MED-PHOENIX: JOURNAL OF NATIONAL MEDICAL COLLEGE

ORIGINAL ARTICLE

PREVALENCE OF ENTEROCOCCUS SPECIES AND THEIR ANTIBIOGRAM IN A TERTIARY CARE HOSPITAL OF GANDAKI PROVINCE, NEPAL

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Date of Submission : April 4, 2025
Date of Acceptance : April 14, 2025
Date of Publication : August 6, 2025

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Citation:
Pokhrel S, Regmi SM, Ghimire K, Paudel S, Aryal B, Jha RK, Shiwakoti Y, Yadav RK. Prevalence of Enterococcus Species and their Antibiogram in a Tertiary Care Hospital of Gandaki Province, Nepal. Medphoenix. 2025;10(1):21-27.

DOI:https://doi.org/10.3126/medphoenix.v10i1.82625

Conflict of interest: None, ***Funding:*** None

Publisher: *National Medical College Pvt. Ltd.*
MedPhoenix - Journal of National Medical College (JNMC); 2025,10(1), available at www.jnmc.com.np

ISSN:2631-1992 (Online); ISSN:2392-425X (Print)

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ABSTRACT

Introduction: *Enterococcus species, part of the normal intestinal flora, have emerged as significant pathogens causing healthcare-associated infections worldwide. Their intrinsic and acquired resistance to multiple antibiotics, including last-resort agents, poses therapeutic challenges, especially in low-resource settings such as Nepal. However, data on their prevalence and resistance patterns in Gandaki Province are scarce. The aim of the study is to determine the prevalence of Enterococcus species in clinical samples and evaluate their antimicrobial susceptibility patterns in a tertiary care hospital in Gandaki Province, Nepal.*

Materials and methods: *A descriptive cross-sectional study was conducted from October 2024 to March 2025 at the Department of Microbiology, Western Regional Hospital, Pokhara Academy of Health Sciences. Ninety-three Enterococcus isolates were recovered from various clinical specimens. Species identification and antibiotic susceptibility testing were performed using standard microbiological methods and the Kirby-Bauer disk diffusion technique following CLSI guidelines.*

Results: *Enterococcus faecalis was the predominant species (93.5%), followed by E. faecium (6.5%). Both species showed 100% susceptibility to vancomycin. High resistance was noted against penicillin, ciprofloxacin, and ampicillin. E. faecalis exhibited high sensitivity to linezolid, teicoplanin, and nitrofurantoin, while E. faecium showed considerable resistance to penicillin and HLG but remained fully sensitive to vancomycin, linezolid, and nitrofurantoin.*

Conclusion: *E. faecalis is the most common Enterococcus species in clinical samples from Gandaki Province, with notable resistance to commonly used antibiotics. Continuous antimicrobial resistance surveillance and targeted stewardship interventions are essential to optimize treatment and control multidrug-resistant Enterococcus infections. We recommend strengthening antimicrobial stewardship programs and ongoing surveillance to monitor resistance trends and guide effective therapy against Enterococcus infections.*

Keywords: *Antibiotic Resistance, Enterococcus faecalis, Enterococcus faecium, Gandaki Province, Prevalence, Susceptibility*

INTRODUCTION

Enterococci are Gram-positive bacteria that typically occur in pairs or short chains. They are facultative anaerobes, which means they can survive and grow in both the presence and absence of oxygen. These bacteria naturally live in the human gastrointestinal tract as harmless commensals, contributing to the normal microbial flora without causing disease under usual conditions. However, when introduced into hospital settings or immune-compromised patients, *Enterococci* can become opportunistic pathogens responsible for serious and sometimes life-threatening infections. Among the various species of *Enterococcus, Enterococcus faecalis* is the most commonly isolated pathogen and accounts for approximately 85 to 90 percent of enterococcal infections. In contrast, *Enterococcus faecium* is less frequently isolated, causing about 5 to 10 percent of these infections, but it is often associated with higher levels of antibiotic resistance and more severe clinical outcomes.[1]

Current scenario

One of the most significant and growing challenges in clinical microbiology today is the remarkable ability of *Enterococcus* species to develop resistance against multiple antibiotics. This resistance is not limited to commonly prescribed drugs but extends to last-resort agents such as vancomycin and linezolid. These drugs are critical in treating severe infections, especially when other standard treatments have failed or are ineffective. The emergence of resistance to these last-resort agents is particularly alarming, as it severely limits the available therapeutic options for clinicians and poses a serious threat to patient outcomes. The rapid adaptability of *Enterococcus* species in acquiring resistance mechanisms complicates infection control and treatment strategies worldwide. This challenge underscores the urgent need for continuous monitoring and development of novel antimicrobial agents to combat these resilient pathogens.[2] This resistance, especially among *E. faecium*, poses significant treatment challenges.[3] Recent studies from different parts of the world, including Poland[4,5], Nigeria[6], and South Asia[7,8,9,10] have highlighted the high prevalence of multidrug-resistant enterococcal infections. Similar trends have been observed in studies from Nepal, where *E. faecalis* is predominantly isolated, and resistance to commonly used antibiotics is widespread.[11,12,13]

