Section: Microbiology

Original Research Article

SPECIES DISTRIBUTION, VIRULENCE FACTORS AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF ENTEROCOCCUS ISOLATES FROM VARIOUS CLINICAL SAMPLES IN A TERTIARY CARE HOSPITAL

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ABSTRACT

Background: Enterococci are important opportunistic pathogens increasingly associated with healthcare-associated infections. Their intrinsic resistance to several antimicrobial agents and ability to acquire resistance determinants have contributed to their emergence as significant nosocomial pathogens. Knowledge of species distribution, virulence factors and antimicrobial susceptibility patterns is essential for appropriate therapeutic management and infection control. **Objectives:** To isolate and identify Enterococcus species from various clinical samples, determine species distribution, evaluate virulence factors, and study antimicrobial susceptibility patterns among the isolates. **Materials and Methods:** A cross-sectional study was conducted in the Department of Microbiology, Chengalpattu Medical College, Tamil Nadu, India, from March 2016 to February 2017. A total of 200 non-duplicate Enterococcus isolates obtained from urine, pus, blood and body fluids were included. Identification and speciation were performed using standard microbiological and biochemical methods. Hemolysin and gelatinase production were evaluated as virulence factors. Antimicrobial susceptibility testing was performed by Kirby-Bauer disc diffusion method according to CLSI 2016 guidelines. **Results:** Among 200 Enterococcal isolates, *E. faecalis* was the predominant species (67%), followed by *E. faecium* (30%). Most isolates were recovered from urine specimens (64.5%), followed by pus (17.5%), blood (14%) and body fluids (4%). Hemolysin production was observed in 56 (28%) isolates, while gelatinase production was detected in 27 (13.5%) isolates. Linezolid demonstrated 100% susceptibility against all Enterococcal isolates. Vancomycin and teicoplanin showed susceptibility rates above 95%. High resistance was observed against penicillin, erythromycin, ciprofloxacin and high-level aminoglycosides. **Conclusion:** *E. faecalis* remains the predominant Enterococcus species isolated from clinical specimens. The high prevalence of multidrug resistance among Enterococci emphasizes the need for routine antimicrobial surveillance and implementation of effective antimicrobial stewardship programs.

INTRODUCTION

Enterococci are Gram-positive, facultative anaerobic cocci that form a part of the normal microbiota of the gastrointestinal tract of humans and animals. Although traditionally regarded as harmless commensals, these organisms have emerged as important opportunistic pathogens responsible for a wide range of community-acquired and healthcare-associated infections. Their remarkable ability to survive under adverse environmental conditions, tolerate disinfectants, and acquire resistance determinants has contributed significantly to their increasing clinical importance in recent decades.[1-3] Among more than 50 recognized Enterococcus species, Enterococcus faecalis and Enterococcus faecium are the most frequently isolated from human infections and account for the majority of clinically significant cases. These organisms are commonly implicated in urinary tract infections, wound infections, intra-abdominal infections, bacteremia,

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endocarditis, neonatal sepsis, and other invasive infections.[4,5] The incidence of Enterococcal infections has increased substantially in hospitalized patients, particularly among those with prolonged hospital stays, underlying chronic illnesses, immunosuppression, indwelling medical devices, and prior exposure to broad-spectrum antibiotics.[6] The emergence of Enterococci as major nosocomial pathogens is largely attributed to their intrinsic resistance to several antimicrobial agents and their extraordinary ability to acquire and disseminate additional resistance genes through plasmids and transposons.[7] Resistance to β-lactams, aminoglycosides, macrolides, and glycopeptides has increasingly limited therapeutic options.[8] Furthermore, Enterococci possess various virulence factors that contribute to their pathogenicity and persistence within the host. Among these, hemolysin and gelatinase play important roles in tissue invasion, colonization, biofilm formation, and evasion of host defense mechanisms.[9,10] The expression of such virulence determinants may influence the severity and outcome of Enterococcal infections.[11] Antimicrobial resistance among Enterococci has become a major global health concern. High-level aminoglycoside resistance abolishes the synergistic bactericidal effect achieved by combining aminoglycosides with cell wall-active agents, thereby complicating treatment strategies. In addition, increasing resistance to commonly used antibiotics such as penicillin, erythromycin, fluoroquinolones, and tetracyclines has been reported worldwide.[12,13] The emergence and spread of multidrug-resistant Enterococci have led to increased morbidity, mortality, length of hospital stay, and healthcare costs.[14,15] Knowledge of the local epidemiology and antimicrobial susceptibility patterns of Enterococcal isolates is essential for guiding empirical therapy and implementing effective infection control measures. Since resistance patterns may vary between geographical regions and healthcare settings, continuous surveillance is necessary to monitor changing trends and identify emerging resistance.[16] Evaluation of virulence characteristics along with antimicrobial susceptibility profiles can provide valuable insights into the pathogenic potential of circulating strains and help formulate appropriate therapeutic and preventive strategies.[17,18] The present study was undertaken to isolate and identify Enterococcus species from various clinical samples obtained in a tertiary care hospital, determine their species distribution, assess the production of important virulence factors such as hemolysin and gelatinase, and evaluate their antimicrobial susceptibility patterns. Understanding these characteristics is crucial for optimizing patient management and preventing the dissemination of resistant Enterococcal strains within healthcare facilities.

