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Original Research Article

HIGH-LEVEL GENTAMICIN RESISTANCE IN URINARY ENTEROCOCCI: SPECIES-SPECIFIC ANTIMICROBIAL SUSCEPTIBILITY PATTERNS AND IMPLICATIONS FOR ANTIMICROBIAL STEWARDSHIP

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ABSTRACT

Background: Enterococci are important opportunistic pathogens associated with urinary tract infections, particularly in healthcare settings. The emergence of high-level gentamicin resistance (HLGR) presents a therapeutic challenge because it can eliminate the synergistic bactericidal activity of gentamicin when combined with cell-wall-active antibiotics. Understanding species-specific resistance patterns is essential for antimicrobial susceptibility surveillance and rational antibiotic selection. The objective is to determine the frequency of high-level gentamicin resistance among urinary Enterococcus isolates, evaluate species-specific antimicrobial susceptibility patterns, and assess their implications for antimicrobial stewardship. **Materials and Methods:** A laboratory-based, descriptive observational study was conducted at the Department of Microbiology, Jaipur National University Institute for Medical Sciences and Research Centre, Jaipur, Rajasthan, India, from June to November 2024. A total of 205 urinary Enterococcus isolates were examined. Species identification was performed using conventional cultural and biochemical methods. Antimicrobial susceptibility testing was conducted using the Kirby–Bauer disk diffusion method, following Clinical and Laboratory Standards Institute guidelines. HLGR was detected phenotypically using high-content gentamicin disks (120 µg). Data were summarized using frequencies and percentages. **Result:** Among 205 urinary enterococcal isolates, Enterococcus faecalis was the predominant species (52.20%), followed by E. faecium (26.83%), E. avium (7.32%), E. dispar (7.32%), and E. durans (6.34%). Female patients accounted for 57.56% of the isolates. Overall, 146 isolates (71.22%) exhibited HLGR. Species-specific HLGR frequencies were 71.03% in E. faecalis, 70.91% in E. faecium, 73.33% in E. avium, 73.33% in E. dispar, and 69.23% in E. durans. **Conclusion:** A high frequency of HLGR was observed across urinary Enterococcus species, highlighting the potential limitations of gentamicin-based synergistic therapy in selected serious enterococcal infections.

INTRODUCTION

Urinary tract infections (UTIs) remain among the most frequently encountered bacterial infections in clinical practice and represent a significant burden on healthcare systems worldwide. Although Gram-negative organisms, particularly Escherichia coli, account for most UTIs, Gram-positive pathogens such as Enterococcus species have gained increasing clinical importance, especially among hospitalized patients and individuals with underlying medical conditions. Enterococci are facultatively anaerobic, Gram-positive cocci that normally inhabit the human gastrointestinal tract but can act as opportunistic pathogens when host defenses are compromised. They are associated with a broad spectrum of infections, including UTIs, bloodstream infections, intra-abdominal infections, and infective endocarditis. Their ability to persist in hospital environments, colonize urinary catheters, form biofilms, and acquire antimicrobial resistance determinants contributes to their importance as healthcare-associated pathogens.[1,2]

Among the species associated with human infections, Enterococcus faecalis and Enterococcus faecium account for the majority of clinically

significant enterococcal isolates. Although E. faecalis is generally more frequently recovered from urinary specimens, E. faecium presents a particular therapeutic challenge because of its greater tendency to acquire resistance to multiple antimicrobial agents. Differences in antimicrobial susceptibility between these species have important consequences for empirical antibiotic selection, definitive treatment, and infection prevention. E. faecium frequently exhibits resistance to ampicillin and other commonly prescribed antibiotics, whereas E. faecalis generally retains comparatively greater susceptibility to several conventional therapeutic agents. However, the emergence of multidrug-resistant strains in both species has reduced the reliability of conventional treatment approaches and increased the importance of species-level identification and antimicrobial susceptibility testing.[2,3]

A major concern in enterococcal infections is their characteristic resistance to aminoglycosides. Enterococci possess intrinsic low-level resistance to aminoglycosides because these antibiotics penetrate the bacterial cell relatively poorly. Under appropriate circumstances, combining an aminoglycoside with an active cell-wall-targeting agent, such as ampicillin or vancomycin, can enhance aminoglycoside uptake and produce synergistic bactericidal activity. This principle has historically formed the basis of combination therapy for selected serious enterococcal infections. However, the acquisition of high-level gentamicin resistance (HLGR) can eliminate this synergistic effect, thereby limiting an important therapeutic option. HLGR is commonly associated with the bifunctional aminoglycoside-modifying enzyme AAC(6′)-Ie-APH(2″)-Ia, encoded by the aac(6′)-Ie-aph(2″)-Ia gene. This resistance determinant can be carried on mobile genetic elements, facilitating its dissemination among enterococcal populations. Consequently, HLGR has important microbiological and therapeutic implications, particularly when urinary infection is accompanied by bacteremia or other invasive complications.[3,4]

The increasing occurrence of HLGR among clinical enterococcal isolates has been documented in different geographical settings, including India. Das et al. investigated 182 clinical enterococcal isolates in northern India and reported that 39% exhibited high-level gentamicin resistance, with significantly greater resistance among E. faecium isolates than among E. faecalis. Furthermore, the aac(6′)-Ie-aph(2″)-Ia gene was detected in 67 of the 71 HLGR isolates, demonstrating the substantial contribution of this resistance mechanism. The investigators also observed greater resistance to other antimicrobial agents among HLGR isolates, suggesting that high-level gentamicin resistance may coexist with broader multidrug resistance. These findings emphasize the need to evaluate HLGR alongside susceptibility to other clinically relevant antibiotics rather than considering gentamicin resistance as an isolated characteristic.[5]

