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Original Research Article

Molecular Detection of Virulence Genes and the High-Level Gentamicin Resistance Gene aac(6′)-Ie-aph(2″)-Ia in Enterococcus faecalis and Enterococcus faecium Isolated from Clinical Specimens

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Abstract

Background: Enterococcus faecalis and Enterococcus faecium are important healthcare-associated pathogens with a high capacity to acquire antimicrobial-resistance and virulence determinants. High-level gentamicin resistance (HLGR) is clinically important because it eliminates aminoglycoside synergy with appropriate cell-wall-active agents. The bifunctional aminoglycoside-modifying enzyme gene aac(6′)-Ie-aph(2″)-Ia is a major molecular determinant of HLGR, while virulence-associated genes such as asa1, gelE, cylA, esp, and hyl may contribute to persistence and pathogenicity. To determine the prevalence of HLGR and to characterize the distribution of aac(6′)-Ie-aph(2″)-Ia and selected virulence-associated genes among clinical isolates of E. faecalis and E. faecium.

Materials and Methods: This observational cross-sectional laboratory-based study was conducted at a tertiary-care hospital in Central India from January 2024 to December 2025. A total of 370 non-duplicate clinical Enterococcus isolates were included. HLGR was detected phenotypically using a 120-µg high-content gentamicin disk according to CLSI criteria. The 141 phenotypically HLGR isolates were subjected to conventional endpoint PCR for detection of aac(6′)-Ie-aph(2″)-Ia and the virulence-associated genes asa1, gelE, cylA, esp, and hyl. Species-wise differences were analyzed statistically, with $p<0.05$ considered significant.

Results: Among 370 isolates, 276 (74.6%) were E. faecalis and 94 (25.4%) were E. faecium. HLGR was detected in 141 (38.1%) isolates and was significantly more frequent in E. faecium than E. faecalis (56.4% vs 31.9%; $p<0.001$). The aac(6′)-Ie-aph(2″)-Ia gene was detected in 132/141 (93.6%) HLGR isolates. Among virulence genes, esp was most frequent (62.4%), followed by gelE (57.4%), asa1 (52.5%), cylA (29.8%), and hyl (25.5%). asa1, gelE, and cylA were significantly more frequent in E. faecalis, whereas hyl was significantly more frequent in E. faecium. The species-wise distribution of esp and aac(6′)-Ie-aph(2″)-Ia was not statistically significant.

Conclusion: HLGR was common among clinical enterococcal isolates, particularly E. faecium. The aac(6′)-Ie-aph(2″)-Ia gene was the predominant molecular determinant of HLGR in both species. Virulence-gene distribution showed clear species-specific differences, with asa1, gelE, and cylA predominating in E. faecalis and hyl being more frequent in E. faecium. These findings support the combined use of phenotypic HLGR screening and targeted molecular characterization for surveillance of clinically significant enterococci.

Keywords: Enterococcus faecalis; Enterococcus faecium; high-level gentamicin resistance; HLGR; aac(6′)-Ie-aph(2″)-Ia; virulence genes; asa1; gelE; cylA; esp; hyl; PCR.

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Introduction

Enterococci are Gram-positive facultatively anaerobic cocci that constitute part of the normal gastrointestinal microbiota and Enterococci are important opportunistic and healthcare-associated pathogens, with Enterococcus faecalis and Enterococcus faecium responsible for most clinically significant infections. They are associated with urinary tract infections, bloodstream infections, infective endocarditis, wound infections, intra-abdominal infections, and device-associated

infections. Their clinical significance is further increased by their ability to survive under adverse environmental conditions and persist in healthcare settings. [1,2,3,4]

The management of enterococcal infections is complicated by intrinsic resistance to several antimicrobial agents and their remarkable ability to acquire additional resistance determinants through plasmids, transposons, and other mobile genetic elements. [2,3,4,5] This ability is particularly important in hospital-associated E. faecium, which frequently exhibits resistance to multiple antimicrobial classes. [2,4]

High-level gentamicin resistance (HLGR) is clinically important because it abolishes the synergistic bactericidal activity of gentamicin with appropriate cell-wall-active agents.[5] The predominant molecular mechanism is the bifunctional aminoglycoside-modifying enzyme encoded by aac(6′)-Ie-aph(2″)-Ia; however, other genes such as aph(2″)-Ib, aph(2″)-Ic, and aph(2″)-Id may also mediate HLGR.[5,6] The study protocol similarly identifies aac(6′)-Ie-aph(2″)-Ia as the principal molecular target for HLGR detection.

