International Journal of Life Sciences, Biotechnology and Pharma Research Vol. 13, No. 6, June 2024 Online ISSN: 2250-3137
Print ISSN: 2977-0122

DOI: 10.69605/ijlbpr_13.6.2024.61

ORIGINAL RESEARCH

Evaluation of Microbiological, Epidemiological and Clinical profile of Enterococcal infectionsat a Tertiary Care Hospital in India

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Received: 11 May, 2024 Accepted: 08 June, 2024

ABSTRACT

Introduction: *Enterococci* are opportunistic pathogens that can cause various infections, especially in hospital settings. They are often resistant to many antibiotics, making them difficult to treat. **Aim & objectives:** This study aimed to evaluate the microbiological, epidemiological, and clinical profile of enterococcal infections at a tertiary care hospital in India. **Methodology:** We collected 11,846 clinical specimens from June 2022 to May 2023 and identified 100 enterococcal isolates using the VITEK-2 compact system. We also collected demographic and clinical data of the patients from the hospital information system. **Result:** The results showed that *Enterococcusfaecium* (64%) was the most common speciesfollowed by*Enterococcusfaecalis* (36%). Blood (67%) was the most common specimenfollowed by swab (13%). Diabetes mellitus (38%) was the most common comorbid conditionfollowed by chronic kidney disease (10%). The mortality rate was 26%. Tigecycline (100%) was the most sensitive antibiotic followed by linezolid (89%), vancomycin (86%) and teicoplanin (84%). Gentamicin High Level AminoglycosideResistance (HLAR) was 87% and vancomycin resistance was 14%. **Conclusion:** *E. faecium* was the most common speciesfollowed by *E. faecalis*.*E. faecium* was more prevalent in individuals aged >40 years. Gender did not significantly influence the distribution.*E. faecium* was dominant in blood specimens indicating its systemic nature. Clinical outcomes did not significantly differ between *E. faecium* and *E. faecalis* groups. Enterococcal infections were associated with high morbidity and mortality. *E.faecium* showed limited sensitivity to penicillin. *E. faecalis* showed higher sensitivity to nitrofurantoin in urine isolatessuggesting its potential efficacy in urinary tract infections.Antibiotics such as tigecycline, linezolid, vancomycinand teicoplanin were effective for both species and are recommended as empirical therapy.

Key words: Antibiotic susceptibility, Demographic factors, Clinical outcomes, *Enterococcus faecium*, *Enterococcus faecalis*

INTRODUCTION

Enterococci are Gram-positive facultative anaerobic cocci and part of normal flora in the gastrointestinal and biliary tract(1) They can be bothcommensal and pathogenic in humans and can cause various infections, especially in hospital settings(2). Enterococcal infections are often difficult to treat due to their intrinsic and acquired resistance to many antibiotics, including aminoglycosides, β-lactams, and glycopeptides(3).

Among the enterococcal species, *E. faecalis* and *E. faecium* are the most frequently isolated from clinical samples, accounting for more than 90% of the cases. However, other species, such as *E. casseliflavus*, *E. gallinarum*, and *E. durans*, have also been reported to cause infections in humans(4).*E. faecalis* and *E. faecium*have acquired resistance to vancomycin whereas some *Enterococcus* species such as *E.casseliflavus* and *E. gallinarum*have intrinsic resistance to vancomycin.*E. faecium*is usually more resistant than *E. faecalis.*Hence, species identification is necessary for efficient management of patients.

The epidemiology, risk factors, and clinical outcomes of enterococcal infections vary depending on the type

DOI: 10.69605/ijlbpr_13.6.2024.61

of infection, the host characteristics, and the local antibiotic resistance patterns. Therefore, it is important to monitor the prevalence and diversity of enterococcal species, their antimicrobial susceptibility profiles, and their association with clinical features and outcomes in different settings.