Studies focusing specifically on urinary enterococci have also demonstrated considerable variation in resistance patterns. Goel et al., in a study of community-acquired enterococcal UTIs in northern India, reported high-level gentamicin resistance in 47.6% of E. faecium isolates compared with 32.7% of E. faecalis isolates. More recently, Bhat et al. analyzed 159 urinary enterococcal isolates from a tertiary care hospital in Karnataka and reported an overall HLGR rate of 59.1%. The study also identified resistance to benzylpenicillin and nitrofurantoin in 30.9% and 23.3% of isolates, respectively. In another investigation involving urine and blood specimens from western Gujarat, Chotaliya et al. documented high-level aminoglycoside resistance in 66.67% of E. faecium isolates and 54.55% of E. faecalis isolates. Collectively, these findings demonstrate that antimicrobial resistance varies across healthcare settings and between enterococcal species, reinforcing the need for updated local surveillance data.[6–8]

Accurate laboratory detection of HLGR is essential for interpreting the potential effectiveness of aminoglycoside-based combination therapy. Routine aminoglycoside susceptibility testing using conventional interpretive criteria is not appropriate for enterococci because intrinsic low-level resistance differs fundamentally from acquired high-level resistance. Dedicated high-level gentamicin testing is therefore necessary when determining the likelihood of synergistic activity with cell-wall-active antimicrobial agents. Khan et al. emphasized the importance of standardized antimicrobial susceptibility testing and appropriate interpretation of resistance phenotypes in enterococci. Species identification, reliable phenotypic testing, and accurate reporting of antimicrobial susceptibility are essential components of microbiological diagnosis. Importantly, the clinical significance of HLGR depends on the infection syndrome; loss of gentamicin synergy is particularly relevant to selected invasive infections and does not independently establish the need for combination therapy in uncomplicated cystitis.[9]

From an antimicrobial stewardship perspective, the emergence of HLGR necessitates a more selective and evidence-based approach to antimicrobial prescribing. Treatment decisions should consider the enterococcal species, site and severity of infection, patient characteristics, and antimicrobial susceptibility profile. For susceptible isolates causing lower UTIs, agents such as amoxicillin or nitrofurantoin may provide appropriate treatment options, while fosfomycin may be considered in selected circumstances according to the organism and available susceptibility evidence. In contrast, complicated or systemic infections may require different antimicrobial strategies based on clinical severity and microbiological findings. Unnecessary

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administration of broad-spectrum or reserve antibiotics should be avoided because inappropriate prescribing can promote further antimicrobial resistance and restrict future therapeutic options.[1,9]

Another important consideration is the distinction between symptomatic UTI and asymptomatic bacteriuria. The isolation of enterococci from urine does not necessarily indicate active infection, particularly among patients with indwelling urinary catheters, older adults, and individuals with underlying urinary tract abnormalities. Treating asymptomatic bacteriuria without an established indication may increase antibiotic exposure without providing clinical benefit. The Infectious Diseases Society of America recommends against treating asymptomatic bacteriuria in most patient populations, with important exceptions including pregnancy and selected invasive urological procedures. Integrating clinical assessment with urine culture results and susceptibility testing is therefore essential to prevent unnecessary antimicrobial use and support responsible antibiotic prescribing.[10]

Despite increasing awareness of antimicrobial resistance among enterococci, substantial variation persists in the reported frequency of HLGR and associated susceptibility patterns. Differences in geographical location, patient populations, healthcare exposure, species distribution, and laboratory methodology may contribute to this variability. Furthermore, aggregate enterococcalantibiograms may conceal clinically important differences between E. faecalis and E. faecium. Continued evaluation of species-specific resistance patterns is therefore necessary to improve local surveillance and guide appropriate antimicrobial selection. Against this background, the present study aims to determine the frequency of high-level gentamicin resistance among enterococci isolated from urine specimens, compare antimicrobial susceptibility profiles across individual enterococcal species, and examine the implications of these resistance patterns for antimicrobial stewardship. The findings are expected to provide locally relevant microbiological evidence to support rational antibiotic selection, improve laboratory-guided treatment decisions, and strengthen institutional antimicrobial resistance surveillance.[6–9]

MATERIALS AND METHODS

A laboratory-based, descriptive observational study was conducted in the Bacteriology Laboratory of the Department of Microbiology, Jaipur National University Institute for Medical Sciences and Research Centre (JNUIMSRC), Jaipur, Rajasthan, India, over six months, from June 2024 to November 2024. The study investigated the distribution of Enterococcus species isolated from urine specimens, their antimicrobial susceptibility profiles, and the occurrence of high-level gentamicin resistance (HLGR).

Clinical urine specimens submitted for routine microbiological examination were obtained from patients attending outpatient departments (OPDs), inpatient departments (IPDs), and intensive care units (ICUs) across different clinical specialties. The microbiological investigation involved conventional culture methods, phenotypic species identification, antimicrobial susceptibility testing, and high-level aminoglycoside resistance screening.

The primary outcome was the proportion of urinary enterococcal isolates exhibiting HLGR. Secondary outcomes included the distribution of individual Enterococcus species, species-specific antimicrobial susceptibility profiles, and differences in HLGR occurrence across the identified species. The study also examined resistance patterns relevant to laboratory-informed antimicrobial stewardship. No antimicrobial prescribing intervention or clinical treatment outcome was evaluated.

