RESEARCH PAPER

Burden Of High-Level Resistance To Aminoglycosides And Vancomycin In Clinical Isolates Of Enterococci

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ABSTRACT

Background:

Enterococci resistant to vancomycin and high-level aminoglycoside drugs are an emerging problem in hospital settings. This study aimed to determine the antibiotic susceptibility of enterococci isolated from various clinical samples with reference to high level aminoglycoside (HLAR) and vancomycin.

Methods:

High-level gentamycin (HLG) and High-level streptomycin (HLS) and Vancomycin Resistant Enterococci (VRE) were determined by disc diffusion method and confirmed by automated technique VITEK 2 according to the Clinical and Laboratory Standards Institute (CLSI) guideline.

Result:

A total of 7952 clinical samples were analysed, from which 167 Enterococcus isolates were recovered. Among these isolates, 76.05% originated from inpatients, while 23.95% were from outpatients. Urine specimens constituted the majority of the samples (77.25%) and showed the highest levels of antimicrobial resistance. Overall, resistance rates among the Enterococcus isolates were notably high, with 67.07% exhibiting resistance to high-level gentamicin (HLG), 61.68% to high-level streptomycin (HLS), and 37.72% to vancomycin (VA). When stratified by patient setting, isolates from inpatients demonstrated significantly higher resistance rates compared to outpatients: HLG resistance was 72.44% versus 50%, HLS resistance was 53.54% versus 50%, and vancomycin resistance was 43.3% versus 22.5%, respectively. Gender-based analysis revealed that female patients carried a greater number of Enterococcus isolates and showed higher resistance to high-level aminoglycosides (HLAR). Conversely, male patients exhibited a higher prevalence of vancomycin-resistant isolates, with a resistance rate of 48.44%. The age group 41–60 years showed the highest overall resistance rates among all age categories. Among hospital wards, the General Medicine and Obstetrics & Gynaecology departments accounted for the highest number of resistant Enterococcus isolates, indicating potential hotspots for antimicrobial resistance within the healthcare setting.

Conclusion:

Enterococcus infections are prevalent among hospitalized patients, particularly females aged 21–40 years. The isolates exhibited high resistance to key antibiotics, including gentamicin, streptomycin, and vancomycin. These findings highlight the need for ongoing surveillance, stringent infection control measures, and prudent antibiotic use to control the spread of multidrug-resistant Enterococcus in healthcare settings.

Keywords: High-Level Resistance, Aminoglycosides, Vancomycin, Clinical Isolates, Enterococci

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Introduction

Enterococci are Gram-positive bacteria commonly found in nature. They primarily reside on the mucous membranes of humans and animals but can also be present in environments such as water, soil, dairy products, and plants(1). Under certain conditions, these bacteria can cause various infections. They are recognized as a major cause of hospital-acquired (nosocomial) infections, particularly affecting patients in healthcare settings(2).

Enterococci have been identified as significant contributors to urinary tract infections (UTIs), bloodstream infections (bacteremia), and other clinical issues, mainly in hospitals. Effective treatment of serious enterococcal infections often relies on the bactericidal synergy between beta-lactam

and glycopeptide antibiotics. However, this synergy is lost when the bacteria develop high-level resistance to either antibiotic class. Multiple studies have reported that vancomycin resistance in enterococci is an emerging and serious concern in India[3].

Enterococci have emerged as important opportunistic pathogens responsible for a wide range of nosocomial infections including urinary tract infections, bacteremia, endocarditis, and surgical site infections. These organisms, particularly *Enterococcus faecalis* and *Enterococcus faecium*, exhibit intrinsic resistance to several commonly used antibiotics such as cephalosporins and low-level aminoglycosides, which complicates therapeutic management. Over the past decades, the development of high-level aminoglycoside resistance (HLAR) has further limited the synergistic effect of aminoglycosides with cell wall–active agents, thereby reducing treatment efficacy[2].