AIM &OBJECTIVES

Aim of our study was to evaluate the microbiological, epidemiological, and clinical profile of enterococcal infections at a tertiary care hospital in India. Primary objective of the study was to identify enterococcal species across various clinical specimens based on microbiological characteristics and study their antimicrobial susceptibility pattern. We also wanted to evaluate epidemiology, risk factors, and clinical outcomes of enterococcal infections.

METHODOLOGY

After ethical approval from Institutional review board, we conducted an Ambispective observational cross-sectional study at a tertiary care hospital in Ahmedabad, India from June 1, 2022 to May 31, 2023.

A total of 11,846 clinical specimens received from both outpatient and inpatient departments for bacterial culture and sensitivity testing in microbiology laboratory.

Gram staining was performed and samples were inoculated on nutrient agar, blood agar, and MacConkey agar, and incubated them at 37°C for 18-24 hours.Gram staining was performed on any growth on the nutrient and blood agar. All isolates of Gram-positive cocci were tested for catalase.

We used the automated VITEK-2 Compact system for further identification and antimicrobial susceptibility testing. We used the GP card for identification, and the AST P628 card for antimicrobial susceptibility testing of Gram-positive organisms.

The AST P628 card included the following antibiotics: Penicillin, Vancomycin, Teicoplanin, Linezolid, Daptomycin, Erythromycin, Tetracycline, Gentamicin (high level), Tigecycline, Ciprofloxacin, Levofloxacin, and Nitrofurantoin. We used Tetracycline, Ciprofloxacin, Levofloxacin, and Nitrofurantoin only for urine isolates and Erythromycin for non-urine isolates. Tigecycline's interpretation is not given in CLSI M100 guideline. Hence, we used EUCAST 2022 guideline[5]

Since Ampicillin was not included in the AST P628 card, we tested for Ampicillin susceptibility manually using the Kirby-Bauer disc diffusion method. We interpreted this drug as per the CLSI M100 32nd edition guideline [6].

We collected demographic and clinical details of patients from the Hospital Information System (HIS) for analysis.

For statistical significance, we calculate p-value using Fisher's exact test with 95% confidence interval.

RESULT

Demographic and Clinical Characteristics

Age and Gender Distribution:

Table 1 displays the age, gender, specimen, and clinical risk factor-wise distribution of *E. faecium* and *E. faecalis* isolates. The analysis revealed significant associations in certain categories. *Age:* The distribution of *E. faecium* and *E. faecalis* isolates differed significantly between age groups (P = 0.0263). A higher percentage of *E. faecium* isolates was observed in individuals aged >40 years (84.37%), compared to those aged <40 years (15.63%). *Gender:* No significant gender-based differences were observed in the distribution of *E. faecium* and *E. faecalis* isolates (P = 1).

Specimens:

Significant differences were observed based on specimen type (P = 0.0021). *E. faecium* was more prevalent in blood specimens (78.12%) compared to other specimen types (21.87%).

Clinical Risk Factors:

Diabetes mellitus appears to be the most common comorbid condition. However, no significant differences were observed in the distribution of isolates based on various clinical risk factors.

Clinical Outcome

Table 1 also presents the clinical outcomes associated with *E. faecium* and *E. faecalis* infections. There were no significant differences in the rates of discharge, discharge against medical advice, or death between the two groups.

Hospital Stays and ICU Stay

The mean hospital stay was 11.7 ± 8.6 (mean ±SD) days for *E. faecium* and 11.94 ± 11.63 (mean ±SD) daysfor *E. faecalis*. ICU stay duration was 12.67 ± 6.5 (mean ±SD) days for *E. faecium* and 8.23 ± 10.6 (mean ±SD) days for *E. faecalis*.

Antibiotic Susceptibility Patterns

Overall Antibiotic Sensitivity:

Table 2 summarizes the antibiotic susceptibility patterns of *E. faecium* and *E. faecalis* isolates.

Tigecycline, Linezolid, Vancomycin, Teicoplanin:

Both *E. faecium* and *E. faecalis* exhibited high sensitivity 100% to tigecycline, 89% to linezolid, 86% vancomycin, and 84% teicoplanin across all specimen types.