Current options

Antimicrobial resistance (AMR) is becoming an increasingly serious global health threat. In low-income countries like Nepal, the problem is made worse by things like the unregulated use of antibiotics, limited access to proper diagnostic tools, and inadequate infection control practices. Experts warn that by 2050, antimicrobial resistance (AMR) could have a major impact on both global health and the world economy.[11]

Alternative options

With rising resistance to conventional antibiotics, alternative drugs like linezolid, daptomycin, and tigecycline have become important for treating resistant *Enterococcus* infections. However, their high cost and limited availability pose challenges in India and similar settings. Antimicrobial stewardship programs are increasingly emphasized to optimize antibiotic use and curb resistance. These programs focus on appropriate prescribing, infection control, and surveillance of local resistance patterns to guide therapy effectively[27].

Limitations and lacking of previous studies

Due to the scarcity of comprehensive data from Gandaki Province, Nepal, this study specifically aims to fill the existing knowledge gap by evaluating the prevalence of *Enterococcus* species in various clinical samples collected from patients. Understanding how frequently these bacteria occur in infections within this particular geographic region is crucial for effective diagnosis and treatment. Additionally, the study investigates the antimicrobial susceptibility patterns of these isolates to determine how they respond to different antibiotics commonly used in clinical practice. This information is vital for guiding clinicians in selecting appropriate therapies and for developing targeted antimicrobial stewardship programs. By focusing on this under-researched area, the study seeks to provide valuable baseline data that can inform healthcare policies and help curb the spread of antibiotic resistance locally.

Future prospective with aim and objectives

The goal is to help improve treatment decisions and support efforts to control antimicrobial resistance in the region.

This hospital-based study found that *Enterococcus faecalis* was the most common species and showed high resistance to commonly used antibiotics. The findings emphasise the importance of ongoing antimicrobial resistance surveillance and the adoption of focused antibiotic stewardship initiatives to enhance patient care.

MATERIALS AND METHODS

Study Design and Ethical approval

A descriptive cross-sectional study was carried out in the Department of Microbiology at Pokhara Academy of Health Sciences, Pokhara, Nepal. The study spanned October 2024 to March 2025, following ethical clearance from the Institutional Review Committee (Ref: 63/081 dated 1st October 2024).

Sample Size Determination

The required sample size was determined based on a 2019 study that indicated a 40% prevalence of *Enterococcus* species in clinical samples from a tertiary care hospital in Eastern Nepal[23]. The following formula was used:

$$n = (Z^2 \times p \times q) / d^2$$

Where:
n = sample size
Z = 1.96 (for 95% confidence interval)
p = 0.40 (expected prevalence)
q = 1 - p = 0.60
d = 0.10 (margin of error)

Thus,
$$n = (1.96^2 \times 0.40 \times 0.60) / (0.10)^2 = 92.2 \approx 93$$

A total of 93 samples were calculated to be necessary for the study.

Sample collection

Clinical samples, including urine, blood, pus, wound swab, bile, and high vaginal swab, were collected from both inpatient and outpatient departments. Patients of both sexes (female and male) and all age groups were included. Samples from respiratory sites, as well as those that were inadequately labeled, cracked, or transported in damaged containers, were excluded from the study.

Only clinically significant *Enterococcus* isolates were included. Isolates were considered clinically significant if they were obtained from sterile sites (blood), and urine with significant colony count or from specimens with corresponding clinical signs and symptoms of infection, as determined by the treating clinicians and microbiology laboratory criteria. Samples suspected to be contaminants, such as isolates from non-sterile sites without clinical correlation or those regarded as colonizers, were excluded from the analysis.

Culture and Identification

Each sample underwent Gram staining, followed by culture on Blood agar, MacConkey agar, Chocolate agar, and CLED agar. Plates were incubated at 37°C for 18 to 24 hours. Colonies indicative of *Enterococcus* species were initially identified by their Gram-positive cocci appearance (in pairs or short chains), catalase negativity, and colony morphology.

Biochemical Tests for Species Differentiation

Further confirmation of the genus *Enterococcus* involved bile esculin hydrolysis, tolerance to 6.5% NaCl, and growth at 45°C. Species differentiation between *E. faecalis* and *E. faecium* was done through a series of biochemical tests, including hippurate hydrolysis, Voges-Proskauer (VP) test, motility, pigment production, and carbohydrate fermentation profiles involving mannitol, sorbitol, pyruvate, lactose, sucrose.