Study Population and Sample Size: The study population comprised patients of all age groups whose urine specimens were submitted for bacteriological investigation of suspected urinary tract infection to the Department of Microbiology, Jaipur National University Institute for Medical Sciences and Research Centre, Jaipur, Rajasthan, India, between June and November 2024. Specimens were received from the outpatient departments, inpatient wards, and intensive care units of different clinical specialties. The analytical population consisted of urinary isolates identified as Enterococcus species using the conventional microbiological procedures described in the study protocol.

The minimum sample size was estimated using the single-population proportion formula, assuming a 95% confidence level, a 5% absolute margin of error, and an anticipated proportion of enterococcal isolates of 10.62% among culture-positive urine specimens, as reported.

$n = Z^2\alpha/2 \times p(1 - p) / d^2$

$n = (1.96)^2 \times 0.1062 \times (1 - 0.1062) / (0.05)^2$

$n = 145.86 \approx 146$ isolates

Here, n denotes the required sample size; Zα/2 is the standard normal value of 1.96 at the 95% confidence level; p is the expected proportion (0.1062); and d is the prespecified absolute precision (0.05). Although the calculated minimum sample size was 146 isolates, the final study dataset comprised 205 urinary Enterococcus isolates. All 205 isolates were included in the descriptive microbiological analysis and constituted the denominator for calculating the overall frequency of high-level gentamicin resistance.

Inclusion and exclusion Criteria: Urine specimens from patients of all age groups, sexes, and clinical departments were considered eligible for inclusion. According to the original study protocol, eligible specimens were required to demonstrate a bacterial count of at least \(10^5\) colony-forming units per

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millilitre (CFU/mL), together with clinical evidence of one or more symptoms suggestive of urinary tract infection.

Specimens originating from non-urinary anatomical sites, including pus, sputum, cerebrospinal fluid, and stool, were excluded. The original protocol also excluded urine cultures yielding bacterial counts below \(10^4\) CFU/mL. The handling of specimens with bacterial counts between \(10^4\) and \(10^5\) CFU/mL was not specified in the thesis and requires clarification.

The analysis was restricted to isolates identified as belonging to the genus Enterococcus. Whether repeated isolates from the same patient were excluded could not be established from the methodology and should be verified using the original laboratory records.

Urine Specimen Collection and Processing: Urine specimens were collected using the clean-catch midstream technique in sterile, wide-mouthed, leak-proof containers. Male patients were instructed to provide early morning midstream urine samples. Female patients were instructed to separate the labia and discard the initial portion of urine before collecting the midstream specimen.

Following receipt in the bacteriology laboratory, specimens were subjected to macroscopic, microscopic, and cultural examination. Macroscopic assessment included evaluation of urine colour, clarity, turbidity, and the presence of visible blood.

Microscopic examination was performed using wet-mount preparations and Gram-stained smears. For wet-mount examination, approximately 50 µL of uncentrifuged urine was placed on a clean glass slide and covered with a coverslip. The preparation was examined microscopically for leukocytes, microorganisms, and other cellular elements. Gram-stained smears prepared from urine sediment were examined to assess bacterial morphology and Gram reaction.

Isolation and Culture of Enterococci: Urine specimens underwent semiquantitative bacteriological culture using a calibrated-loop approach to estimate bacterial growth in CFU/mL. The culture media included HiChrome urinary tract infection (UTI) agar, blood agar, and MacConkey agar. Inoculated media were incubated aerobically at approximately 37°C for 24 hours, as described in the laboratory workflow.

Presumptive enterococcal colonies were selected based on their morphological characteristics. On HiChrome UTI agar, enterococci produced small, round, convex colonies with blue coloration. On blood agar, colonies were typically small, smooth, greyish-white, and predominantly non-haemolytic. Growth on MacConkey agar was characterized by small, smooth, pinkish colonies.

Suspected colonies were subjected to Gram staining and further biochemical identification. The presence of Gram-positive cocci arranged predominantly in pairs or short chains supported presumptive identification as enterococci. Final genus and species identification relied on the phenotypic characteristics described below.

Phenotypic Identification and Speciation: Presumptive Enterococcus isolates were identified using conventional microbiological and biochemical procedures. Genus-level characterization included Gram staining, catalase reaction, bile esculin hydrolysis, and the ability to grow in broth containing 6.5% sodium chloride.

Catalase testing was performed using 3% hydrogen peroxide. The absence of immediate bubble formation was interpreted as a negative reaction. Bile esculin hydrolysis was assessed by observing blackening of the medium following incubation. Salt tolerance was evaluated by detecting bacterial growth in the presence of 6.5% sodium chloride.

Further species-level differentiation was performed using a combination of arginine dihydrolase activity, Voges–Proskauer reaction, mannitol motility testing, and carbohydrate utilization profiles. Fermentation reactions involving lactose, sucrose, mannitol, arabinose, raffinose, and pyruvate were used in the biochemical identification algorithm.

Species assignments were based on the combined biochemical characteristics rather than an individual reaction. The study identified five species: E. faecalis, E. faecium, E. avium, E. dispar, and E. durans.

The original investigation used phenotypic identification without documented confirmation by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), polymerase chain reaction, or sequencing. Accordingly, the identification method is described as conventional phenotypic speciation rather than molecularly confirmed identification.

Antimicrobial Susceptibility Testing: Antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion method on Mueller–Hinton agar. The procedure followed the interpretive criteria described in the Clinical and Laboratory Standards Institute (CLSI) M100 guidelines, 34th edition, published in 2024.