Penicillin, Daptomycin, Ampicillin:

E. faecalis displayed higher sensitivity to penicillin (25%), daptomycin (19%), and ampicillin (71.42%) compared to *E. faecium.* Penicillin showed poor sensitivity in *E. faecium* isolates.

High Level Gentamicin, Erythromycin, Nitrofurantoin, Tetracycline, Ciprofloxacin, Levofloxacin:

Variable sensitivity was observed for high level gentamicin (13%), erythromycin (2.24%), nitrofurantoin (63.63%),tetracycline (9.09%),

DOI: 10.69605/ijlbpr_13.6.2024.61

ciprofloxacin (0%) and levofloxacin (0%). Notably, *E. faecalis* displayed higher sensitivity to nitrofurantoin in urine isolates.

In conclusion, our study provides valuable insights into the demographic and clinical factors associated with *E. faecium* and *E. faecalis* infections, as well as their antibiotic susceptibility patterns. These findings contribute to our understanding of the epidemiology and management of Enterococcal infections.

Table 1: Age, Gender, Specimen and Clinical Risk factor wise distribution of *E. faecium* & *E. faecalis*

		***E. Faecium*(n=64) n (%)**	***E. Faecalis* (n=36) n (%)**	***Enterococcus* Total (n=100) n (%)**	**P value[#]**
Age (years)	**< 40**	**10** (15.63%)	**13** (36.11%)	**23**(23%)	**0.0263**
	>40	**54** (84.37%)	**23** (63.88%)	**77**(77%)	
Gender	**Male**	**35** (54.68%)	**19** (52.78%)	**54**(54%)	**1**
	Female	**29** (45.31%)	**17** (47.22%)	**46**(46%)	
Specimens	**Blood**	**50** (78.12%)	**17** (47.22%)	**67**(67%)	
	Other than blood	**14** (21.87%)	**19** (52.78%)	**33**(33%)	**0.0021**
Clinical Risk Factors	**Diabetes mellitus**	**24** (37.5%)	**14** (38.88%)	**38**(38%)	**1**
	Chronic kidney diseases	**8** (12.5%)	**2** (5.55%)	**10**(10%)	**0.3226**
	Cardio-vascular diseases	**6** (9.38%)	**4** (11.11%)	**10**(10%)	**0.7438**
	Chronic lung disease	**3** (4.68%)	**2** (5.55%)	**5**(5%)	**1**
	Chronic liver diseases	**2** (3.12%)	**2** (5.55%)	**4**(4%)	**0.6175**
	Cerebro-vascular diseases	**3** (4.68%)	**0**	**3**(3%)	**0.5512**
	Rheumatoid arthritis	**2** (3.12%)	**0**	**2**(2%)	**0.5345**
	Active malignancy	**1** (1.56%)	**0**	**1**(1%)	**1**
	Dementia	**0**	**1** (2.77%)	**1**(1%)	**0.36**
	Hepatitis B	**1** (1.56%)	**0**	**1**(1%)	**1**
	Trigeminal neuralgia	**1** (1.56%)	**0**	**1**(1%)	**1**
	Epilepsy	**1** (1.56%)	**0**	**1**(1%)	**1**
Clinical Outcome	**Discharge**	**29** (45.31%)	**24**(66.67%)	**53**(53%)	**-**
	Discharge Against Medical Advice	**16** (25%)	**5** (13.88%)	**21**(21%)	**-**
	Death (Mortality)	**19** (29.69%)	**7** (19.44%)	**26**(26%)	**0.3441**
Hospital stays (in days) (mean ± SD)		**11.7 ± 8.6**	**11.94 ± 11.63**	**11.8 ± 9.7**	**0.1849**
ICU stay (in days) (mean ± SD)		**12.67 ± 6.5**	**8.23 ± 10.6**	**9.4 ±7.6**	**0.1696**

We used Fisher's exact test to calculate p-value.