MATERIALS AND METHODS

Study Design and Setting

A cross-sectional study was conducted in the Department of Microbiology, Chengalpattu Medical College and Hospital, Tamil Nadu, India, from March 2016 to February 2017.

Study Population

A total of 200 non-duplicate Enterococcus isolates recovered from various clinical specimens, including urine, pus/wound swabs, blood, and body fluids, were included in the study.

Isolation and Identification of Enterococci

Clinical specimens were processed using standard microbiological techniques. Samples were cultured on Blood agar, MacConkey agar, Nutrient agar, and CLED agar (for urine samples) and incubated aerobically at 37°C for 18–24 hours. Presumptive Enterococcus isolates were identified based on colony morphology, Gram staining, catalase test, bile esculin hydrolysis, PYR test, growth in 6.5% NaCl, heat tolerance at 60°C for 30 minutes, and growth at 10°C and 45°C.

Species Identification

Species-level identification was performed according to the Facklam and Collins conventional biochemical scheme using carbohydrate fermentation tests (mannitol, arabinose, raffinose, sorbitol, sucrose, and pyruvate), arginine hydrolysis, motility testing, and pigment production.

Detection of Virulence Factors

Hemolysin production was assessed on 5% sheep blood agar by observing complete hemolysis around colonies. Gelatinase production was determined on gelatin agar by the presence of a clear zone surrounding bacterial growth.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed by the Kirby–Bauer disk diffusion method on Mueller–Hinton agar and interpreted according to CLSI 2016 guidelines. The antibiotics tested included penicillin, ampicillin, erythromycin, doxycycline, ciprofloxacin, levofloxacin, nitrofurantoin, high-level gentamicin, high-level streptomycin, vancomycin, teicoplanin, and linezolid.

Detection of High-Level Aminoglycoside Resistance

High-level aminoglycoside resistance (HLAR) was detected using high-level gentamicin (120 µg) and high-level streptomycin (300 µg) discs as per CLSI recommendations.

Detection of Vancomycin Resistance

Vancomycin resistance was screened using Brain Heart Infusion agar containing 6 µg/mL vancomycin. Confirmation was performed by broth microdilution method to determine the minimum inhibitory concentration (MIC) of vancomycin according to CLSI guidelines.

Molecular Detection of vanA and vanB Genes

Genomic DNA was extracted using a commercial bacterial genomic DNA extraction kit. Polymerase

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chain reaction (PCR) was performed for the detection of vanA and vanB genes using specific primers. Amplified products were analyzed by agarose gel electrophoresis and visualized under UV illumination.

Statistical Analysis

Data were entered into Microsoft Excel and analyzed using descriptive statistics. Results were expressed as frequencies and percentages and presented in tables and figures.

