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PREVALENCE AND ANTIBIOTIC SUSCEPTIBILITY PATTERN OF ENTEROCOCCAL SPECIES ISOLATED FROM CLINICAL SPECIMENS IN A TERTIARY CARE CENTRE

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ABSTRACT

Background: Enterococci are increasingly recognized as significant nosocomial pathogens, with rising antimicrobial resistance complicating treatment. Understanding their prevalence and antibiotic susceptibility patterns is critical for effective management.

Objectives: To determine the prevalence of *Enterococcus* species isolated from clinical specimens and to evaluate their antibiotic susceptibility patterns in a tertiary care hospital.

Methods: A prospective observational study was conducted from January to August 2017 at the Institute of Microbiology, Madurai Medical College. A total of 396 clinical specimens from patients suspected of urinary tract infections, sepsis, wound infections, meningitis, and lower respiratory tract infections were processed. Enterococci were isolated, identified using conventional microbiological methods, and antibiotic susceptibility was determined using the Kirby-Bauer disc diffusion method as per CLSI guidelines.

Results: Out of 396 specimens, 368 showed bacterial growth, and 104 (28.26%) isolates were identified as *Enterococcus* species. *E. faecalis* was predominant (74.03%), followed by *E. faecium* (25.96%). The majority of isolates were from urine (46.15%), pus (28.84%), blood (9.6%), and wound swabs (15.38%). *E. faecium* exhibited higher resistance to antibiotics compared to *E. faecalis*. Ampicillin, ciprofloxacin, and doxycycline showed reduced sensitivity, whereas glycopeptides (vancomycin and teicoplanin) retained high efficacy. Vancomycin-resistant enterococci (VRE) constituted 3.8% of isolates.

Conclusions: *Enterococcus* species, particularly *E. faecalis*, remain prevalent nosocomial pathogens with significant antimicrobial resistance. Glycopeptides remain effective treatment options, but emerging resistance underscores the need for continuous surveillance, antibiotic stewardship, and strict infection control measures.

Keywords: *Enterococcus* species, Antibiotic susceptibility, Nosocomial infections, Vancomycin-resistant enterococci, Antimicrobial resistance

1. INTRODUCTION

Enterococci are facultative anaerobic, gram-positive cocci that typically appear as diplococci or in short chains on Gram stain. They are a part of the normal flora of the human gastrointestinal tract, oral cavity, and female genitourinary tract, and are also found in various animal hosts [1, 2]. Historically considered low-virulence organisms, Enterococci have emerged over the past few decades as significant opportunistic and nosocomial pathogens, particularly in immunocompromised patients and those undergoing prolonged hospitalization [3]. Enterococcal infections have been increasingly reported in clinical settings worldwide due to their ability to survive under adverse environmental conditions, including exposure to various disinfectants and antibiotics [4]. Their intrinsic resistance to several antimicrobial agents, such as cephalosporins and low-level aminoglycosides, coupled with their capacity to acquire and disseminate resistance genes through plasmids and transposons, contributes to their resilience in hospital environments [5, 6]. The clinical spectrum of enterococcal infections includes urinary tract infections (UTIs), bacteremia, endocarditis, intra-abdominal and pelvic infections, wound and surgical site infections, and central nervous system infections [7, 8]. Among the species, *E. faecalis* is the most frequently isolated, accounting for approximately 80–90% of enterococcal infections in humans, followed by *E. faecium*, which accounts for 5–15% of cases [9, 10]. Other species, such as *E. gallinarum*, *E. casseliflavus*, *E. durans*, *E. hirae*, and E. *mundtii*, are less commonly associated with human disease [11, 12]. One of the most clinically challenging aspects of treating enterococcal infections is their variable and often high levels of resistance to commonly used antibiotics. Intrinsic resistance mechanisms include poor uptake of aminoglycosides, low-affinity penicillin-binding proteins, and limited permeability to β-lactam antibiotics [13, 14]. Acquired resistance mechanisms, which are of greater concern, involve genetic mutations and horizontal gene transfer, especially in the case of resistance to high-level aminoglycosides and glycopeptides such as vancomycin [15]. Enterococci are the second most common cause of hospital-acquired urinary tract infections and the third most frequent cause of nosocomial bloodstream infections [16, 17]. In addition, they are responsible for 10%–20% of infective endocarditis cases [18]. Due to the increasing incidence of antimicrobial resistance, particularly to vancomycin and high-level aminoglycosides, management of enterococcal infections has become increasingly complicated [19].