Table 2: Antibiotic Susceptibility Pattern of *E. faecium* & *E. faecalis*

Antibiotic Name	**Overall sensitivity (n=100) n (%)**	***E. faecium* sensitivity (n=64) n (%)**	***E. faecalis* sensitivity (n=36) n (%)**	**Blood**			**Other than Blood (Swab, Urine, Pus, Tissue, Tracheal secretion, Central line tip)**		
				Overall sensitivity (n=67) n (%)	***E. faecium* sensitivity (n=50) n (%)**	***E. faecalis* sensitivity (n=17) n (%)**	**Overall sensitivity (n=33) n (%)**	***E. faecium* sensitivity (n=14) n (%)**	***E. faecalis* sensitivity (n=19) n (%)**
Tigecycline	**100** (100%)	**64** (100%)	**36** (100%)	**67** (100%)	**50** (100%)	**17** (100%)	**33** (100%)	**14** (100%)	**19** (100%)
Linezolid	**89** (89%)	**53** (82.81%)	**36** (100%)	**56** (83.58 %)	**39** (78%)	**17** (100%)	**33** (100%)	**14** (100%)	**19** (100%)
Vancomycin	**86** (86%)	**51** (79.69%)	**35** (97.22%)	**55** (82.09 %)	**38** (76%)	**17** (100%)	**31** (93.93%)	**13** (92.85 %)	**18** (94.73%)

DOI: 10.69605/ijlbpr_13.6.2024.61

Teicoplanin	**84** (84%)	**48** (75%)	**36** (100%)	**52** (77.61%)	**35** (70%)	**17** (100%)	**32** (96.96%)	**13** (92.85%)	**19** (100%)
Penicillin	**29** (29%)	**4** (6.25%)	**25** (69.44%)	**13** (19.40%)	**4** (8%)	**9** (52.94%)	**16**(48.48%)	**0** (0%)	**16** (84.21%)
Daptomycin	**19** (19%)	**0**(0%)	**19** (52.78%)	**9**(13.43%)	**0**(0%)	**9** (52.94%)	**10** (30.30%)	**0** (0%)	**10** (52.63%)
Ampicillin	**18 (n=41)** (43.90%)	**8 (n=27)** (29.63%)	**10 (n=14)** (71.42%)	**10 (n=29)** (34.48%)	**5 (n=20)** (25%)	**5 (n=9)** (55.56%)	**8 (n=12)** (66.66%)	**3 (n=7)** (42.86%)	**5 (n=5)** (100%)
High level Gentamicin	**13** (13%)	**9** (14.06%)	**4** (11.11%)	**10** (14.93%)	**9** (18%)	**1** (5.88%)	**3** (9.09%)	**0** (0%)	**3** (15.78%)
Erythromycin	**2 (n=89)** (2.24%)	**0 (n=58)** (0%)	**2 (n=31)** (6.45%)	**1**(1.49%)	**0** (0%)	**1** (5.88%)	**1 (n=22)**(4.55%)	**0 (n=8)** (0%)	**1 (n=14)** (7.14%)

For Urine isolates only

Antibiotic Name	**Overall sensitivity (n=11) n (%)**	***E. faecium* sensitivity (n=6) n (%)**	***E. faecalis* sensitivity (n=5) n (%)**	**Blood**			**Other than Blood (Urine)**		
				Overall sensitivity (n=0) n (%)	***E. faecium* sensitivity (n=0) n (%)**	***E. faecalis* sensitivity (n=0) n (%)**	**Overall sensitivity (n=11) n (%)**	***E. faecium* sensitivity (n=6) n (%)**	***E. faecalis* sensitivity (n=5) n (%)**
Nitrofurantoin	**7** (63.63%)	**2** (33.33%)	**5** (100%)	-	-	-	**7** (63.63%)	**2** (33.33%)	**5** (100%)
Tetracycline	**1 (**9.09%)	**1 (**16.67%)	**0** (0%)	-	-	-	**1 (**9.09%)	**1** (16.67%)	**0**(0%)
Ciprofloxacin	**0** (0%)	**0** (0%)	**0** (0%)	-	-	-	**0** (0%)	**0** (0%)	**0** (0%)
Levofloxacin	**0** (0%)	**0** (0%)	**0** (0%)	-	-	-	**0** (0%)	**0** (0%)	**0** (0%)