Antibiotic Susceptibility Testing

The Kirby-Bauer disc diffusion method was employed for antibiotic susceptibility testing in line with Clinical and Laboratory Standards Institute (CLSI) guidelines. The antibiotics tested included:

- Penicillin (10 units)
- Vancomycin (30 µg)
- Teicoplanin (30 µg)
- Linezolid (30 µg)
- Ciprofloxacin (5 µg)
- High-level Gentamicin (120 µg)
- Nitrofurantoin (300 µg)
- Ampicillin (10 µg)
- Chloramphenicol (30 µg)
- Erythromycin (15 µg)

A 0.5 McFarland standard was used to prepare bacterial suspensions, followed by lawn culture on Mueller-Hinton agar. Antibiotic discs (HiMedia, India) were then applied, and plates were incubated at 37°C for 18 to 24 hours. Zones of inhibition were measured using a Vernier caliper. Based on CLSI guidelines, isolates were categorized as sensitive or resistant.

No MIC testing or E-test was conducted. Additionally, no specialized VRE screening methods such as growth on bile esculin azide agar with vancomycin were performed.

Quality Control

For quality control, *Enterococcus faecalis* ATCC 29212 and *Staphylococcus aureus* ATCC 25923 were used as reference strains.

Data Analysis

The gathered data were examined using the Statistical Package for the Social Sciences (SPSS) version 20 for Windows. To analyze parametric variables, the chi-squared test was applied when suitable. A result was considered statistically significant if the *p*-value was less than 0.05.

RESULTS

Age-wise Distribution of Patients with Enterococcal Infections

A total of 93 *Enterococcus* isolates were obtained from various clinical samples. The age group with the highest representation was 20-29 years, comprising 38 patients (40.86%), while the lowest representation was seen in patients less than 1 year of age, with 4 cases (4.30%). Other age groups included 2–14 years (5.38%), 15–19 years (5.38%), 30–39 years (18.28%), 40–49 years (17.20%), 50–59 years (5.38%), and ≥60 years (13.98%). No statistically significant association was found between age groups and *Enterococcus* infection prevalence (chi-square test, $p = 0.37$).

Table 1: Age wise distribution of patients having Enterococcal infections

Age Groups (years)	No. of Patient	Percentage
<1	4	4.30%
2–14	5	5.38%
15–19	5	5.38%
20–29	38	40.86%
30–39	17	18.28%
40–49	16	17.20%
50–59	5	5.38%
≥60	13	13.98%
Total	93	100%

Gender Distribution of Patients with Enterococcal Infections

Of the 93 patients, 55 (59.1%) were female and 38 (40.9%) were male, indicating a female predominance. The difference in gender distribution was statistically significant (chi-square test, $p = 0.04$).

Table 2: Gender Distribution of Patients with Enterococcal Infections

Gender	No. of Patients	Percentage
Female	55	59.1%
Male	38	40.9%
Total	93	100%

Species Distribution According to Clinical Samples

Enterococcus faecalis was the predominant species isolated, with 63 isolates (92.6%) from urine samples, 17 isolates (94.4%) from high vaginal swabs (HVS), 5 isolates (100%) from pus, and 2 isolates (100%) from blood samples. *Enterococcus faecium* was less frequently isolated, accounting for 5 isolates (7.4%) from urine and 1 isolate (5.6%) from HVS; it was not detected in pus or blood samples. A statistically significant difference was observed in species distribution among different sample types (chi-square test, p = 0.02).

Table 3: Species distribution according to clinical species

Samples	No. of *E. faecalis* isolated	No. of *E. faecium* isolated
Urine (n = 68)	63	5
HVS (n = 18)	17	1
Pus (n = 5)	5	0
Blood (n = 2)	2	0
Total (n = 93)	87	6

Antibiotic Susceptibility Patterns Among *E. faecalis* and *E. faecium*

The antibiotic susceptibility testing revealed that *E. faecalis* exhibited high resistance to penicillin (90.8%), ciprofloxacin (87.4%), and ampicillin (92.0%). It remained highly sensitive to vancomycin (100%), linezolid (97.7%), teicoplanin (95.4%), and nitrofurantoin (96.6%). In contrast, *E. faecium* showed complete resistance to penicillin (100%) and high resistance to ciprofloxacin (62.5%) and high-level gentamicin (87.5%). However, all *E. faecium* isolates were fully sensitive to vancomycin, linezolid, and nitrofurantoin (100% each), with good sensitivity to teicoplanin (87.5%) and ampicillin (75.0%). Significant differences in resistance patterns between *E. faecalis* and *E. faecium* were observed for penicillin ($p = 0.01$), ciprofloxacin ($p = 0.03$), and gentamicin ($p = 0.04$), while no significant differences were found for vancomycin and linezolid ($p > 0.05$).