In addition to aminoglycoside resistance, the emergence of vancomycin-resistant enterococci (VRE) has become a serious global healthcare concern. Resistance to vancomycin is primarily mediated by alteration of the peptidoglycan target site (D-Ala-D-Ala to D-Ala-D-Lac), reducing antibiotic binding affinity. VRE infections are associated with increased morbidity, mortality, prolonged hospital stay, and higher healthcare costs. The coexistence of HLAR and vancomycin resistance in enterococci represents a significant therapeutic challenge, emphasizing the need for continuous surveillance and antimicrobial stewardship programs.

This study determined the antibiotic susceptibility patterns of *Enterococcus* species isolated from various clinical samples, with particular emphasis on high-level aminoglycoside resistance (HLAR) and vancomycin resistance. The results provide valuable guidance for clinicians in selecting effective empirical combination therapies, such as the use of a cell wall inhibitor in conjunction with an aminoglycoside.

MATERIALS AND METHODS

This study was conducted on 167 isolates of *Enterococcus* species obtained from various clinical specimens, including urine,pus,blood and high vaginal swab collected over a 12-month period from July 2024 to June 2025. Samples were processed immediately after collection. Identification of *Enterococcus* isolates was performed using standard microbiological protocols, including Gram staining, colony morphology assessment, catalase testing, bile solubility, growth in sodium chloride, bile esculin hydrolysis, and sugar fermentation tests[4].

Antibiotic sensitivity testing of enterococci was performed using Kirby-Bauer disc diffusion method on Mueller-Hinton agar[4].

HLAR and VRE in enterococci was detectedby disc diffusion method and confirmed by automated technique VITEK 2 according to the CLSI guideline[5].

RESULT

A total of 167 Enterococcal isolates were included in the present study. Of these, 127 isolates (76.05%) were obtained from inpatients (IPD), while 40 isolates (23.95%) were recovered from outpatients (OPD)(Table1). This distribution indicates a markedly higher prevalence of Enterococcus infections among hospitalized individuals. Analysis of antimicrobial resistance patterns revealed that high-level gentamicin (HLG) resistance was observed in 92 IPD isolates (72.44%) and 20 OPD isolates (50%). Similarly, high-level streptomycin (HLS) resistance was detected in 68 IPD isolates (53.54%) and 20 OPD isolates (50%). Vancomycin resistance was also significantly higher among inpatients, with 55 isolates (43.30%) showing resistance compared to 9 isolates (22.5%) from outpatients (Table2). These findings collectively indicate a greater burden of antimicrobial resistance in the inpatient population, likely due to prolonged hospitalization, invasive procedures, and increased antibiotic exposure.

Table 1) Showing number of Enterococci isolates according to the patient department.

Department	**Enterococci isolates**
IPD	127(76.05%)
OPD	40(23.95%)

(Table 2) Percentage of HLG, HLS, and VA resistance from IPD and OPD patients.

Department	**HLG**	**HLS**	**VA**
IPD	72.44% (92)	53.54% (68)	43.30% (55)

OPD	50% (20)	50% (20)	22.5% (9)

Gender-wise analysis of the 167 Enterococcal isolates demonstrated a higher prevalence of infections among female patients, who accounted for 103 isolates (61.7%), compared to 64 isolates (38.32%) recovered from male patients (Table 3). Antimicrobial resistance patterns also showed notable gender-related variations. Among females, high-level gentamicin resistance (HLG) was observed in 72.4% of isolates, while high-level streptomycin resistance (HLS) was detected in 60.2%. Vancomycin resistance (VA) was present in 32.04% of female isolates. In contrast, isolates from males exhibited comparatively lower rates of HLG (59.4%) and HLS (58.1%), but vancomycin resistance was significantly higher (48.44%) in this group(Table4). These findings indicate gender-specific differences in both infection prevalence and resistance patterns, potentially influenced by anatomical factors, infection susceptibility, or differences in clinical exposure and antibiotic usage.