DISCUSSION

***Enterococcus* isolation rate:** Our study found that 2.94% of all clinical samples were positive for *Enterococcus*, which is similar to a previous study[7] in India. However, other studies have reported higher[8,9,10] or lower rates[11], ranging from 2.68% to 54.73%. This may reflect the variable prevalence of *Enterococcus* as a hospital-associated infection in different settings.

***Enterococcus* species distribution:** We identified *E. faecium* as the most common species (64%), followed by *E. faecalis* (36%). This is consistent with some studies[11,12], but not others[7,8,9,10,13,14,15], where *E. faecalis* was more prevalent. The predominance of *E. faecium* and *E. faecalis* may be due to their ability to form biofilms, resist antibiotics and immune system attacks, produce various virulence factors that help them attach, evade, and inflame. As present study site is Tertiary care hospital having ICU beds >25% and bacteraemiais more in ICU patients. *E. faecium* is emerges as predominant isolate. This is similar to finding with previous study. [16]

Gender distribution: The absence of a gender-based difference suggests that *Enterococcus* infections do not exhibit gender predilection. Some studies show higher male prevalence [7,9,10,14,15], and some studies[8,13] suggest that females are more prone to enterococcal infections, especially UTIs, due to anatomical, hormonal, sexual, and hygiene factors. But none of these studies have calculated p value to obtain statistically significant.

Age distribution: Our study revealed a significant association between age and the distribution of *E. faecium* and *E. faecalis* isolates. The higher

DOI: 10.69605/ijlbpr_13.6.2024.61

prevalence of *E. faecium* in individuals aged >40 years may suggest age-related susceptibility or potential exposure patterns. This finding aligns with previous studies[8,10] indicating age as a relevant factor in *Enterococcus* infections. In some studies [9,14], younger age groups were more affected. The age distribution may be influenced by underlying conditions, healthcare exposure, invasive devices, and antibiotic use.

Specimen distribution: The predominance of *E. faecium* in blood specimens highlights its systemic nature, possibly indicating a higher propensity for bloodstream infections. This aligns with previous studies reporting the importance of blood cultures in detecting *Enterococcus* infections. However, other studies[8,9,10,13,14] have reported urine as the most common specimen, followed by pus or blood. This may indicate that different types of enterococcal infections are prevalent in different settings, such as UTIs, wound infections, or bloodstream infections.

Risk factors: Clinical risk factors, however, did not significantly influence the distribution of *E. faecium* and *E. faecalis*, suggesting a multifactorial nature of *Enterococcus* infections not solely dependent on specific risk factors.

Clinical Outcomes and Hospital Stay: The comparable rates of discharge, discharge against medical advice, and death between *E. faecium* and *E. faecalis* groups suggest similar clinical outcomes for patients infected with either species. The mean hospital stay was similar for both the species, did not reach statistical significance. The mean ICU stays was slightly longer for *E. faecium* and also did not reach statistical significance. These findings underscore the importance of early recognition and appropriate management for both *Enterococcus* species.Other studies reported similar[15,17] or different[18,19.20]results for hospital stays and higher [17,21] for ICU stays.This suggests that the duration of hospital and ICU stay may depend on various factors, such as type and severity of infection, comorbidities, treatment options, and outcomes.

Antibiotic profile: The antibiotic susceptibility patterns revealed important insights into the management of *E. faecium* and *E. faecalis* infections. High sensitivity to tigecycline, linezolid, vancomycin, and teicoplanin suggests these antibiotics remain effective choices. However, the limited sensitivity to penicillin in *E. faecium* isolates underscores the importance of selecting antibiotics based on susceptibility profiles.