Molecular detection complements phenotypic susceptibility testing by identifying specific resistance determinants and providing information on the local molecular epidemiology of HLGR. [5,7,8] The epidemiological importance of aac(6′)-Ie-aph(2″)-Ia is increased by its association with transferable genetic elements, which may facilitate horizontal dissemination among enterococcal strains. [5,8]

In addition to antimicrobial resistance, enterococci possess several virulence-associated genes that may contribute to colonization, persistence, biofilm formation, and tissue interaction. Important determinants include aggregation substance (asa1), gelatinase (gelE), cytolysin (cylA), enterococcal surface protein (esp), and the hyaluronidase-associated gene (hyl). These five genes were selected for PCR-based molecular characterization in the present study.

The asa1 gene is associated with aggregation and adherence, whereas gelE encodes an extracellular protease involved in degradation of host substrates and biofilm-related processes. cylA is involved in cytolysin activation, while esp has been associated with adherence, colonization, and biofilm formation. The hyl determinant has been reported more frequently among some hospital-associated E. faecium isolates. [6,9,10]

Previous studies have demonstrated species-related differences in virulence-gene distribution. Vankerckhoven et al. reported differences in asa1, gelE, cylA, esp, and hyl among enterococcal species, while Hasani et al. demonstrated frequent coexistence of HLGR and virulence-associated genes among clinical isolates. [6,9] Indian studies have also documented substantial occurrence of aminoglycoside-resistance determinants and virulence genes among clinical enterococci. [7,8,10]

The coexistence of resistance and virulence-associated determinants may have epidemiological importance; however, the presence of a gene does not necessarily indicate its expression or greater clinical virulence. [6,9] Molecular characterization can therefore complement phenotypic methods in understanding the resistance and virulence profiles of clinically significant HLGR enterococci.

Because information on the combined molecular distribution of HLGR and virulence genes remains limited in Central India, the present study aimed to detect aac(6′)-Ie-aph(2″)-Ia and the virulence-associated genes asa1, gelE, cylA, esp, and hyl among phenotypically HLGR E. faecalis and E. faecium isolated from clinical specimens.

Materials and Methods

Study Design and Setting: This observational cross-sectional laboratory-based study was conducted in the Department of Microbiology, Index Medical College Hospital and Research Centre, Malwanchal University, Indore, Madhya Pradesh, India, from January 2024 to December 2025. The final sample consisted of 370 clinical Enterococcus isolates.

Study Population: A total of 370 non-duplicate clinical isolates of E. faecalis and E. faecium recovered from urine, blood, pus, wound swabs, and other relevant clinical specimens were included. Phenotypic HLGR was detected in 141 isolates, which constituted the molecular study group.

Among the 141 HLGR isolates:

- E. faecalis: 88
- E. faecium: 53

Isolation and Identification: Clinical specimens were processed using standard microbiological procedures. Enterococci were identified by colony morphology, Gram staining, catalase test, pyrrolidonyl arylamidase test, bile-esculin hydrolysis, growth in 6.5% sodium chloride, and appropriate carbohydrate-fermentation reactions. Arabinose fermentation was used as a supporting test for species differentiation. Species identification was additionally correlated with molecular confirmation using species-specific ddl gene targets.

Antimicrobial Susceptibility and Detection of HLGR: Antimicrobial susceptibility testing was carried out according to the applicable Clinical and Laboratory Standards Institute criteria (CLSI).[11] HLGR was screened phenotypically using a 120-µg high-content gentamicin disk.

The study protocol also recognized a gentamicin MIC ≥500 µg/mL as indicative of HLGR when an MIC-based method was used. Only phenotypically HLGR isolates were included in the molecular resistance-gene analysis.

Molecular Detection

DNA Extraction: Phenotypically pure isolates were subcultured on 5% sheep-blood agar and incubated aerobically at 35–37°C for 18–24 hours. Genomic DNA was extracted from overnight pure cultures using the DNeasy Blood & Tissue Kit (QIAGEN, Hilden, Germany; Cat. No. 69504) according to the manufacturer's protocol for Gram-positive bacteria. Extracted DNA was stored at -20°C until PCR analysis.

Target Genes: Conventional endpoint PCR was performed for detection of:

High-level gentamicin-resistance gene: aac(6′)-Ie-aph(2″)-Ia

Virulence-associated genes: asa1, gelE, cylA, esp, and hyl.

Primer Sequences

Table 1: Primer sequences used for molecular detection

Target gene	Function	Primer sequence (5′→3′)	Amplicon
aac(6′)-Ie-aph(2″)-Ia	HLGR	F: CAGAGCCTTGGGAAGATGAAG; R: CCTCGTGTAATTCATGTTCTGGC	348 bp
asa1	Aggregation substance	F: GCACGCTATTACGAACTATGA; R: TAAGAAAGAACATCACCACGA	375 bp
gelE	Gelatinase	F: TATGACAATGCTTTTTGGGAT; R: AGATGCACCCGAAATAATATA	213 bp
cylA	Cytolysin	F: ACTCGGGGATTGATAGGC; R: GCTGCTAAAGCTGCGCTT	688 bp
esp	Enterococcal surface protein	F: AGATTTCATCTTTGATTCTTGG; R: AATTGATTCTTTAGCATCTGG	510 bp
hyl	Hyaluronidase-associated determinant	F: ACAGAAGAGCTGCAGGAAATG; R: GACTGACGTCCAAGTTTCCAA	276 bp

The aac(6′)-Ie-aph(2″)-Ia primers were based on Vakulenko et al. [5], while primers for asa1, gelE, cylA, esp, and hyl were based on Vankerckhoven et al. [6] The sequences and expected amplicon sizes correspond to those documented in the study protocol.