Routine identification and antimicrobial susceptibility testing of enterococcal isolates are essential for effective patient management and infection control [20]. Moreover, understanding the local prevalence and resistance trends is critical to guide empirical therapy and reduce the spread of multidrug-resistant strains in healthcare settings. This study aims to evaluate the prevalence and antibiotic susceptibility patterns of Enterococcal species from clinical isolates in a tertiary care hospital.

2. MATERIALS AND METHODS

Study Design

This was a prospective observational study designed to determine the prevalence and antibiotic susceptibility patterns of *Enterococcus* species isolated from various clinical specimens.

Study Period and Location

The study was conducted over a period of eight months, from January 2017 to August 2017, at the Institute of Microbiology, Madurai Medical College, attached to the Government Rajaji Hospital (GRH), Madurai, which is a major tertiary care referral centre in Tamil Nadu, India.

Sample Size and Population

A total of 396 clinical specimens were collected from patients attending both inpatient and outpatient departments. These patients presented with clinical symptoms suggestive of:

- Urinary Tract Infection (UTI)
- Sepsis

- Wound infections
- Meningitis
- Lower Respiratory Tract Infections (LRTI)

The samples were collected across various age groups and both genders.

Inclusion Criteria

- Patients of all age groups (neonates to elderly).
- Both sexes.
- Patients presenting with clinical signs and symptoms of the above-mentioned infections.

Exclusion Criteria

- Stool samples – due to the presence of large numbers of commensal organisms, making identification of Enterococci unreliable.
- Sputum samples – often contaminated with oropharyngeal flora, which may confound the results.

Types of Specimens Collected

The following types of specimens were included in the study:

- Urine
- Blood
- Pus
- Wound swabs
- Sterile body fluids (e.g., cerebrospinal fluid, pleural fluid, ascitic fluid)
- Bronchoalveolar lavage (BAL)

All samples were collected using standard sterile techniques, and processed immediately in the microbiology laboratory.

Collection and Processing of Specimens

Specimens collected included urine, blood, pus, wound swabs, and various sterile body fluids. All samples were collected using sterile techniques and processed immediately upon arrival at the microbiology laboratory. Each sample was inoculated onto appropriate culture media, namely Nutrient Agar (NA), MacConkey Agar (MAC), and Blood Agar Plates (BA). The culture plates were incubated at 37°C for 24 to 48 hours under aerobic conditions. Growth was observed, and colony morphology was noted for preliminary identification.

Identification of Enterococcus Species

Preliminary identification of Enterococcal isolates was based on colony morphology, Gram staining, and biochemical characteristics. On Gram staining, Enterococci appeared as Gram-positive cocci arranged in pairs or short chains. They were catalase-negative, which distinguishes them from Staphylococci. Bile esculin hydrolysis was used to confirm Enterococcus by the characteristic blackening of the medium. Further confirmation was done using the 6.5% NaCl salt tolerance test, where *Enterococcus* species show positive growth. Speciation was done based on biochemical tests such as fermentation of mannitol, sorbitol, sucrose, and arabinose, as well as growth on potassium tellurite agar. Based on these reactions, isolates were identified primarily as *E. faecalis* or *E. faecium*.

Antibiotic Susceptibility Testing

Antibiotic susceptibility testing was performed using the Kirby-Bauer disk diffusion method, following CLSI 2017 guidelines. Mueller-Hinton agar was used as the testing medium. Bacterial inoculum was standardized to 0.5 McFarland turbidity, and the surface of the agar was evenly inoculated using a sterile swab.

The antibiotic discs used included:

- Ampicillin (10 µg)

- Penicillin (10 units)
- Ciprofloxacin (5 µg)
- Doxycycline (30 µg)
- Teicoplanin (30 µg)
- Vancomycin (30 µg)
- High-Level Gentamicin (HLG – 120 µg)
- High-Level Streptomycin (HLS – 300 µg)

Plates were incubated at 37°C for 18–24 hours, and zones of inhibition were measured in millimeters. Interpretation of results was done using CLSI breakpoints to categorize the isolates as sensitive, intermediate, or resistant.