For preparation of the bacterial inoculum, approximately four to five well-isolated colonies from a fresh culture were suspended in 5 mL of peptone water and incubated at 37°C for 3–4 hours. The turbidity of the resulting bacterial suspension was adjusted to the equivalent of a 0.5 McFarland standard.

A sterile cotton swab was immersed in the standardized inoculum, and excess suspension was removed by pressing the swab against the inner wall of the tube. The inoculum was evenly distributed over the entire surface of Mueller–Hinton agar by swabbing in three directions, rotating the plate approximately 60° between applications. The agar surface was allowed to dry before antimicrobial disks were applied.

Antimicrobial disks were placed on the inoculated agar using sterile forceps or a disk dispenser. Plates were incubated at approximately 35°C for 16–18

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hours. Following incubation, the diameters of growth-inhibition zones were measured in millimetres and interpreted using the applicable CLSI criteria.

The antimicrobial panel comprised ampicillin (10 µg), penicillin (10 µg), vancomycin (30 µg), teicoplanin (30 µg), linezolid (30 µg), doxycycline (30 µg), tetracycline (30 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), nitrofurantoin (300 µg), and fosfomycin (200 µg), together with high-content gentamicin (120 µg) and streptomycin (300 µg) disks.

The interpretation of individual antimicrobial results was dependent on the organism–antimicrobial combination and the availability of valid breakpoints. Urine-specific agents were considered separately from agents applicable to systemic infections. Fosfomycin susceptibility was reported for E. faecalis, therefore, its susceptibility proportions should be presented using the corresponding species-specific denominator rather than the entire isolate population.

Phenotypic Detection of High-Level Gentamicin Resistance: All enterococcal isolates were screened for high-level gentamicin resistance using the disk diffusion method with a 120 µg gentamicin disk on Mueller–Hinton agar. The bacterial inoculum was standardized to 0.5 McFarland turbidity and distributed uniformly across the agar surface. Following placement of the high-content gentamicin disk, the plates were incubated at approximately 37°C for 16–18 hours.

The inhibition zones were interpreted according to the high-level aminoglycoside resistance screening criteria described by CLSI and supported by the multicentre methodological evaluation conducted.

The following interpretive criteria were applied.

Zone diameter	Interpretation
≤6 mm	High-level gentamicin resistant
7–9 mm	Inconclusive; confirmatory testing required
≥10 mm	No high-level gentamicin resistance detected

Isolates meeting the resistant criterion were classified as HLGR-positive. The significance of this phenotype relates principally to the potential loss of synergistic bactericidal activity between gentamicin and an appropriate cell-wall-active antimicrobial agent.

The study additionally evaluated high-level streptomycin resistance using 300 µg disks, permitting the assessment of resistance to another aminoglycoside. HLGR remained the primary outcome of the investigation.

The original study relied on phenotypic disk diffusion screening. Minimum inhibitory concentration (MIC) testing and molecular detection of aminoglycoside resistance genes were not documented. Therefore, resistance mechanisms were not directly characterized, and no molecular confirmation is claimed.

Data Collection and Study Variables: Demographic and microbiological information was documented using the structured study proforma and laboratory records. The available variables included patient age, sex, clinical department, inpatient or outpatient status, species identification, and antimicrobial susceptibility results.

The primary outcome variable was HLGR status, categorized according to the high-content gentamicin screening result. Secondary microbiological variables included susceptibility to the other tested antimicrobials and high-level streptomycin resistance.

Species-specific distributions were considered for E. faecalis, E. faecium, E. avium, E. dispar, and E. durans. The proportion of HLGR isolates for each species was determined using the number of isolates belonging to that species as the denominator.

Demographic and departmental distributions were summarized to describe the patient population from which the urinary isolates originated. Because the original dataset did not establish clinical outcomes, individual antibiotic exposure, catheterization status, or antibiotic prescription patterns, these variables were not included as measured outcomes.

Statistical Analysis: The specified descriptive statistical analysis using Microsoft Excel and IBM SPSS software. Categorical variables, including sex, clinical department, Enterococcus species, antimicrobial susceptibility, and HLGR status, were summarized using frequencies and percentages. Quantitative variables, where available, were intended to be summarized using appropriate measures of central tendency and dispersion.

RESULTS

During June–November 2024, 205 Enterococcus isolates recovered from urine specimens were included in the reported analysis. Of these, 146 (71.22%) had high-level gentamicin resistance (HLGR), while 59 (28.78%) were negative by the high-level gentamicin screening assay.

Table 1: Demographic distribution of patients with urinary Enterococcus isolates (N = 205)

Characteristic	n	%
Age group (years)		
0–10	21	10.24
11–20	27	13.17
21–30	28	13.66
31–40	27	13.17
41–50	39	19.02
51–60	35	17.07

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>60	28	13.66
Age total	205	100.00
Sex		
Male	87	42.44
Female	118	57.56
Sex total	205	100.00

The largest age category was 41–50 years (39/205, 19.02%), followed by 51–60 years (35/205, 17.07%). Female patients contributed 118 isolates (57.56%), compared with 87 (42.44%) from male patients. These percentages describe the distribution within the sampled isolates; they are not population-based UTI incidence estimates.

