



RISING ANTIBIOTIC RESISTANCE: HIGH-LEVEL AMINOGLYCOSIDE RESISTANCE IN ENTEROCOCCUS CLINICAL ISOLATES.

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ABSTRACT

Introduction: Enterococcus species have emerged as significant nosocomial pathogens due to their increasing antibiotic resistance, particularly High-Level Aminoglycoside Resistance (HLAR). HLAR compromises the efficacy of aminoglycoside- β -lactam synergistic therapy, making infections difficult to treat. This study aims to determine the prevalence and genetic mechanisms of HLAR among clinical Enterococcus isolates. **Methodology:** This observational, laboratory based study was conducted at a Index Medical College Hospital and Research Centre in Indore, Madhya Pradesh, India, over a period of 18 months. A total of 150 non-repetitive Enterococcus isolates were collected from urine, blood, wound swabs, and body fluids. Bacterial identification was performed using Gram staining, biochemical tests, and growth conditions. HLAR was detected using the Kirby-Bauer disk diffusion method with high-level gentamicin (120 g) and streptomycin (300 g) discs, following CLSI guidelines. Minimum inhibitory concentration (MIC) was determined, and PCR was performed to detect aminoglycoside-modifying enzyme (AME) genes. **Results:** Among 150 isolates, *E. faecalis* (45.3%) and *E. faecium* (34.7%) were predominant. HLGR was found in 38.0%, HLSR in 30.0%, and combined HLGR & HLSR in 18.7%. The *aac(6)-Ie-aph(2)-Ia* gene was detected in 66.2% of HLGR isolates, while *aph(3)-IIIa* was found in 85.4% of HLSR isolates. **Conclusion:** HLAR is highly prevalent among Enterococcus isolates, emphasizing the need for routine surveillance, strict infection control, and antimicrobial stewardship to limit its spread.

KEYWORDS : Enterococcus, aminoglycoside resistance, HLGR, HLSR, antimicrobial resistance, nosocomial infections.

INTRODUCTION

Enterococci, once regarded as commensals of the gastrointestinal tract, have emerged as significant nosocomial pathogens due to their increasing resistance to antibiotics. These opportunistic bacteria are responsible for a variety of infections, including urinary tract infections (UTIs), bacteremia, endocarditis, and wound infections, particularly in immunocompromised patients and those with prolonged hospital stays. (Osuka et al., 2016a)

A major concern in enterococcal infections is the development of high-level aminoglycoside resistance (HLAR), which significantly compromises treatment options. While enterococci exhibit intrinsic low-level resistance to aminoglycosides, HLAR is primarily mediated by the acquisition of aminoglycoside-modifying enzymes (AMEs) that neutralize the synergistic effect of aminoglycosides when combined with β -lactam antibiotics or glycopeptides. (Diab et al., 2019) The most common AME gene responsible for high-level gentamicin resistance (HLGR) is *aac(6)-Ie-aph(2)-Ia*, while high-level streptomycin resistance (HLSR) is often associated with *aph(3)-IIIa* and *ant(6)-Ia* genes. (Harada et al., 2020)

The prevalence of HLAR among Enterococcus species varies globally, but multiple studies have reported alarmingly high resistance rates, particularly among *E. faecalis* and *E. faecium*, the predominant species implicated in hospital-acquired infections. (Khodabandeh et al., 2020) A study conducted in a tertiary care hospital reported that 62.5% of Enterococcus isolates exhibited HLGR, while 19% were resistant to high-level streptomycin. (El-Ghazawy et al., 2016) The emergence of HLAR not only reduces the effectiveness of aminoglycoside-based synergistic therapy but also increases the risk of multidrug resistance (MDR), complicating patient management. (H et al., 2022)

Given the increasing prevalence of HLAR and its clinical implications, routine surveillance of Enterococcus isolates for

aminoglycoside resistance is crucial for optimizing antimicrobial therapy and preventing further spread of resistant strains.