RESULTS

Demographic Characteristics of the Study Population

A total of 200 non-duplicate Enterococcus isolates were recovered during the study period. The majority of isolates were obtained from adults (89.5%), while pediatric patients accounted for 10.5% of cases. The highest number of isolates was observed in the 31–45 years age group (30.5%), followed by 46–60 years (28.0%). Male patients constituted 58.5% of cases, with a male-to-female ratio of 1.4:1. [Figure 1]

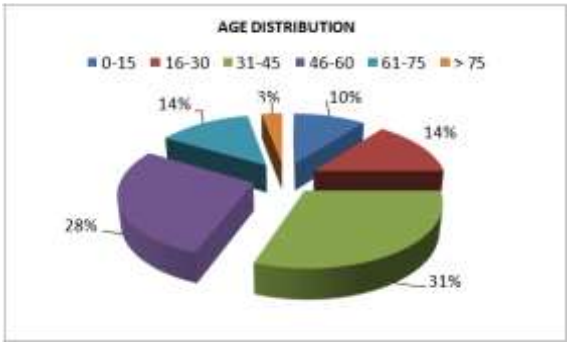

Figure 1: Age and Sex Distribution of Patients with Enterococcal Isolates

Distribution of Clinical Specimens

Urine was the predominant source of Enterococcal isolates, contributing 64% of all isolates. Pus/wound swabs accounted for 18%, followed by blood cultures (14%) and body fluids (4%) (Figure 2). These findings indicate that Enterococci were most frequently associated with urinary tract infections in the study population.

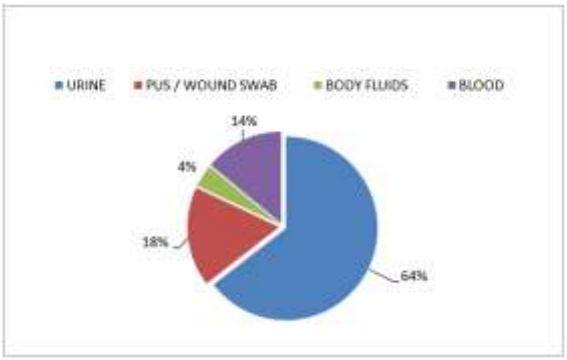

Figure 2: Distribution of Clinical Specimens

Species Distribution of Enterococci

Among the 200 isolates, *E. faecalis* was the predominant species accounting for 67% of isolates, followed by E. faecium (30%). Other species including *E. raffinosus, E. avium,* and *E. durans* were isolated infrequently. [Table 1]

Table 1: Species Distribution of Enterococcal Isolates

Species	Number (%)
E. faecalis	134 (67.0)
E. faecium	60 (30.0)
E. raffinosus	3 (1.5)
E. avium	2 (1.0)
E. durans	1 (0.5)
Total	200 (100)

Distribution of Enterococcal Species Among Clinical Specimens

E. faecalis was the most common species isolated from urine, pus, blood, and body fluids. Similarly, *E. faecium* was recovered from all specimen types but in lower proportions (Table 2). Rare Enterococcal species were isolated mainly from urine and blood specimens.

Table 2: Distribution of Enterococcal Species in Various Clinical Samples

Species	Urine	Pus	Blood	Body Fluids	Total
E. faecalis	85	24	19	6	134
E. faecium	39	11	8	2	60
Other species	5	0	1	0	6

Ward-wise Distribution of Enterococcal Isolates

The highest number of isolates was recovered from the Medicine ward (22.5%), followed by Surgery (14.5%), ICU (12.5%), and Urology (12.5%) (Table 3). The distribution suggests a significant burden of Enterococcal infections among hospitalized patients, particularly in high-risk clinical settings.

Table 3: Ward-wise Distribution of Isolates

Ward	Number (%)

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Medicine	45 (22.5)
Surgery	29 (14.5)
ICU	25 (12.5)
Urology	25 (12.5)
Others	76 (38.0)
Total	200 (100)

Associated Risk Factors

Urinary catheterization and diabetes mellitus were the most common risk factors, each observed in 8.5% of patients. Burns, septicemia, and postoperative wound infections were also associated with Enterococcal infections. [Table 4]

Table 4: Associated Risk Factors

Risk Factor	Number (%)
Urinary Catheterization	17 (8.5)
Diabetes Mellitus	17 (8.5)
Burns	11 (5.5)
Septicemia	10 (5.0)
Postoperative Wound Infection	5 (2.5)

Virulence Factors of Enterococci

Among the 200 isolates, hemolysin production was detected in 56 (28%) isolates, while gelatinase production was observed in 27 (13.5%) isolates. Hemolysin production was more common in *E.* faecalis than E. faecium. [Figure 3]