Table 2: Clinical department distribution of urinary Enterococcus isolates (N = 205)

Department	n	%
General Medicine	73	35.61
General Surgery	27	13.17
Obstetrics and Gynaecology	38	18.54
Emergency Medicine	31	15.12
Paediatrics	21	10.24
Respiratory Medicine	11	5.37
Orthopaedics	2	0.98
Ear, Nose and Throat	2	0.98
Total	205	100.00

General Medicine accounted for the largest proportion of isolates (73/205, 35.61%), followed by Obstetrics and Gynaecology (38/205, 18.54%) and Emergency Medicine (31/205, 15.12%). Together, these three departments accounted for 142 of 205 isolates (69.27%).

Table 3: Distribution of Enterococcus species isolated from urine (N = 205)

Species	n	%
E. faecalis	107	52.20
E. faecium	55	26.83
E. avium	15	7.32
E. dispar	15	7.32
E. durans	13	6.34
Total	205	100.00

Enterococcus faecalis was the predominant species (107/205, 52.20%), followed by E. faecium (55/205, 26.83%). The remaining 43 isolates (20.98%) were identified as E. avium, E. dispar, or E. durans. Species identification was based on phenotypic methods reported.

Table 4: Reported antimicrobial susceptibility and high-level aminoglycoside screening results

Agent / screening phenotype	Reported S / screen-negative (n)	Tested N	%
Ampicillin	136	205	66.34
Doxycycline	56	205	27.32
Ciprofloxacin	17	205	8.29
Nitrofurantoin	192	205	93.66
Fosfomycin	62	107	57.94
Vancomycin	205	205	100.00
Teicoplanin	205	205	100.00
Linezolid	205	205	100.00
Penicillin*	135	205	65.85
Tetracycline	27	205	13.17
Levofloxacin	17	205	8.29
High-level gentamicin screen negative	59	205	28.78
High-level streptomycin screen negative	149	205	72.68

Note. S, susceptible as reported. High-level gentamicin/streptomycin results denote absence of high-level resistance on screening, not ordinary systemic susceptibility. Fosfomycin was reported only for E. faecalis (62/107).

All 205 isolates were reported susceptible to vancomycin, teicoplanin and linezolid. Nitrofurantoin susceptibility was reported for 192/205 isolates (93.66%), whereas ciprofloxacin and levofloxacin were each reported susceptible for 17/205 (8.29%). Fosfomycin susceptibility was reported for 62/107 E. faecalis isolates (57.94%). High-level gentamicin screening was negative in 59/205 isolates (28.78%); therefore, 146/205 (71.22%) met the HLGR definition.

Species-specific reported susceptibility counts are shown in [Table 5]. Values are presented as n (%) with each species as its denominator; the absence of fosfomycin testing for the remaining species is explicitly indicated.

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Table 5: Species-specific susceptible counts and high-level aminoglycoside screening phenotypes: n (%)

Antimicrobial	E. faecalis(n=107)	E. faecium(n=55)	E. avium(n=15)	E. dispar(n=15)	E. durans(n=13)
Ampicillin	72 (67.3)	41 (74.5)	3 (20.0)	12 (80.0)	8 (61.5)
Doxycycline	27 (25.2)	16 (29.1)	6 (40.0)	4 (26.7)	3 (23.1)
Ciprofloxacin	8 (7.5)	7 (12.7)	2 (13.3)	0 (0.0)	0 (0.0)
Nitrofurantoin	101 (94.4)	52 (94.5)	13 (86.7)	15 (100.0)	11 (84.6)
Fosfomycin	62 (57.9)	Not tested	Not tested	Not tested	Not tested
Vancomycin	107 (100.0)	55 (100.0)	15 (100.0)	15 (100.0)	13 (100.0)
Teicoplanin	107 (100.0)	55 (100.0)	15 (100.0)	15 (100.0)	13 (100.0)
Linezolid	107 (100.0)	55 (100.0)	15 (100.0)	15 (100.0)	13 (100.0)
Penicillin*	71 (66.4)	39 (70.9)	5 (33.3)	8 (53.3)	9 (69.2)
Tetracycline	14 (13.1)	9 (16.4)	1 (6.7)	2 (13.3)	1 (7.7)
Levofloxacin	7 (6.5)	7 (12.7)	2 (13.3)	0 (0.0)	1 (7.7)
High-level gentamicin screen negative	31 (29.0)	16 (29.1)	4 (26.7)	4 (26.7)	4 (30.8)
High-level streptomycin screen negative	81 (75.7)	33 (60.0)	13 (86.7)	13 (86.7)	9 (69.2)

Note. Columns use species-specific denominators. “Not tested” indicates no value was reported.

Screening-negative HLG/HLS does not constitute conventional therapeutic susceptibility.

Table 6: Frequency of high-level gentamicin resistance among urinary Enterococcus species (N = 205)

Species	Total n	HLGR n (%)	No HLGR n (%)
E. faecalis	107	76 (71.03)	31 (28.97)
E. faecium	55	39 (70.91)	16 (29.09)
E. avium	15	11 (73.33)	4 (26.67)
E. dispar	15	11 (73.33)	4 (26.67)
E. durans	13	9 (69.23)	4 (30.77)
Total	205	146 (71.22)	59 (28.78)

Note. HLGR-positive counts were obtained from data. HLGR-negative counts and percentages were calculated as total isolates minus HLGR-positive isolates.