The variability in sensitivity to other antibiotics necessitates a cautious approach in antibiotic selection, considering the specific species and infection site. The higher sensitivity of *E. faecalis* to nitrofurantoin in urine isolates suggests its potential efficacy in urinary tract infections caused by this species and this finding was similar to one study [13]

Vancomycin resistance: We detected vancomycin resistance in 14% of our enterococcal isolates, which is similar to one study[16], but different from others[7,8,9,10,13,14,15,22], where the rates ranged from 0% to 31%. The variation in vancomycin resistance may be due to geographic location, prolonged hospitalization, hospitalization in ICU, exposure to equipment or devices contaminated with VRE, duration of antibiotic therapy and other factors[23]. Vancomycin resistance is mediated by the alteration of the peptidoglycan precursors, the modification of the existing peptidoglycan, the decrease of the cell wall permeability, or the mutation of the vancomycin-binding proteins.

Other Antibiotics susceptibility: We observed the highest susceptibility in our enterococcal isolates to tigecycline (100%), followed by linezolid (89%), vancomycin (86%), and teicoplanin (84%). This is consistent with one study[7], where vancomycin, linezolid, and teicoplanin showed (100%) sensitivity, followed by high-level gentamicin (92%). However, other studies[9,10,14,15] have reported lower sensitivity rates to these antibiotics, especially vancomycin and linezolid. Tigecycline, linezolid, vancomycin, and teicoplanin are among the last-resort antibiotics for treating enterococcal infections. Therefore, it is important to monitor their sensitivity patterns and prevent their misuse or overuse.

Gentamicin HLAR: We found gentamicin HLAR in 87% of our enterococcal isolates, which is higher than other studies[7,8,9,10,13,14,15], where the rates ranged from 8% to 63.53%. Gentamicin HLAR affects the synergy between gentamicin and cell wall-active agents, such as penicillin or ampicillin, which are often used as combination therapy for enterococcal infections.

Our study has several limitations, including its Ambispective nature. The single-centre design may limit generalizability. Additionally, the lack of genotypic analysis restricts our understanding of strain-specific characteristics. Future research endeavours should consider genotypic analyses and multicentre studies to further elucidate strain-specific characteristics and enhance the applicability of our findings.

This study enhances our knowledge of the factors influencing the prevalence, outcomes, and antibiotic susceptibility of *Enterococcus faecium* and *Enterococcus faecalis* infections. The data generated herein provide a foundation for informed decision-making in the clinical management of these infections, fostering a more targeted and effective approach to patient care. Further research endeavours will undoubtedly contribute to ongoing efforts aimed at refining our strategies for combating *Enterococcus* infections.

CONCLUSION

The study evaluated the microbiological, epidemiological, and clinical profile of enterococcal infections at a tertiary care hospital in India. The study found that *E. faecium* was the most common enterococcal species, followed by *E. faecalis*. Our

DOI: 10.69605/ijlbpr_13.6.2024.61

findings suggest an age-related association, with *E. faecium* being more prevalent in individuals aged >40 years. Gender, however, did not significantly influence the distribution of *Enterococcus* species. The dominance of *E. faecium* in blood specimens highlights its systemic nature, while the lack of association with specific clinical risk factors underscores the complex multifactorial nature of *Enterococcus* infections. Clinical outcomes, including discharge rates, discharge against medical advice, and mortality, did not significantly differ between *E. faecium* and *E. faecalis* groups. The comparable hospital and ICU stay durations emphasize the need for prompt recognition and appropriate management for both species. The antibiotic susceptibility patterns reveal that tigecycline, linezolid, vancomycin, and teicoplanin remain effective choices for both *E. faecium* and *E. faecalis*. However, caution is warranted, especially regarding the limited sensitivity of *E. faecium* to penicillin. The variable sensitivity to other antibiotics necessitates a tailored approach, taking into consideration the specific *Enterococcus* species and the site of infection. Notably, the higher sensitivity of *E. faecalis* to nitrofurantoin in urine isolates suggests its potential efficacy in urinary tract infections caused by this species.

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