PCR Reaction Mixture: Each target was amplified in a separate conventional PCR reaction with a final volume of 25 µL, containing 12.5 µL of 2× PCR master mix, 0.5 µL each of forward and reverse primers (10 µM), 2 µL template DNA, and 9.5 µL nuclease-free water.

PCR Amplification: PCR amplification was carried out using a programmable thermal cycler with the following optimized cycling profile:

Table 2:

Step	Temperature	Time	Cycles
Initial denaturation	95°C	5 min	1
Denaturation	94°C	30 sec	35
Annealing	Assay-specific	30 sec	35
Extension	72°C	45–60 sec	35
Final extension	72°C	7 min	1
Hold	4°C	Until retrieval	—

The protocol states that annealing temperatures were optimized by gradient PCR before testing.

The exact final annealing temperature for each primer should be inserted from the laboratory record before submission.

Agarose-Gel Electrophoresis: Amplified PCR products were separated on 1.5–2.0% agarose gel in TAE or TBE buffer. Five microlitres of PCR product were loaded with loading dye and a 100-bp DNA ladder was run simultaneously. Electrophoresis was carried out at approximately 80–120 V. Bands were visualized using a gel documentation system. A result was considered positive only when a band corresponding to the expected amplicon size was detected, the positive control produced the appropriate band, and the no-template control showed no amplification. Invalid runs were repeated.

Statistical Analysis: Data were analysed using descriptive and inferential statistics. Categorical variables were presented as frequencies and percentages. Species-wise differences in gene distribution were analysed using the chi-square test with continuity correction where appropriate. Fisher's exact test was considered for small expected frequencies. A two-sided p value <0.05 was considered statistically significant.

Results

Distribution of Enterococcal Species and High-Level Gentamicin Resistance: A total of 370 non-duplicate clinical Enterococcus isolates were included in the study. Of these, 276 (74.6%) were identified as E. faecalis and 94 (25.4%) as E. faecium.

High-level gentamicin resistance (HLGR) was detected in 141/370 isolates (38.1%).

Among the 141 HLGR isolates, 88 were E. faecalis and 53 were E. faecium. The species-specific prevalence of HLGR was 31.9% (88/276) in E. faecalis and 56.4% (53/94) in E. faecium, showing a significantly higher prevalence of HLGR among E. faecium (p<0.001).

Table 3: Species-wise prevalence of high-level gentamicin resistance

Species	Total isolates	HLGR isolates n (%)	HLG-susceptible isolates n (%)
E. faecalis	276	88 (31.9)	188 (68.1)
E. faecium	94	53 (56.4)	41 (43.6)
Total	370	141 (38.1)	229 (61.9)

χ^2=16.82, df=1, p<0.001, This demonstrates a significant association between species and HLGR status.

