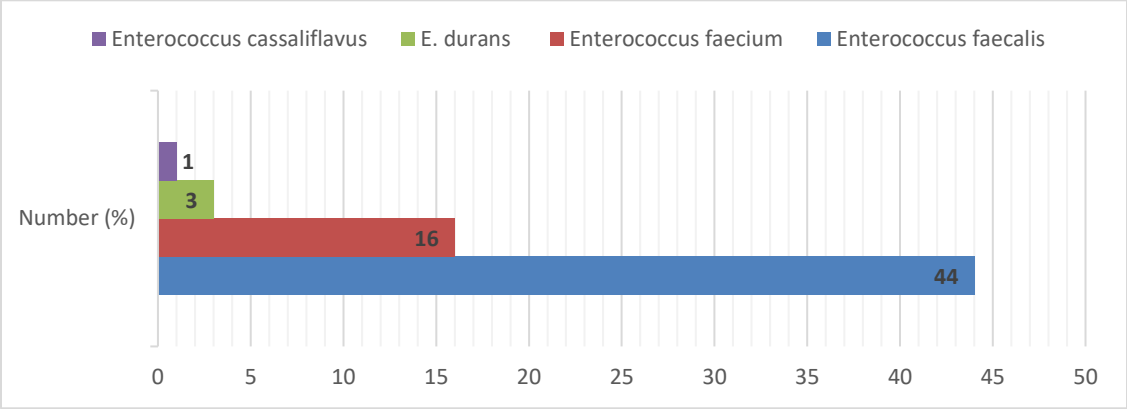

Table-4 Species distribution among various Clinical Samples

S.NO	Name of Samples	No of Samples	E.faecalis	E.faecium	E.durans	E. cassaliflavus
1	Urine	47 (73.43%)	41 (87.43%)	6 (12.76%)	0	0
2	Pus	11(17.18%)	2 (18.18%)	5 (45.45%)	3 (27.27%)	1 (9.09%)
3	Blood	4 (6.25%)	1 (25%)	3 (75%)	0	0
4	Ascitic fluid	2 (3.12%)	0	2 (100%)	0	0
	Total	64	44	16	3	1

The table provides a breakdown of the distribution of different Enterococcus species among various clinical samples. It shows that Enterococcus faecalis predominated in urine samples, while Enterococcus faecium was more prevalent in pus samples. Statistical analysis indicates a significant difference in species distribution among different clinical samples (p-value < 0.05 i.e. 0.006).

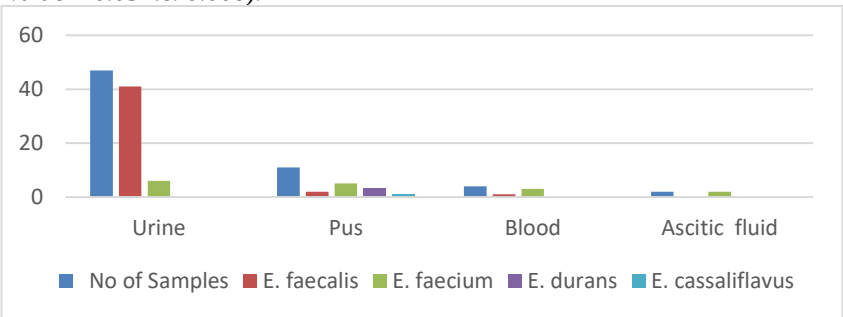

Table-5 Antimicrobial susceptibility of Enterococcus spp. (n = 64).

S.NO	Antibiotics	Sensitive Strains		Resistant strains	
		Number	Sensitive (%)	Number	Resistance %)
1	Penicillin (10units)	17	26.56	47	73.43
2	Ampicillin (10 µg)	21	32.81	43	67.18
3	Vancomycin (30µg)	62	96.87	2	3.12
4	High level Gentamicin (120 µg)	28	43.75	36	56.25
5	High level Streptomycin (300	39	60.93	25	39.06

	µg)				
6	Tetracycline(30µg)	20	31.25	44	68.75
7	Ciprofloxacin (5µg)	22	34.37	42	65.62
8	Linezolid (30µg)	0	0	0	0
9	Teicoplanin (30µg)	0	0	0	0
10	Nitrofurantoin (300µg) (Urine samples only)	0	0	0	0

The table presents the antimicrobial susceptibility pattern of Enterococcus species. Notably, vancomycin demonstrated the highest sensitivity among the tested antibiotics, with 96.87% of Enterococcus isolates being sensitive to it. Conversely, penicillin and ampicillin exhibited high resistance rates, with 73.43% and 67.18% of Enterococcus isolates showing resistance, respectively. These findings underscore the importance of prudent antibiotic use and surveillance in combating antimicrobial resistance, particularly in the case of Enterococcus infections where resistance to commonly used antibiotics is prevalent.