Detection of High-Level Aminoglycoside Resistance (HLAR)

To assess the presence of high-level aminoglycoside resistance, all Enterococcus isolates were tested using high-concentration gentamicin (120 µg) and streptomycin (300 µg) discs. Isolates that were resistant to either or both were recorded as HLGR (high-level gentamicin resistant) and HLSR (high-level streptomycin resistant). These findings are clinically significant, as HLAR renders combination therapy with aminoglycosides and cell wall-active agents ineffective.

Quality Control

To ensure accuracy and consistency of laboratory procedures, quality control was maintained using standard control strains. *E. faecalis* ATCC 29212 was used to validate both identification and antibiotic susceptibility testing procedures. Additionally, all media were checked for sterility and performance prior to use.

Data Collection and Statistical Analysis

All data were compiled using Microsoft Excel. Results were analyzed descriptively, and frequencies and percentages were calculated to determine the distribution of Enterococcus species across various specimen types and to assess their antibiotic susceptibility patterns. These statistics helped in evaluating the prevalence of resistance trends within the hospital setting.

3. RESULTS

A total of 396 clinical specimens were processed during the study period (Fig. 1). Of these, 368 specimens (92.9%) showed bacterial growth, while the remaining 28 (7.1%) showed no growth after standard incubation. Out of the 368 culture-positive samples, 104 isolates (28.26%) were identified as *Enterococcus species* based on Gram staining, biochemical reactions, and growth characteristics.

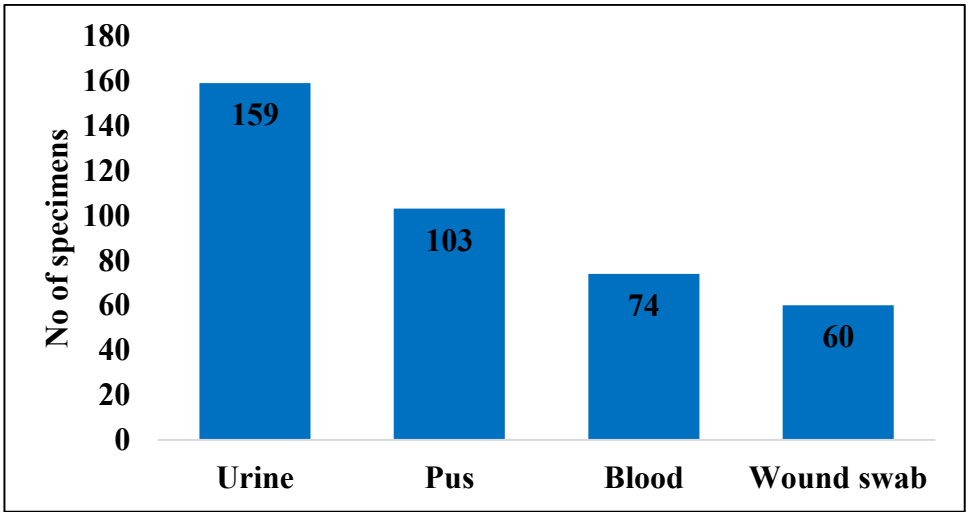

Fig: 1 Distribution of Clinical Specimens

Distribution of Enterococcal Isolates by Sample Type

Among the 104 Enterococcus isolates, the highest number were obtained from urine samples (48 isolates, 46%), followed by pus (30 isolates, 29%), wound swabs (16 isolates, 15%), and blood samples (10 isolates, 10%) (Fig.2). This distribution highlights that urinary tract infections are the most common clinical condition associated with enterococcal infections in the hospital setting, followed by wound and soft tissue infections.