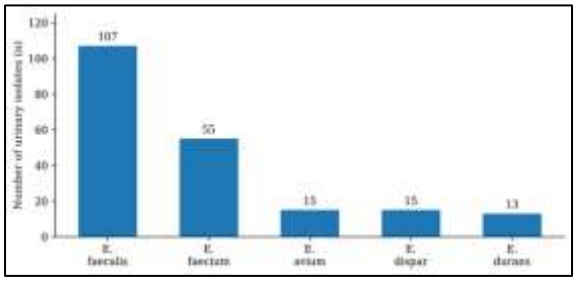


Figure 1: Species distribution among 205 urinary Enterococcus isolates. Bars show the number of isolates identified as E. faecalis, E. faecium, E. avium, E. dispar or E. durans; values above bars indicate the corresponding counts.

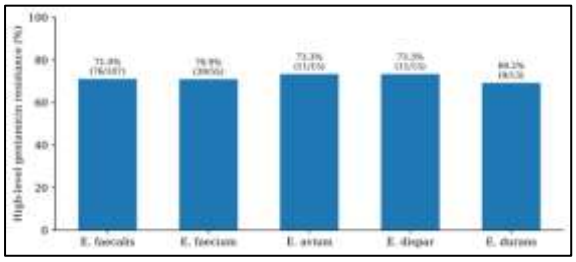


Figure 2: Species-specific high-level gentamicin resistance in urinary Enterococcus isolates. Numbers above bars show resistant isolates divided by the total for each species.

Of the 146 HLGR-positive isolates, 76 were E. faecalis, 39 E. faecium, 11 E. avium, 11 E. dispar and nine E. durans. Species-specific HLGR proportions ranged from 69.23% in E. durans to

73.33% in E. avium and E. dispar. These proportions are descriptive; no statistically supported interspecies difference can be claimed from the data as supplied.

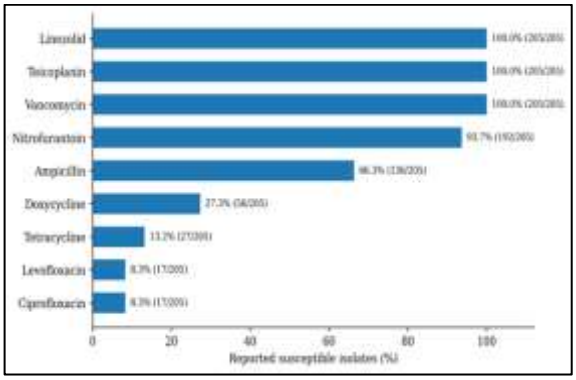


Figure 3: Reported antimicrobial susceptibility among urinary Enterococcus isolates. Horizontal bars show the proportion of 205 isolates recorded as susceptible to each displayed agent. Fosfomycin was excluded because the thesis only reports data for E. faecalis; penicillin was excluded because its pooled and species-level totals disagree.

DISCUSSION

The present study investigated the species distribution, antimicrobial susceptibility profiles, and high-level gentamicin resistance (HLGR) among 205 urinary Enterococcus isolates obtained at a tertiary care hospital in Jaipur, Rajasthan, India, between June and November 2024. The principal finding was the high frequency of HLGR, detected

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in 146 (71.22%) isolates. Enterococcus faecalis was the predominant species (52.20%), followed by E. faecium (26.83%). Although all isolates were reported susceptible to vancomycin, teicoplanin, and linezolid, substantial resistance was observed to fluoroquinolones and tetracyclines. These findings highlight the importance of species-level identification and high-level aminoglycoside resistance screening in routine microbiological surveillance. They also emphasize the need for locally generated susceptibility data to support evidence-based antimicrobial stewardship, particularly given the variations in resistance patterns reported by Pradhan et al,.[11] and Gandham et al,.[14]

The age distribution demonstrated that the largest proportion of isolates originated from patients aged 41–50 years (19.02%), followed by those aged 51–60 years (17.07%). These findings are broadly comparable with observations reported by Gandham et al. [14], who investigated 320 urinary enterococcal isolates in Pune, India, and found that patients aged 41–60 years contributed the largest proportion (34.1%). Similarly, Mahajan et al,.[16] reported a predominance of enterococcal isolates in the 41–50-year age group in their investigation of clinical specimens. The concentration of isolates among middle-aged and older patients may reflect differences in healthcare utilization, underlying medical conditions, previous antimicrobial exposure, or urinary tract abnormalities. However, these potential explanations were not directly examined in the present investigation. Therefore, the observed age distribution should be interpreted as a characteristic of the sampled population rather than evidence of increased age-specific infection risk.[14,16]

Regarding sex distribution, female patients accounted for 118 (57.56%) isolates, whereas 87 (42.44%) isolates were obtained from male patients. This pattern is comparable with the investigation by Bhat et al,.[7] which also demonstrated a female predominance among patients with urinary enterococcal isolates. In contrast, Mahajan et al,.[16] reported a male predominance in their clinical isolate population, indicating that sex distributions may differ across institutions and study populations. Anatomical and physiological differences between males and females, together with underlying urinary disorders, may influence the epidemiology of urinary infections. Nevertheless, differences in specimen submission rates and clinical characteristics may also contribute to the observed distribution. Because the present study did not include the total numbers of male and female patients undergoing urine culture, sex-specific infection rates could not be determined.[7,16]

Departmental analysis revealed that General Medicine contributed the highest number of isolates (35.61%), followed by Obstetrics and Gynaecology (18.54%) and Emergency Medicine (15.12%). The predominance of isolates from General Medicine was comparable with Gandham et al,.[14]who reported that 34.7% of urinary enterococcal isolates originated from the Medicine Department. Sengupta et al. [20] also identified the Medicine Department as the leading contributor to enterococcal isolates in a tertiary care hospital in eastern India. Patients admitted to medical departments may have conditions requiring repeated hospitalization, prolonged antimicrobial treatment, or urinary instrumentation, potentially increasing their exposure to resistant organisms. However, as individual hospitalization duration, catheterization status, and previous antibiotic exposure were not systematically documented in the present study, these factors cannot be established as explanations for the departmental distribution.[14,20]