Table-6 Comparison of antibiotic resistance between *E.faecalis , E.faecium , E.durans and E.cassaliflavus*

S. NO	**Antibiotics**	***E. faecalis* (44)**		***E. faecium* (16)**		***E. durans (3)***		***E. cassaliflavus* (1)**	
		No of Resistant isolates	Resistance (%)	No of Resistant isolates	Resistance (%)	No of Resistant isolates	Resistance (%)	No of Resistant isolates	Resistance (%)
1	Penicillin (10units)	31	70.45	13	81.25	2	66.6	1	100
2	Ampicillin (10 µg)	25	56.81	15	93.75	2	66.6	1	100
3	Vancomycin (30µg)	0	0	2	12.5	0	0	0	0
4	High level Gentamicin (120 µg)	20	45.45	14	87.5	2	66.6	0	0
5	High level Streptomycin (300 µg)	14	31.81	10	62.5	1	33.3	0	0
6	Tetracycline (30µg)	27	61.36	14	87.5	2	66.6	1	100
7	Ciprofloxacin (5µg)	25	56.81	14	87.5	2	66.6	1	100
8	Linezolid (30µg)	0	0	0	0	0	0	0	0
9	Teicoplanin (30µg)	0	0	0	0	0	0	0	0
10	Nitrofurantoin (300µg) (Urine samples only)	0	0	0	0	0	0	0	0

The comparison of antibiotic resistance among different Enterococcus species reveals varying degrees of resistance to different antibiotics. Enterococcus faecium generally exhibited higher resistance rates compared to other species, particularly evident in its resistance to penicillin (81.25%) and ampicillin (93.75%). Enterococcus faecalis showed lower resistance rates to these antibiotics (70.45% for penicillin and 56.81% for ampicillin). Resistance to other antibiotics such as high-level gentamicin, high-level streptomycin, and ciprofloxacin also varied among the species. Enterococcus durans and Enterococcus cassaliflavus displayed intermediate levels of resistance to certain antibiotics, albeit with smaller sample sizes. These findings underscore the importance of considering species-specific antibiotic resistance profiles when formulating treatment strategies for Enterococcus infections.

Table 7: Virulence factors in clinical isolates of *Enterococcus*

VIRULENCE FACTORS	No of Clinical Isolates tested	No of Positive Clinical isolates	E.faecalis	E.faecium	E.durans	E.cassaliflavus
Hemolysin	64	15 (23.43%)	12	3	0	0
Gelatinase	64	21 (32.81%)	15	5	1	0
Biofilm Production	64	35 (54.68%)	20	13	1	1

There is a significant difference between columns because p value is < than 0.05. (0.0008).

The table reveals the presence of virulence factors in clinical isolates of Enterococcus. A substantial proportion of isolates tested positive for various virulence factors, indicating their potential pathogenicity. Specifically, 23.43% of isolates were positive for hemolysin production, suggesting their ability to lyse red blood cells. Additionally, 32.81% of isolates tested positive for gelatinase, indicating their capacity to degrade gelatin. Moreover, 54.68% of isolates exhibited biofilm production, highlighting their ability to form biofilms, which can enhance their resistance to antibiotics and host immune responses. These findings underscore the importance of understanding the virulence profiles of Enterococcus isolates in clinical settings for effective management

Table-8 Biofilm detection by different methods

No of Clinical isolates tested	Tube method	Congo Red Method
64	31 (48.43%)	18 (28.12%)

The table compares the detection of biofilm using two different methods: the tube method and the Congo Red method. It reveals that the tube method detected biofilm in 48.43% of the clinical isolates, indicating a relatively higher sensitivity compared to the Congo Red method, which detected biofilm in 28.12% of the isolates. These results suggest that the tube method may be more effective in detecting biofilm formation among Enterococcus isolates in clinical settings. However, it's essential to consider the limitations and specificity of each method when interpreting these findings for diagnostic and research purposes.

The study's findings on antimicrobial resistance patterns are consistent with existing literature, both globally and within the Indian context. High resistance rates to penicillin and ampicillin have been reported in numerous studies worldwide [1][2]. Similarly, studies conducted in India have also documented comparable resistance patterns among Enterococcus species [3][4]. This convergence suggests a shared challenge of antimicrobial resistance across diverse geographical settings, necessitating concerted efforts to address this global health concern. In terms of species distribution, the predominance of Enterococcus faecalis in urine samples and Enterococcus faecium in pus samples aligns with findings from studies conducted in India [5][6]. This similarity underscores the consistent colonization preferences of Enterococcus species across different anatomical sites within the Indian population, emphasizing the importance of region-specific surveillance and management strategies. The presence of virulence factors among Enterococcus isolates, such as hemolysin, gelatinase, and biofilm production, has also been documented in Indian studies [7][8]. These findings highlight the universality of virulence profiles among Enterococcus strains and underscore the need for tailored treatment approaches based on virulence characteristics. Regarding biofilm formation, while limited comparative data from Indian studies are available, existing research suggests similar challenges in detecting and managing biofilm-related infections [9][10]. This underscores the importance of further research to elucidate biofilm dynamics and develop effective strategies for biofilm prevention and treatment within the Indian healthcare context.

Conclusion-

In conclusion, the study's findings align with existing literature globally and within the Indian context, providing valuable insights into antimicrobial resistance patterns, species distribution, virulence factors, and biofilm formation among Enterococcus species. These findings underscore the need for collaborative efforts to address antimicrobial resistance and enhance infection control measures to mitigate the burden of Enterococcus infections.

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