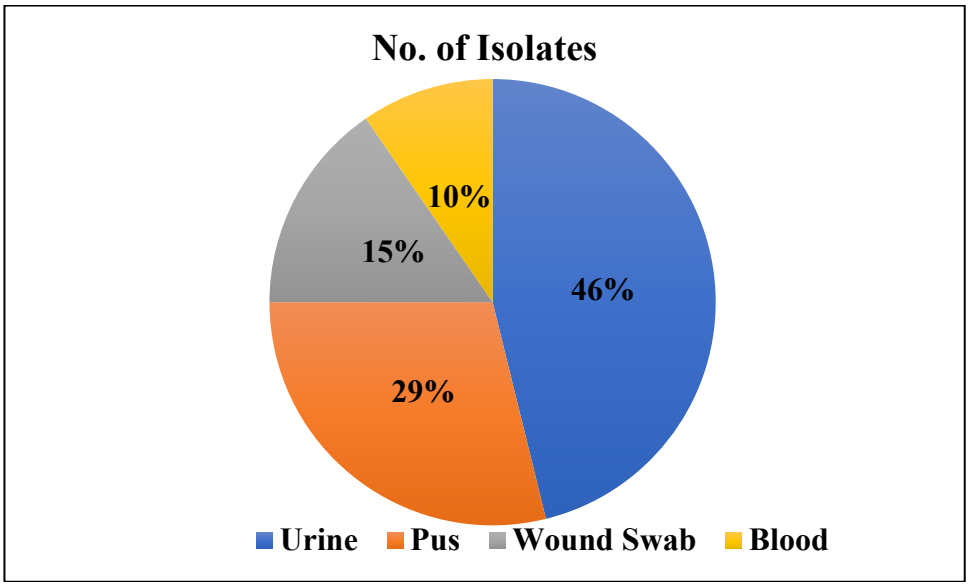

Fig: 2 Specimen-Wise Distributions of Enterococcus Isolates

Species Distribution among Enterococcus Isolates

Upon speciation, *E. faecalis* emerged as the predominant species, with 77 isolates (74.03%), while *E. faecium* accounted for 27 isolates (25.96%) (Fig.3). No other species were isolated during the study period.

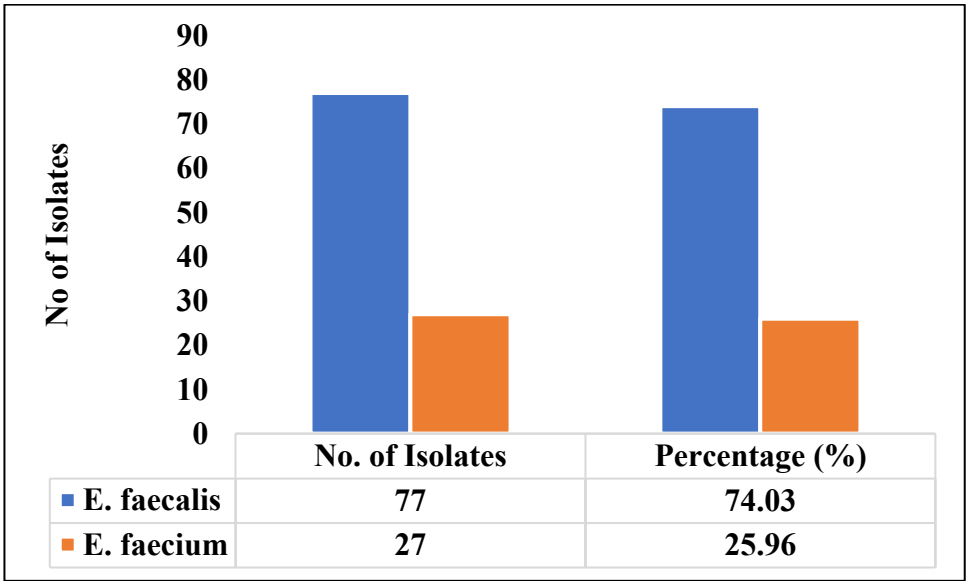

Fig: 3 Species Distribution of Enterococcus (n=104)

Age and Gender Distribution

The majority of enterococcal infections occurred in the 13–45 years age group (38 cases, 36.5%), followed by patients aged 46–60 years (24 cases, 23%) and those over 60 years (22 cases, 21.15%) (Table 1). Pediatric cases (under 12 years) comprised 17.3% of infections. In terms of gender, 66 cases (63.46%) were male, and 38 cases (36.53%) were female (Table 2).