(Table 3) Gender wise distribution

Females (%)	**Males (%)**
61.7% (103)	38.32% (64)

(Table 4) Percentage of Resistance (HLG, HLS and VA)

Gender	**HLG**	**HLS**	**VA**
Female	72.4%	60.2%	32.4%
Male	59.4%	58.1%	48.44%

Age-wise distribution of the 167 Enterococcal isolates showed that the 21–40 years age group accounted for the highest number of cases, contributing 85 isolates (50.89%). This was followed by the 41–60 years group with 43 isolates (25.74%), while the 0–20 years and >60 years age groups contributed 11.97% and 11.37% of cases, respectively. These findings indicate that Enterococcal infections were most prevalent among young to middle-aged adults, whereas the elderly population exhibited a comparatively lower proportion of cases (Table 5).

Antimicrobial susceptibility testing (AST) further revealed significant age-related variability in resistance patterns. The 41–60 years age group demonstrated the highest levels of antimicrobial resistance across all categories. In this group, high-level gentamicin resistance (HLG) was observed in 83.7% of isolates, representing the most substantial resistance burden. This was followed by high-level streptomycin resistance (HLS) at 67.4%, indicating considerable high-level aminoglycoside resistance. Additionally, vancomycin resistance (VA) was detected in 44.2% of isolates, highlighting a high prevalence of multidrug-resistant Enterococci within this population.

In comparison, the 21–40 years group exhibited moderate resistance levels (HLG: 57.3%, HLS: 50.6%, VA: 36.5%), while the 0–20 years group showed the lowest resistance levels overall (HLG: 60%, HLS: 50%, VA: 10%). The >60 years group displayed relatively high rates of HLS (63.2%) and moderate levels of HLG (64.2%) and vancomycin resistance (37.4%). Overall, these results suggest that middle-aged to older adults may be at greater risk of acquiring drug-resistant Enterococcal infections, potentially due to increased antibiotic exposure, chronic comorbid conditions, and more frequent healthcare interactions (Table 6).

(Table 5) shows age wise distribution of samples and their percentage

Age Group(years)	**No. of samples**	**Percentage**
0-20	20	11.97%
21-40	85	50.89%
41-60	43	25.74%
>60	19	11.37%

(Table 6)

Age group	HLG	HLS	VA
0-20	60%	50%	10%
21-40	57.3%	50.6%	36.5%
41-60	83.7%	67.4%	44.2%
>60	64.2%	63.2%	37.4%

Analysis of specimen-wise distribution showed that urine samples contributed the majority of Enterococcal isolates, accounting for 129 out of 167 isolates (77.25%). This was followed by pus samples, which contributed 15.57% (26/167), while blood samples accounted for 4.79% (8/167). A smaller proportion of isolates, 2.39% (4/167), was obtained from high vaginal swabs (HVS) (Table 7). This distribution reflects the predominance of Enterococcal infections in the urinary tract, consistent with their role as common uropathogens.

A detailed assessment of antimicrobial resistance patterns across different clinical specimens revealed marked variations. Urine isolates, representing the largest group, exhibited the highest levels of resistance overall. High-level gentamicin resistance (HLG) was detected in 79.1% (102/129) of isolates, while high-level streptomycin resistance (HLS) was observed in 65.9% (85/129). Vancomycin resistance (VA) was also substantial, recorded in 41.08% (53/129) of urinary isolates. These findings indicate a significant burden of multidrug resistance in urinary Enterococcal infections and raise concerns regarding treatment options.

(Table 7) Showing the percentage of isolated enterococci according to the sample

Sample	Percentage (%)
Urine	77.25% (129)
Pus	15.75% (26)
Blood	4.79% (8)
HVS	2.39% (4)

In pus samples, resistance levels were comparatively lower but still clinically important. Vancomycin resistance was the most prominent, observed in 26.9% (7/26) of isolates, followed by HLS in 23.1% (6/26) and HLG in 19.23% (5/26). The presence of vancomycin-resistant strains in wound and soft-tissue infections highlights the need for careful therapeutic decision-making in such cases.