Species identification demonstrated the predominance of E. faecalis (107/205; 52.20%), followed by E. faecium (55/205; 26.83%). These findings are consistent with the general distribution reported in several investigations of urinary enterococci. Bhat et al,.[15] in a study of 75 urinary isolates, identified E. faecalis in 57.3% and E. faecium in 16.0%. Similarly, Parameswarappa et al,.[18] reported E. faecalis and E. faecium frequencies of 63.3% and 36.7%, respectively, among 150 urinary enterococcal isolates. The present investigation additionally identified E. avium (7.32%), E. dispar (7.32%), and E. durans (6.34%). Bhat et al,.[15] also documented less frequently encountered species, including E. dispar and E. durans, indicating that the urinary enterococcal population may extend beyond the two predominant species. Nevertheless, conventional biochemical identification can be challenging for uncommon enterococcal species, and confirmatory identification using MALDI-TOF mass spectrometry or molecular methods would strengthen the reliability of these findings.[15,18]

The overall antimicrobial susceptibility results demonstrated complete reported susceptibility to vancomycin, teicoplanin, and linezolid among all 205 isolates. This finding corresponds with Bhat et al,.[7]who observed 100% susceptibility to these three antimicrobial agents among 159 urinary enterococcal isolates in Karnataka. However, the absence of detected resistance in the current sample should not be interpreted as evidence that resistance is absent from the wider patient population. Sengupta et al,.[20] reported vancomycin-resistant enterococci in 6.47% of 371 clinical isolates, together with two linezolid-resistant isolates. Salem-Bekhit et al,.[19] also documented vancomycin resistance in 3.9% of enterococcal isolates collected from clinical specimens in Saudi Arabia. These differences underline the importance of continued susceptibility surveillance and appropriate confirmatory testing when glycopeptide or oxazolidinone resistance is suspected.[7,19,20]

Nitrofurantoin demonstrated a high reported susceptibility proportion of 93.66% (192/205), suggesting that it may remain a useful option for

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appropriately selected lower urinary tract infections caused by susceptible enterococci. This finding is more favourable than that of Bhat et al,.[7] who documented nitrofurantoin resistance in 23.3% of urinary enterococcal isolates. The difference may reflect variations in geographical location, species distribution, patient population, or antimicrobial exposure. However, nitrofurantoin achieves therapeutically relevant concentrations primarily in urine and should not be used for suspected pyelonephritis or systemic enterococcal infection. Its clinical use must therefore be based on the infection site, renal function, patient characteristics, and confirmed susceptibility. The relatively favourable in vitro findings in the present study support its consideration for antimicrobial stewardship but do not establish clinical treatment effectiveness, as patient outcomes were not evaluated.[7,11]

In contrast, susceptibility to ciprofloxacin and levofloxacin was markedly limited, with only 17 (8.29%) isolates reported susceptible to each antimicrobial agent. These findings are consistent with Gandham et al,.[14] who reported ciprofloxacin resistance in 96.8% of HLGR enterococcal isolates. The low fluoroquinolone susceptibility observed in the present study suggests that these agents would be unreliable for empirical treatment of suspected enterococcal UTI in this setting. Moreover, doxycycline and tetracycline demonstrated reported susceptibility proportions of 27.32% and 13.17%, respectively. Such findings highlight the importance of avoiding empirical antimicrobial selection based solely on traditional prescribing practices without considering local resistance data. Nevertheless, the absence of information on prior antimicrobial exposure prevents conclusions regarding the factors responsible for these resistance patterns.[14,21]

An important observation was the species-specific variation in beta-lactam susceptibility. Ampicillin susceptibility was reported in 67.29% of E. faecalis and 74.55% of E. faecium isolates, while only 20.00% of E. avium isolates were susceptible. The relatively high ampicillin susceptibility reported among E. faecium isolates deserves careful examination because this species frequently exhibits resistance to beta-lactam antibiotics in hospital-associated populations. For example, Kraszewska et al,.[21] in a six-year analysis of urinary enterococci from hospitalized patients in Poland, reported beta-lactam resistance in 99.3% of E. faecium isolates. Differences in regional epidemiology and isolate populations may explain some variation; however, species identification, antimicrobial disk interpretation, and susceptibility records should be rechecked before drawing conclusions regarding the unusual susceptibility pattern observed in the current study.[21]

The most clinically significant finding of the present investigation was the high frequency of HLGR, identified in 146 (71.22%) urinary enterococcal isolates. This proportion was slightly higher than the 68.4% reported by Gandham et al,.[14] and considerably higher than the 59.1% reported by Bhat et al,.[7] Pradhan et al,.[11] observed HLGR in 41.0% of 61 urinary enterococcal isolates, further demonstrating the substantial variation between healthcare settings. Parameswarappa et al,.[18] also reported a high frequency of high-level aminoglycoside resistance among urinary enterococci, although their combined HLAR measurement should not be considered directly equivalent to gentamicin-specific resistance. Such differences may be influenced by antimicrobial consumption, infection-control practices, patient characteristics, and institutional microbial ecology. However, the present cross-sectional laboratory investigation was not designed to identify the determinants of HLGR or establish whether resistance increased over time.[7,11,14,18]

Species-wise analysis revealed remarkably similar HLGR frequencies among the identified enterococci. HLGR was detected in 71.03% of E. faecalis, 70.91% of E. faecium, 73.33% of E. avium, 73.33% of E. dispar, and 69.23% of E. durans isolates. The close similarity between E. faecalis and E. faecium contrasts with Goel et al,.[6] who reported greater HLGR among E. faecium isolates (47.6%) than among E. faecalis isolates (32.7%). The findings suggest that high-level gentamicin resistance was widely represented across the phenotypically identified enterococcal species in the present sample. Nevertheless, the relatively small number of isolates belonging to less common species limits the precision of these estimates. Furthermore, without inferential statistical testing, the observed differences cannot be interpreted as statistically significant.