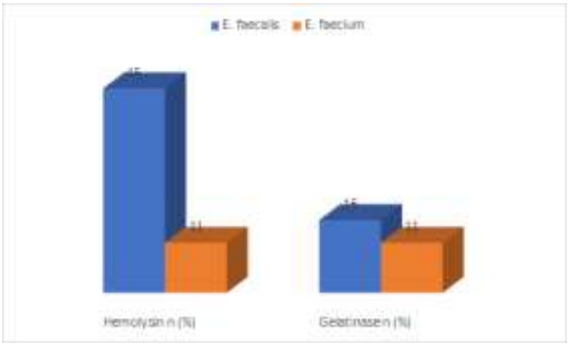

Figure 3: Production of Virulence Factors

Antimicrobial Susceptibility Pattern

Linezolid demonstrated 100% susceptibility against both *E. faecalis* and *E. faecium* isolates. Vancomycin and teicoplanin also showed high activity, with susceptibility rates exceeding 95% (Table 5). Resistance was highest against penicillin, erythromycin, ciprofloxacin, and high-level aminoglycosides.

Table 5: Antimicrobial Susceptibility Pattern of Major Enterococcal Species

Antibiotic	E. faecalis Sensitive (%)	E. faecium Sensitive (%)
Linezolid	100	100
Teicoplanin	99.3	96.7
Vancomycin	98.5	95.0
Ampicillin	40.3	35.0
Penicillin	22.4	13.3

High-Level Aminoglycoside Resistance (HLAR)

High-level aminoglycoside resistance was observed in 38.1% of *E. faecalis* and 50% of E. faecium isolates. Combined resistance to both gentamicin and streptomycin was more frequent among *E. faecium*. [Table 6]

Table 6: High-Level Aminoglycoside Resistance

Resistance Pattern	E. faecalis n (%)	E. faecium n (%)
HLGR alone	20 (14.9)	6 (10.0)
HLSR alone	44 (32.8)	14 (23.3)
HLAR (Both)	51 (38.1)	30 (50.0)

Prevalence of Vancomycin-Resistant Enterococci (VRE)

Five isolates (2.5%) were confirmed as vancomycin-resistant Enterococci. Of these, three were E. faecium and two were E. faecalis. VRE isolates were recovered from urine (40%), pus (40%), and blood (20%) specimens. [Table 7]

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Table 7: Distribution of VRE Isolates

Specimen	VRE Isolates
Urine	2
Pus	2
Blood	1
Total	5

Susceptibility Pattern of VRE Isolates

All VRE isolates remained susceptible to linezolid and tigecycline. Additionally, all E. faecium VRE isolates were susceptible to quinupristin/dalfopristin, indicating these agents as effective therapeutic options for VRE infections. [Table 8]

Table 8: Antimicrobial Susceptibility of VRE Isolates

Antibiotic	Susceptibility (%)
Linezolid	100
Tigecycline	100
Quinupristin/Dalfopristin*	100

Tested only for E. faecium isolates.