Blood isolates showed a distinct and concerning pattern, with equal resistance rates for HLG, HLS, and VA—each at 37.5% (3/8). This uniform resistance suggests that bloodstream infections are more likely to involve resistant strains, potentially complicating patient management and requiring advanced therapeutic interventions.

Among HVS isolates, resistance rates were comparatively lower. HLG was detected in 25% (1/4), HLS in 50% (2/4), and importantly, no vancomycin resistance was observed. The absence of VA in HVS samples may reflect reduced selection pressure or lower exposure to resistant strains at this anatomical site (Table 8).

(Table 8) Percentage of HLAR and VRE according to sample

Sample	HLG	HLS	VA
Urine	79.1%	65.9%	41.1%
Pus	19.23%	23.1%	26.9%
Blood	37.5%	37.5%	37.5%
HVS	25%	50%	–

Ward-wise distribution of Enterococcal isolates showed that the highest number of isolates originated from inpatient departments (IPD), reaffirming the higher burden of Enterococcal infections among hospitalized patients compared to outpatients. Within IPD, the Obstetrics and Gynaecology ward contributed the largest proportion of isolates, accounting for 19.16% (32/167). This was followed closely by the Surgery ward with 17.36% (29/167), while the Medicine ward accounted for 13.17% (22/167) of isolates. Other wards, including ICU (11.97%), Respiratory Medicine (8.4%), Orthopaedics

(2.4%), Paediatrics (2.4%), and Private Ward (1.2%), contributed comparatively fewer isolates (Table 9).

(Table 9) Showes the distribution of samples in different wards.

rd	OBS &Gynae	Surgery	Medicine	ICU	Respiratory medicine	Ortho	Paedia	PV W
ıple	32 (19.2%)	29 (17.5%)	22 (13.2%)	20 (11.97%)	14 (8.4%)	4 (2.4%)	4 (2.4%)	2 (1.

Analysis of antimicrobial resistance patterns across hospital wards revealed marked variations. High-level gentamicin resistance (HLG) was most frequently observed in the General Medicine ward (63.63%), followed by the Obstetrics and Gynaecology ward (59.4%), indicating a higher prevalence of resistant strains in these departments. The Surgery ward also showed a similar resistance proportion (59.1%), reflecting a considerable burden of aminoglycoside-resistant Enterococci in surgical settings.

High-level streptomycin resistance (HLS) was highest in the Obstetrics and Gynaecology ward (52.3%), followed closely by the Surgery ward (52.1%), suggesting significant levels of high-level aminoglycoside resistance among isolates from these wards. In comparison, the Medicine ward showed moderately lower HLS rates (45.45%), while the ICU exhibited the lowest resistance levels (35%).

Regarding vancomycin resistance (VA), the General Medicine ward again showed the highest prevalence at 40.90%, followed by the Obstetrics and Gynaecology ward (34.4%). The Surgery ward demonstrated a moderate level of vancomycin resistance (28.1%), while the ICU showed the lowest proportion (20%) (Table 10).

Table 10) Percentage of Resistance (HLG, HLS and VA)

Ward	HLG	HLS	VA
OBS &Gynae	59.4%	52.3%	34.4%
Surgery	59.1%	52.1%	28.1%
Medicine	63.63%	45.45%	40.90%
ICU	45%	35%	20%

Overall Antimicrobial Susceptibility Test (AST) Result

A total of 167 Enterococcal isolates were subjected to antimicrobial susceptibility testing (AST) to assess their resistance patterns against key antibiotics used in clinical management. The overall findings revealed a markedly high prevalence of antimicrobial resistance among the isolates. High-level gentamicin resistance (HLG) was detected in 67.07% of the strains, indicating a significant reduction in the synergistic bactericidal activity typically achieved when gentamicin is combined with cell-wall–active agents. Similarly, high-level streptomycin resistance (HLS) was observed in 61.68% of the isolates, reflecting the widespread presence of high-level aminoglycoside resistance mechanisms, which further compromise combination therapy effectiveness.