Table:1 Age-Wise Distribution of Enterococcus Infections (n=104)

Age Group (Years)	No. of Cases	Percentage (%)
<1	2	1.92
1–12	18	17.3
13–45	38	36.5
46–60	24	23
>60	22	21.15
Total	**104**	**100**

Table: 2 Gender-Wise Distribution of Enterococcus Infections (n=104)

Gender	No. of Cases	Percentage (%)
Male	66	63.46
Female	38	36.53
Total	**104**	**100**

Antibiotic Susceptibility Pattern

The antibiotic susceptibility testing of the 104 enterococcal isolates revealed notable differences in resistance between *E. faecalis* and *E. faecium*. Among the tested antibiotics, ampicillin, teicoplanin, and vancomycin were the most effective against both species. Specifically, 83.1% of *E. faecalis* isolates were sensitive to ampicillin, compared to 70.3% of *E. faecium*. Similarly, teicoplanin showed high effectiveness, with 97.4% of *E. faecalis* and 92.6% of *E. faecium* being sensitive. Vancomycin also retained strong activity, with 94.8% sensitivity among *E. faecalis* and 85.18% among *E. faecium* isolates. On the other hand, ciprofloxacin exhibited moderate effectiveness, with 68.8% of *E. faecalis* and 62.9% of *E. faecium* being sensitive. The lowest susceptibility was observed with doxycycline, where only 38.9% of *E. faecalis* and 40.7% of *E. faecium* isolates responded (Table 3). These findings indicate that while glycopeptide antibiotics like teicoplanin and vancomycin continue to be reliable treatment options, the resistance to commonly used agents such as ciprofloxacin and doxycycline is alarmingly high, especially in *E. faecium*. The higher resistance profile observed in *E. faecium* underscores its growing role as a multidrug-resistant hospital-acquired pathogen. These results highlight the importance of antimicrobial stewardship and the need for regular surveillance of resistance patterns to guide empirical therapy and reduce the spread of resistant enterococcal strains in healthcare settings.

Table: 3 Antibiotic Susceptibility of *Enterococcus* Species

Antibiotic	*E. faecalis* Sensitive (%)	*E. faecium* Sensitive (%)
Ampicillin	83.10%	70.30%
Ciprofloxacin	68.80%	62.90%
Doxycycline	38.90%	40.70%
Teicoplanin	97.40%	92.60%
Vancomycin	94.80%	85.18%

4. DISCUSSION

Enterococci, once regarded as low-virulence commensals have increasingly become significant nosocomial pathogens in hospital settings. According to older CDC data, *Enterococcus* species rank among the second most common causes of hospital-acquired infections [21, 22]. Their growing resistance to multiple antibiotic classes, especially β-lactams, aminoglycosides, and glycopeptides such as vancomycin, has solidified their status as formidable healthcare pathogens. In our study, Enterococci were isolated in 28.26% of all culture-positive specimens, which is substantially higher than the 4.8% prevalence you mentioned in your draft, suggesting substantial burden in our setting. This detection rate lies between the extremes reported in literature: higher than that observed by Sreeja *et al*., 2013 and lower than some reports by Tamboli *et al*., 2017 [23, 24]. The most frequent site of isolation was urine (46.15%), followed by pus, wound swabs, and blood—reinforcing the well-recognized role of enterococci in urinary tract infections (UTIs) and uropathogen prevalence in hospital settings [21,25]. This pattern likely reflects the organism's colonization of the gastrointestinal and genitourinary tracts, which can act as reservoirs for ascending infections. Species distribution in our isolates showed a predominance of *E. faecalis* (74.03%) over *E. faecium* (25.96%). Numerous prior studies similarly report *E. faecalis* as the more common clinical species, often comprising 80–90% of isolates [21, 22]. However, the proportion of *E. faecium* has been rising in many settings owing to its higher propensity for acquiring resistance determinants. A salient finding in our work was the differential resistance pattern between *E. faecium* and *E. faecalis*. *E. faecium* displayed greater resistance across several antibiotic classes, consistent with prior observations [26, 27]. In our data, resistance to ciprofloxacin and doxycycline was substantial: about 31.1% of *E. faecalis* and 37% of *E. faecium* isolates were non-susceptible to ciprofloxacin, and resistance to doxycycline reached 61.1% in *E. faecalis* and 59.25% in *E. faecium*. These levels echo global surveillance trends that report increasing fluoroquinolone and tetracycline resistance in enterococci. Indeed, a study from Amritsar, Punjab, observed that both *E. faecalis* and *E. faecium* showed maximal resistance to ciprofloxacin [26].