DISCUSSION

Enterococci have emerged as important opportunistic pathogens causing hospital-associated infections due to their intrinsic resistance mechanisms and their ability to acquire transferable antimicrobial resistance determinants. In the present study, 200 Enterococcus isolates were recovered from various clinical samples, among which Enterococcus faecalis was the predominant species (67%), followed by E. faecium (30%). Similar predominance of E. faecalis has been reported by Peters et al., Bhatt et al., and Abdeta et al., where E. faecalis constituted the major proportion of clinical isolates.[19-20] In contrast, Mansoor et al. reported E. faecium as the predominant species, suggesting variation in species distribution according to geographical location and hospital settings.[20] In the present study, urine was the most common clinical specimen yielding Enterococcus isolates (64.5%), followed by pus/wound samples (17.5%) and blood (14%). Similar findings were reported by Chakraborty et al. and Gupta et al., where urinary tract infections were the predominant enterococcal infections.[21,22] The predominance of urinary isolates may be attributed to the ability of enterococci to survive in the urinary tract, especially among hospitalized patients with catheterization and underlying medical conditions. The majority of isolates were obtained from adult patients, with the highest isolation rate observed in the 31–45 years age group (30.5%). Male patients showed higher isolation rates (58.5%) compared with females (41.5%). Similar male predominance was observed in studies by Tripathi et al.[23] The increased occurrence among adults may be associated with prolonged hospitalization, antibiotic exposure, invasive procedures, and associated comorbidities. Urinary catheterization and diabetes mellitus were the major associated risk factors observed in this study (8.5% each). Tripathi et al. reported urinary catheterization and diabetes mellitus as important predisposing factors for enterococcal infections.[23] Catheter-associated infections are common due to biofilm formation by enterococci, which promotes persistence and resistance to antimicrobial therapy. Virulence factor analysis showed hemolysin production in 28% and gelatinase production in 13.5% of isolates. E. faecalis showed higher hemolysin production compared with E. faecium. Similar observations were reported by Mansoor et al., who demonstrated the presence of hemolysin and gelatinase among clinical enterococcal isolates.[22] These virulence factors contribute to tissue invasion, host cell damage, and increased pathogenicity. The antimicrobial susceptibility pattern showed high resistance among Enterococcus isolates to commonly used antibiotics. Penicillin showed the lowest susceptibility among both E. faecalis and E. faecium, which was comparable with findings reported by Salem-Bekhit et al.[26] High-level aminoglycoside resistance was observed among isolates, with HLAR detected in 38.05% of E. faecalis and 50% of E. faecium. Similar observations were reported by Choudhury et al.[27] High-level aminoglycoside resistance is clinically significant as it prevents the synergistic effect of aminoglycosides when combined with cell wall-active antibiotics. Linezolid showed 100% susceptibility among all Enterococcus isolates in the present study, followed by teicoplanin and vancomycin. Similar complete susceptibility to linezolid has been reported by Peters et al. and Bhatt et al.[19,20] However, emergence of linezolid resistance has been reported in other studies, emphasizing the importance of continuous antimicrobial surveillance. Vancomycin-resistant Enterococci (VRE) were detected in 5 isolates, giving a prevalence rate of 2.5%. Among these, three isolates were E. faecium and two were E. faecalis. Similar prevalence rates were reported by Gupta et al.[24] All VRE isolates showed vancomycin MIC values ranging from 32–128 µg/ml, confirming high-level resistance. All VRE isolates showed susceptibility to linezolid and tigecycline, while E. faecium isolates were susceptible to quinupristin/dalfopristin. Similar findings were reported by Gupta et al. and Choudhury et al. [24,27] Early detection of VRE through phenotypic and molecular methods, combined with strict infection control measures and antimicrobial stewardship, is

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essential to prevent dissemination of multidrug-resistant Enterococci in healthcare settings.

Limitations

The present study has certain limitations. It was conducted in a single tertiary care hospital over a period of one year; therefore, the findings may not reflect the true prevalence and antimicrobial resistance pattern of Enterococcus species in other healthcare settings or the community. The sample size was limited to 200 isolates and included only selected clinical specimens, which may have influenced the observed distribution of species and resistance rates. Molecular detection was restricted to vanA and vanB genes; other vancomycin resistance determinants such as vanC, vanD, vanE, and vanM were not studied. Other important resistance genes and virulence-associated genes were not analyzed. Patient follow-up, treatment outcome, recurrence, and mortality associated with enterococcal infections were not evaluated. Further multicentric studies with larger sample sizes, long-term surveillance, and advanced molecular techniques are required for better understanding of Enterococcus epidemiology and resistance mechanisms.

CONCLUSION

Enterococci have become significant healthcare-associated pathogens due to their ability to cause infections and develop resistance to multiple antimicrobial agents. In this study, Enterococcus faecalis was the predominant species isolated, followed by E. faecium, with urine being the most common clinical specimen. The isolates demonstrated considerable resistance to commonly used antibiotics, particularly penicillin and aminoglycosides, highlighting the challenge in treatment of enterococcal infections. Virulence factors such as hemolysin and gelatinase production were observed among clinical isolates, contributing to their pathogenic potential. Vancomycin-resistant Enterococci were detected with a prevalence of 2.5%, and molecular analysis showed predominance of the vanA genotype over vanB. All VRE isolates remained susceptible to linezolid and tigecycline. Continuous surveillance, accurate laboratory detection, antimicrobial stewardship, and strict infection control measures are essential to prevent the emergence and spread of multidrug-resistant Enterococcus strains in hospitals.

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