Of particular concern was the high rate of vancomycin resistance, identified in 37.72% of the isolates, demonstrating a substantial proportion of vancomycin-resistant Enterococci (VRE). The presence of VRE is clinically significant due to restricted therapeutic options, increased risk of treatment failure, and the ability of resistant strains to spread rapidly within healthcare environments. These findings underscore the critical need for vigilant antimicrobial stewardship and ongoing surveillance efforts. A detailed distribution of resistance patterns is presented in (Table 11).

(Table 11) Showing AST pattern of Enterococci (HLG, HLS and vancomycin resistance)

Name of antibiotics	Sensitive (%)	Resistant (%)
HLG	32.93%	67.07%
HLS	38.32%	61.68%
Vancomycin	62.28%	37.72%

DISCUSSION

In the present study, a total of 167 Enterococcus isolates were obtained, with the majority recovered from inpatients 76.05% (IPD) as compared to outpatients 23.95% (OPD). This distribution suggests a higher burden of Enterococcus infections among hospitalized individuals, likely due to prolonged hospital stays, invasive procedures, and antibiotic pressure which are common risk factors in inpatient settings.

DISTRIBUTION OF ENTEROCOCCUS IN DIFFERENT DEPARTMENT

In the present study, *Enterococcus* species were most isolated from in-patient department (IPD) patients, followed by out-patient department (OPD) patients. These findings are consistent with those of Basanthi Kumari Pathi et al. (2025) (6), who also reported a higher rate of *Enterococcus* isolation in IPD patients (34.1%) compared to OPD patients (29.1%). Furthermore, high-level aminoglycoside resistance (HLAR) and vancomycin resistance were more frequently observed among isolates from IPD patients than from OPD patients. Similar trends were reported in a study by Thakan et al. (2023) (7), which showed HLAR in 85.6% of isolates and vancomycin-resistant *Enterococcus* (VRE) in 46.66%.

DEMOGRAPHIC DISTRIBUTION OF ISOLATES

In this study, the majority of *Enterococcus* isolates were obtained from female patients (61.7%) compared to males (38.3%). This finding aligns with the results of Adeyemi FM et al. (2021) (8) who reported a higher number of isolates from females 64.7% (22/34) than males 29.4% (10/34). In contrast, Hassan RM et al. (2018) (9) observed a greater number of isolates in males 59.79% (55/92) than females 39.13% (36/92). High-level gentamicin (HLG) and high-level streptomycin (HLS) resistance were more frequently observed in female patients (13.2%), whereas vancomycin resistance was more prevalent among male patients (12.7%), consistent with the findings of Yangzom T et al. (2019) (10).

AGE-WISE DISTRIBUTION OF ISOLATES

In this study, the highest prevalence of *Enterococcus* infection was observed in the 21–40 years age group, accounting for 50.89% of isolates, which is consistent with the findings reported by Mahajan M et al.(11)Notably, high-level resistance to gentamicin (HLG), streptomycin (HLS), and vancomycin was predominantly observed in isolates from patients aged 41–60 years, also reflecting trends reported by Mahajan M et al.(11), where the prevalence of high-level aminoglycoside resistance (HLAR) was 7.5% and vancomycin-resistant *Enterococcus* (VRE) was 5.3%.