Encouragingly, in our study glycopeptides vancomycin and teicoplanin retained robust in vitro activity. Vancomycin susceptibility was 94.8% for *E. faecalis* and 85.18% for *E. faecium*, while teicoplanin susceptibility was even higher. These findings suggest that glycopeptides remain reliable options for serious enterococcal infections in our setting. However, the looming threat of vancomycin-resistant enterococci (VRE) cannot be ignored. A recent nationwide meta-analysis in India reported a pooled VRE prevalence of 12.4% (95% CI: 8.6–17.5), with *E. faecium* more often VRE than *E. faecalis* [28]. Institutional studies have reported VRE rates ranging from 1.7% to 20% in tertiary care hospitals [26, 27, 29-31]. In one tertiary care centre, vancomycin and linezolid-resistant enterococci have already been documented [32]. Likewise, trends from North India show increasing VRE among enterococcal bacteremia isolates [33]. These data emphasize the need for vigilance in our locale. The emergence of high-level gentamicin resistance (HLGR) further complicates therapy. In our cohort, 61.5% of isolates demonstrated HLGR, which greatly reduces the efficacy of synergistic antibiotic combinations (e.g. aminoglycoside + cell wall-active agent). This pattern is consistent with increasing global and national reports attributed to aminoglycoside modifying enzymes, particularly the bifunctional gene aac(6′)-Ie-aph(2″)-Ia [34, 27]. Identification of HLGR is clinically crucial, as standard synergy is invalid. Such resistance dynamics have important clinical implications. *E. faecalis* often remains susceptible to β-lactams such as ampicillin, whereas *E. faecium* more frequently exhibits intrinsic and acquired resistance, partly due to low-affinity penicillin-binding proteins [35]. Therefore, species-level identification is essential for guiding therapy. The high prevalence of multidrug-resistant (MDR) and VRE strains in tertiary care settings is frequently linked to overuse of broad-spectrum antibiotics, inadequate stewardship, and frequent patient transfers from peripheral centres [36]. These contribute to colonization pressure and facilitate inter-facility spread of resistant clones. Inappropriate use of vancomycin, poor compliance with infection control protocols, and heavy antibiotic selection pressure are recognized drivers of VRE emergence [37].

While our study is limited by the absence of clinical outcome correlation and molecular typing of resistance genes beyond HLGR, it nonetheless provides valuable insight into the local epidemiology of enterococcal resistance. These findings can aid in designing institutional empirical therapy guidelines and strengthening antimicrobial stewardship efforts. Going forward, routine molecular surveillance (e.g. for vanA, vanB genes, additional aminoglycoside resistance genes) should be integrated into microbiology workflows. Continued surveillance, strict infection control, antibiotic stewardship, and context-specific empirical therapy policy are essential to curb the spread of multidrug-resistant enterococci.

5. CONCLUSION

This study highlights the significant prevalence of *Enterococcus* species, particularly *E. faecalis*, as important nosocomial pathogens isolated from various clinical specimens, with a predominance in urinary tract infections. The higher antimicrobial resistance observed in *E. faecium* compared to *E. faecalis* underscores the challenges posed by this species in clinical management. Despite the increasing resistance to commonly used antibiotics such as ciprofloxacin, doxycycline, and aminoglycosides, glycopeptide antibiotics like vancomycin and teicoplanin continue to demonstrate good efficacy against most isolates. However, the emergence of vancomycin-resistant enterococci and high-level gentamicin resistance signals an urgent need for ongoing surveillance and robust antibiotic stewardship programs. Implementation of targeted infection control measures, rational antibiotic use, and routine species-level identification will be critical to curbing the spread of multidrug-resistant enterococci and improving patient outcomes in healthcare settings.

Conflict of interest statement

The authors declare that they have no conflicts of interest.

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