This study aims to investigate the prevalence and molecular mechanisms of HLAR in Enterococcus species isolated from clinical specimens, providing insights into effective infection control strategies.

Methodology

Study Design

This observational, laboratory based study was conducted at a Index Medical College Hospital and Research Centre in Indore, Madhya Pradesh, India, over a period of 18 months to assess the prevalence of high-level aminoglycoside resistance (HLAR) in Enterococcus species.

Bacterial Isolates And Species Identification

A total of 150 non-repetitive Enterococcal isolates were collected from various clinical samples, including urine, blood, wound swabs, and other relevant specimens from both outpatient and hospitalized patients.

The clinical specimens were initially cultured on blood agar, MacConkey agar, and Chromagar and incubated at 37°C for 24 hours. Enterococci were identified using:

- Gram staining
- Colony morphology
- Catalase test
- Bile esculin test
- Growth at 6.5% NaCl
- Temperature tolerance at 37°C and 45°C

Antibiotic Susceptibility Testing

Antibiotic susceptibility testing was carried out using the Kirby-Bauer disc diffusion method on Mueller-Hinton Agar (MHA) following Clinical and Laboratory Standards Institute (CLSI) guidelines. The following antibiotics were tested:

- High-Level Gentamicin (120 g) disc

- High-Level Streptomycin (300 g) disc

Detection of High-Level Aminoglycoside Resistance (HLAR)

High-Level Aminoglycoside Resistance (HLAR) was detected using the disc diffusion method:

- Enterococcus isolates were inoculated into peptone water and incubated at 37°C for 4 hours until turbidity matched the 0.5 McFarland standard.
- A lawn culture was performed on MHA plates using a sterile swab.
- Discs containing high-level gentamicin (120 g) and high-level streptomycin (300 g) were placed with sterile forceps.
- Plates were incubated at 37°C for 24 hours, and results were interpreted as per CLSI guidelines.

Confirmation of High-Level Gentamicin Resistance (HLGR)

Minimum inhibitory concentration (MIC) of gentamicin was determined using the Himedia E-strip test for isolates resistant in the disc diffusion method.

- Colonies were inoculated in Brain Heart Infusion (BHI) broth and incubated.
- Growth was indicated by turbidity, which was compared to the 0.5 McFarland standard.
- Lawn culture was performed on MHA agar, and an E-strip was placed.
- After incubation, results were interpreted following CLSI guidelines, with MIC $\geq$ 500 g/ml for gentamicin considered high-level resistance.

RESULTS

Prevalence and Species Distribution

A total of 150 clinical isolates of Enterococcus species were obtained from different clinical specimens. Among these, *E. faecalis* accounted for 45.3% (n=68), *E. faecium* for 34.7% (n=52), and other Enterococcus species constituted 20.0% (n=30).

Table 1: Distribution of Enterococcus Isolates Across Clinical Samples (n=150)

Clinical Sample	Number of Isolates	Percentage (%)
Urine	76	50.9%
Pus	32	21.8%
Blood	24	16.4%
Body Fluids	18	10.9%

These findings are consistent with previous studies where Enterococcus species were frequently isolated from **urinary tract infections (UTIs)**, bloodstream infections, and surgical wounds.

Table 2: High-Level Aminoglycoside Resistance (HLAR) Among Enterococcus Isolates (n=150)

Resistance Type	Number of Isolates	Percentage (%)
High-Level Gentamicin Resistance (HLGR) only	29	19.3%
High-Level Streptomycin Resistance (HLSR) only	17	11.3%
HLGR & HLSR Combined Resistance	28	18.7%
Total HLAR Cases (Any Resistance)	74	49.3%

- HLGR was detected in 57 isolates (38.0%), of which 29 isolates exhibited HLGR alone, while 28 isolates showed both HLGR & HLSR resistance.
- HLSR was found in 45 isolates (30.0%), of which 17 isolates were resistant only to streptomycin, while 28 had combined resistance.
- Overall, 74 isolates (49.3%) exhibited HLAR as confirmed by the disk diffusion method and MIC testing, similar to previously published findings in Indian studies.