Table 4: Antibiotic susceptibility pattern among *E. faecalis* and *E. faecium*

Antibiotics	*E. faecalis* (n=87) Resistance/Susceptible		*E. faecium* (n=6) Resistance/Susceptible	
	Resistance	Sensitive	Resistance	Sensitive
Penicillin	79 (90.8%)	8 (9.2%)	6 (100%)	0 (0%)
High-level Gentamicin	52 (59.8%)	35 (40.2%)	7 (87.5%)	1 (12.5%)
Teicoplanin	4 (4.6%)	83 (95.4%)	1 (12.5%)	5 (87.5%)
Linezolid	2 (2.3%)	85 (97.7%)	0 (0%)	6 (100%)
Ciprofloxacin	76 (87.4%)	11 (12.6%)	5 (62.5%)	1 (12.5%)
Vancomycin	0 (0%)	87 (100%)	0 (0%)	6 (100%)
Nitrofurantoin	3 (3.4%)	84 (96.6%)	0 (0%)	6 (100%)
Ampicillin	80 (92.0%)	7 (8.0%)	2 (25.0%)	4 (75.0%)

DISCUSSION

This study examined the prevalence, species distribution, and antibiotic susceptibility patterns of *Enterococcus faecalis* and *Enterococcus faecium* in clinical samples from a tertiary care hospital in Gandaki Province, Nepal. Of the 93 isolates, *E. faecalis* was the most common species (93.5%), consistent with previous reports from Eastern Nepal and other South Asian countries.[12,23,24] In contrast, *E. faecium* was less frequently isolated (6.5%), which agrees with studies showing its lower occurrence but higher resistance.[1,4,5,16]

Most isolates came from patients aged 20–29 years, and urine was the most frequent source. This pattern reflects other studies showing that urinary tract infections in young adults are a common form of enterococcal infection.[6,15,17] A female predominance was also noted, especially in high vaginal swab samples, which is expected due to anatomical factors.

In terms of resistance, *E. faecalis* showed high resistance to penicillin (90.8%), ciprofloxacin (87.4%), and norfloxacin (92.0%), similar to patterns reported in West Bengal, Assam, and Nigeria.[6,10,16] However, it was fully sensitive to vancomycin (100%) and remained largely sensitive to linezolid (97.7%), teicoplanin (95.4%), and nitrofurantoin (96.6%).[9,24]

E. faecium showed complete resistance to penicillin (100%) and high resistance to ciprofloxacin (62.5%) and high-level gentamicin (87.5%). Still, all *E. faecium* isolates were 100% sensitive to vancomycin, linezolid, and nitrofurantoin, and mostly sensitive to teicoplanin (87.5%) and norfloxacin (75.0%).[3,4,18,19,20]

These findings show the need for regular monitoring of antibiotic resistance and stress the importance of using local antibiogram to guide treatment. Rising resistance reflects a larger global problem of antimicrobial resistance (AMR), especially in low-income countries like Nepal.[11,22,25] Factors such as misuse of antibiotics, availability without prescriptions, and weak infection control may contribute to this.[14,25]

Biochemical differentiation methods used for species identification are described in the Methods section. Accurate species identification remains important due to differences in resistance and clinical relevance.

This study has several limitations. First, it was confined to a single tertiary hospital, which may limit the applicability of the results to other regions of Nepal. Second, although our study reported 100% susceptibility to vancomycin, this finding is based solely on disk diffusion testing, which has known limitations in detecting vancomycin resistance in enterococci. MIC testing or E-tests provide more accurate susceptibility data. Furthermore, we did not perform specialized VRE screening. Therefore, the true prevalence of VRE in our isolates might be underestimated.

Third, the study period was limited to six months and may not capture seasonal variations in infection rates. Additionally, variability in susceptibility testing methods and the limited antibiotic panel used could influence the interpretation of resistance patterns.

CONCLUSION

In conclusion, *E. faecalis* remains the dominant *Enterococcus* species in clinical samples in Gandaki Province, while *E. faecium*, though less prevalent, shows concerning resistance profiles. These findings highlight the importance of local antimicrobial surveillance and targeted stewardship interventions. Future research should incorporate molecular typing and multicentric approaches to obtain a comprehensive understanding of the epidemiology and resistance mechanisms of *Enterococcus* species in Nepal.

ACKNOWLEDGEMENT

We express our sincere gratitude to all the faculty members, laboratory staffs of the Department of Microbiology, as well as the Institutional Review Committee (IRC) of Pokhara Academy of Health Sciences, Pokhara, for their valuable support and assistance in conducting this research.

Sources of Financial Support

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of Interest Statement

The authors declare no conflict of interest.

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