DISTRIBUTION OF ENTEROCOCCUS IN DIFFERENT SAMPLES

In this study, *Enterococcus* species were most commonly isolated from urine samples (77.25% [129/167]), followed by pus samples (15.75% [26/167]). These findings are consistent with those of Nayak PP et al. (2018) (12), who reported a higher rate of *Enterococcus* isolation from urine samples (53.5% [79/150]), followed by pus samples (45.33% [68/150]). High-level aminoglycoside resistance (HLAR) was also more frequently observed in urine samples (41%) than in pus samples (36%), a trend similarly reported by Seema Mittal et al. (2016) (1).

DISTRIBUTION OF ENTEROCOCCUS IN VARIOUS WARDS

The majority of the samples in this study were obtained from inpatients (IPD) rather than outpatients (OPD). Among the IPD cases, the highest isolation rate was observed in the Obstetrics and Gynecology ward (19.2%, 32 isolates), followed by the Surgery ward (17.5%, 29 isolates). High-level gentamicin (HLG) resistance was most prevalent in the General Medicine ward, whereas high-level streptomycin (HLS) resistance was more commonly observed in the Obstetrics and Gynecology ward. Regarding vancomycin resistance, the highest rates were noted in the General Medicine ward. These findings are consistent with those reported by Seema Mittal et al. (2016) (1). From there observation Obstretrics and Gynecology.

DISTRIBUTION OF TOTAL PERCENTAGE OF RESISTANCE FROM ALL THE *Enterococcus* ISOLATES

The high prevalence of resistance among *Enterococcus* isolates observed in this study is a cause for concern, particularly with 67.07% showing high-level gentamicin (HLG) resistance and 61.68% showing high-level streptomycin (HLS) resistance. These results indicate the limited effectiveness of aminoglycosides in treating enterococcal infections, especially when used in synergy with cell wall-active agents. Moreover, vancomycin resistance was detected in 37.72% of isolates, highlighting the emergence of vancomycin-resistant enterococci (VRE), which present a significant therapeutic challenge due to the scarcity of effective treatment options. The resistance patterns observed in this study are consistent with those reported by Yangzom et al. (2019) (10), who found HLG resistance at 30.2%,

HLS resistance at 20.9%, and vancomycin resistance at 13.7%. These findings suggest a growing and geographically widespread trend of multidrug resistance in *Enterococcus* spp.

Methicillin-resistant Staphylococcus aureus and carbapenem-resistant gram-negative bacteria are major causes of osteomyelitis. Given the emergence of multidrug-resistant bacteria in osteomyelitis cases, cleanliness and tailored antibiotherapy should be prioritized(13)

The increasing incidence of antimicrobial resistance complicates management strategies, especially in prosthetic joint infections([14])

These findings are consistent with a recent prospective study conducted in 2025, where 178 clinical isolates of *Enterococcus* showed a high prevalence of HLAR (39.9%), with significant resistance to high-level gentamicin and streptomycin, and an increasing trend of vancomycin resistance among hospital isolates. Furthermore, another 2025 genomic study demonstrated that *E. faecium* isolates exhibited a high burden of multidrug resistance, with widespread presence of vanA-mediated vancomycin resistance genes and aminoglycoside-modifying enzyme genes, indicating the role of mobile genetic elements in the dissemination of resistance. These observations strongly correlate with our study findings and emphasize the growing clinical importance of resistant enterococcal infections([15,16]).

CONCLUSION

The present study demonstrates a substantial prevalence of *Enterococcus* infections predominantly among hospitalized patients, with urine and pus as the principal sources of isolation. High levels of antimicrobial resistance were observed, particularly against high-level gentamicin, high-level streptomycin, and vancomycin, indicating the emergence of multidrug-resistant *Enterococcus* strains. The predominance of isolates from female patients aged 21–40 years, especially from the Obstetrics and Gynaecology ward, identifies a vulnerable patient population. These findings underscore the critical need for continuous surveillance, enhanced infection control practices, and judicious antibiotic stewardship to mitigate the impact and dissemination of resistant *Enterococcus* species in healthcare settings.

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