The clinical importance of HLGR lies in its ability to compromise the synergistic bactericidal activity of gentamicin when administered with an active cell-wall-targeting antimicrobial agent. Enterococci exhibit intrinsic low-level aminoglycoside resistance due to restricted cellular uptake, whereas acquired high-level resistance is commonly mediated by aminoglycoside-modifying enzymes. Diab et al,.[17] demonstrated the presence of the aac(6′)-Ie-aph(2″)-Ia resistance determinant in 66.7% of their HLGR isolates, supporting the importance of this mechanism in clinical enterococcal resistance. However, the current study employed phenotypic screening without molecular characterization. Therefore, the presence of specific aminoglycoside resistance genes cannot be inferred from the observed HLGR phenotype. Furthermore, the implications of lost gentamicin synergy are primarily relevant to selected serious enterococcal infections; HLGR does not independently establish the need for combination therapy in uncomplicated urinary tract infections.[13,17]

In addition to gentamicin resistance, high-level streptomycin screening showed that 149 (72.68%) isolates were negative for high-level streptomycin resistance, corresponding to 56 (27.32%) isolates

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with reported high-level streptomycin resistance. This difference suggests that resistance to gentamicin and streptomycin was not uniformly distributed in the investigated population. Because these resistance phenotypes may involve different mechanisms, separate testing is important when aminoglycoside synergy is clinically relevant. Swenson et al,.[13] demonstrated the usefulness of standardized high-content aminoglycoside disk screening while emphasizing the importance of confirmatory testing for inconclusive results. The current study did not report the number of isolates requiring confirmatory testing or the overlap between gentamicin and streptomycin resistance. Therefore, the prevalence of combined high-level aminoglycoside resistance could not be established from the available summary data.[12,13]

From an antimicrobial stewardship perspective, the findings support routine species-level identification, reliable susceptibility testing, and timely communication of clinically significant resistance results to treating clinicians. The high frequency of HLGR indicates that gentamicin-containing synergistic regimens should not be selected without appropriate microbiological assessment when such treatment is clinically indicated. Conversely, the high reported susceptibility to nitrofurantoin and selected cell-wall-active antibiotics suggests opportunities to preserve broader-spectrum or reserve agents through culture-directed treatment. The reported complete susceptibility to vancomycin, teicoplanin, and linezolid should not encourage their indiscriminate use for uncomplicated urinary infections. Antimicrobial selection must also consider symptoms, the distinction between asymptomatic bacteriuria and infection, infection severity, and the anatomical site involved. These principles are consistent with recommendations for improving antimicrobial prescribing through laboratory-supported decision-making.[10–12]

Several limitations should be acknowledged. First, the study was conducted at a single tertiary care centre over six months, which may limit its generalizability to other institutions and regions. Second, species identification relied on conventional phenotypic methods without molecular or MALDI-TOF confirmation, potentially affecting the identification accuracy of uncommon enterococcal species. Third, resistance was assessed phenotypically, and the genetic determinants responsible for HLGR were not investigated. Fourth, the available thesis data did not provide detailed information regarding prior antimicrobial exposure, catheter use, comorbidities, repeated isolates, treatment outcomes, or the differentiation of complicated and uncomplicated urinary infections. Fifth, some reported aggregate susceptibility figures require reconciliation with the species-specific data, particularly for penicillin. Finally, the investigation did not include a documented comparative inferential analysis or patient-level data sufficient to establish relationships between HLGR and other resistance phenotypes. These limitations should be considered when interpreting the findings and planning future investigations.

Despite these limitations, the study provides useful institution-specific evidence regarding the distribution of urinary enterococci and their resistance patterns. Its major contribution is the demonstration of a substantial HLGR burden across both predominant and less frequently isolated enterococcal species. The findings support strengthening routine laboratory resistance surveillance, incorporating appropriately interpreted HLGR screening into diagnostic workflows, and updating local antibiograms to guide rational antimicrobial selection. Future multicentre investigations incorporating molecular resistance characterization, patient-level clinical information, and longitudinal surveillance would provide a more comprehensive understanding of the epidemiology, clinical significance, and stewardship implications of high-level gentamicin-resistant urinary enterococci.

CONCLUSION

The present study highlights the substantial burden of high-level gentamicin resistance (HLGR) among urinary Enterococcus isolates in a tertiary care hospital in Jaipur, Rajasthan. Among 205 enterococcal isolates, 146 (71.22%) exhibited HLGR, indicating a considerable prevalence of this resistance phenotype in the study population. Enterococcus faecalis was the predominant species (52.20%), followed by Enterococcus faecium (26.83%). High-level gentamicin resistance was observed across all five identified species, with relatively similar resistance proportions, suggesting that this resistance phenotype was not restricted to the two predominant enterococcal species.

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