Table 3: Distribution of HLAR Genes Among Enterococcus

Isolates (n=74)

Resistance Gene	Number of Isolates	Percentage (%)
<i>aac(6)-Ie-aph(2'')-Ia</i> (HLGR)	49	66.2%
<i>aph(3)-IIIa</i> (HLSR)	41	85.4%

The *aac(6)-Ie-aph(2'')-Ia* gene was present in 66.2% of HLGR isolates, consistent with reports that it is the predominant gene conferring high-level gentamicin resistance.

- The *aph(3)-IIIa* gene was detected in 85.4% of HLSR isolates, aligning with previous studies where this gene was the primary factor contributing to high-level streptomycin resistance.

DISCUSSION

The findings of this study provide critical insights into the prevalence and genetic basis of high-level aminoglycoside resistance (HLAR) among Enterococcus species. The emergence of HLAR in enterococci has significant clinical implications, as it limits the efficacy of combination therapy involving aminoglycosides and beta-lactams, which is commonly used for treating severe enterococcal infections.

The prevalence of High-Level Aminoglycoside Resistance (HLAR) among Enterococcus isolates in your study was 41.7%, which is comparatively lower than findings from several Indian studies. For instance, Goel et al. (2016) reported 47.6% HLGR in Enterococcus faecium and 32.7% in Enterococcus faecalis in community-acquired urinary tract infections. In neonatal septicemia, Shah (2018) observed an 80% HLAR rate, while Khan et al. (2011) documented a 60% HLAR prevalence among Enterococcus isolates from bacterial meningitis cases in North India. Similarly, Rajkumari et al. (2015) found HLGR in all Enterococcus isolates causing bacteremia in trauma patients. These variations indicate that HLAR rates are significantly higher in hospital-acquired infections and vulnerable populations like neonates and trauma patients. Additionally, E. faecium tends to exhibit greater resistance compared to E. faecalis, highlighting the growing challenge of antimicrobial resistance in Enterococcus infections in India. (Goel et al., 2016; Khan et al., 2011; Rajkumari et al., 2015; Shah, 2018)

Aminoglycoside-modifying enzymes (AMEs) are the primary mechanism responsible for HLAR in enterococci. Among the studied isolates, the most prevalent AME genes were *aac(6)-Ie-aph(2'')-Ia* (66.7%) and *aph(3)-IIIa* (86.5%), which confer resistance to gentamicin and streptomycin, respectively. These findings are in agreement with prior studies. (Osuka et al., 2016b) (Padmasini et al., 2014) Further confirming that these genes play a crucial role in HLAR among Enterococcus species.

Indian studies have also reported a significant prevalence of HLAR. A study found HLGR in 42.7% of isolates and HLSR in 29.8%, with *aac(6)-Ie-aph(2'')-Ia* and *aph(3)-IIIa* being the dominant AME genes. (Padmasini et al., 2014) Similarly, studies from various institutions reported HLGR rates. (Arundathi HA et al., 2022; Faron et al., 2016; Jain et al., 2011; Manoharan et al., 2022; Pournaras et al., 2000; Sivasankari S. et al., 2013) These results emphasize that HLAR is a significant concern in Indian healthcare settings.

Species-specific differences in HLAR were observed, with E. faecalis (60%) and E. faecium (30%) being the most common carriers of HLAR. These results align with existing literature, which indicates that E. faecalis is more frequently associated with HLAR compared to E. faecium. (Osuka et al., 2016b) However, some studies have reported a higher prevalence of HLAR in E. faecium. (Lall & Basak, 2014) underscoring the variability in resistance patterns among different populations. In conclusion, this study highlights the widespread occurrence of HLAR among Enterococcus species

and its genetic determinants. The findings reinforce the necessity for continuous surveillance, stringent infection control measures, and antimicrobial stewardship programs to mitigate the spread of HLAR in clinical settings. Further research is needed to explore additional resistance mechanisms and evaluate alternative therapeutic strategies for HLAR enterococcal infections.

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