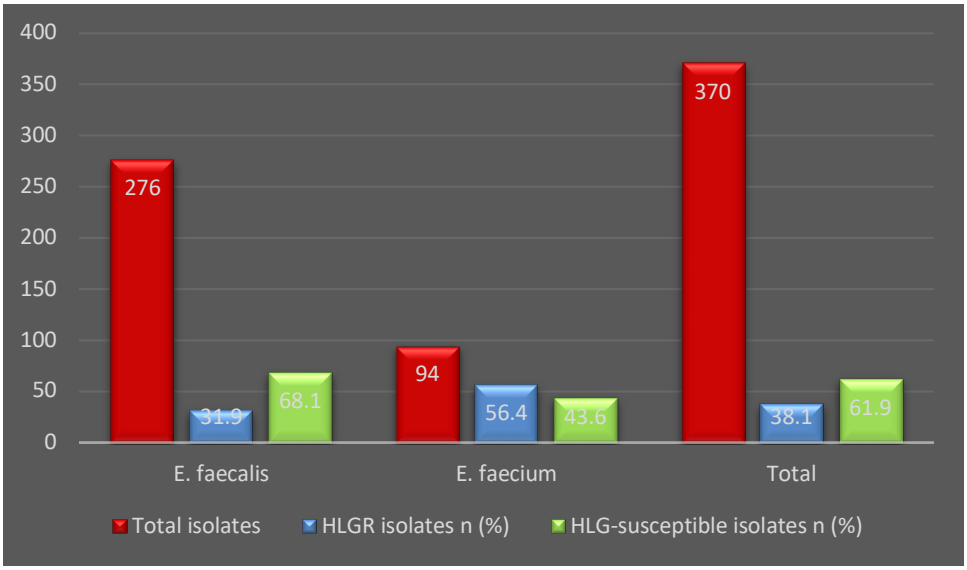

Figure 1: Species-wise prevalence of high-level gentamicin resistance

Molecular Detection of the High-Level Gentamicin Resistance Gene: Among the 141 phenotypically HLGR isolates, the aac(6′)-Ie-aph(2″)-Ia gene was detected in 132 isolates (93.6%), whereas 9 isolates (6.4%) were negative for this target. Thus, aac(6′)-Ie-aph(2″)-Ia represented the predominant molecular determinant of HLGR in the study population.

Table 4: Detection of aac(6′)-Ie-aph(2″)-Ia among phenotypically HLGR isolates

Molecular finding	n	%
aac(6′)-Ie-aph(2″)-Ia detected	132	93.6
aac(6′)-Ie-aph(2″)-Ia not detected	9	6.4
Total	**141**	**100.0**

The high detection rate indicates strong concordance between phenotypic HLGR and the presence of the bifunctional aminoglycoside-resistance gene.

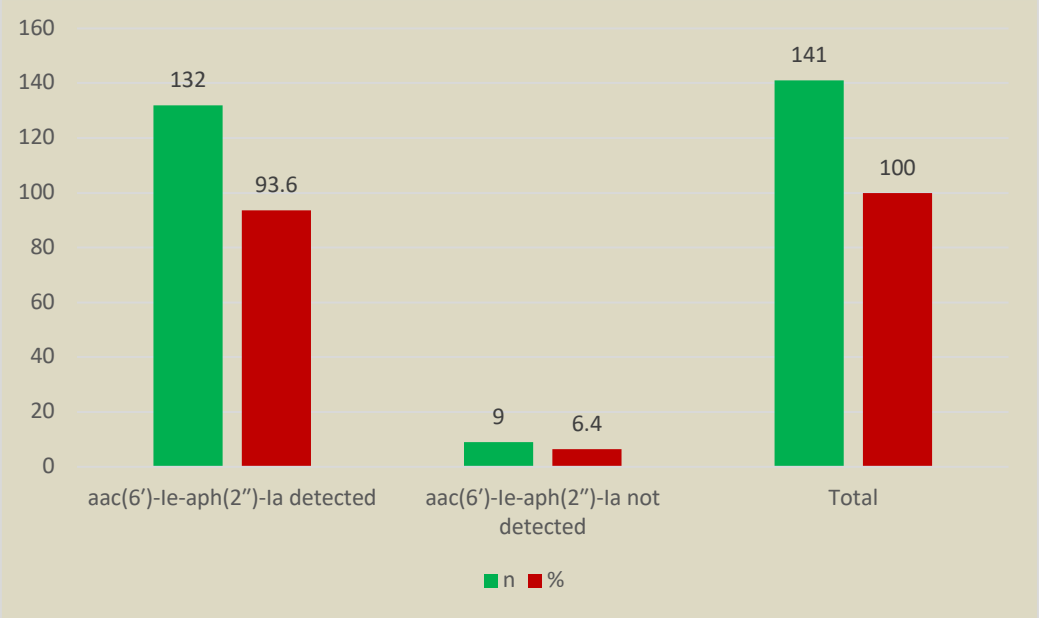

Figure 2: Detection of aac(6′)-Ie-aph(2″)-Ia among phenotypically HLGR isolates

Distribution of Virulence-Associated Genes among HLGR Isolates: Virulence-associated genes were frequently detected among the 141 HLGR isolates. The most common gene was esp, detected in 88 isolates (62.4%), followed by gelE in 81 (57.4%), asa1 in 74 (52.5%), cylA in 42 (29.8%), and hyl in 36 (25.5%).

Table 5: Distribution of virulence-associated genes among HLGR isolates

Virulence gene	Detected n (%)	Not detected n (%)
esp	88 (62.4)	53 (37.6)
gelE	81 (57.4)	60 (42.6)
asa1	74 (52.5)	67 (47.5)
cylA	42 (29.8)	99 (70.2)
hyl	36 (25.5)	105 (74.5)

Thus, genes associated with surface adherence and extracellular enzymatic activity, particularly esp, gelE, and asa1, were commonly present among HLGR isolates.

Species-Wise Distribution of aac(6′)-Ie-aph(2″)-Ia: The aac(6′)-Ie-aph(2″)-Ia gene was detected in 83/88 (94.3%) HLGR E. faecalis and 49/53 (92.5%) HLGR E. faecium isolates. The difference was not statistically significant (p=0.934), indicating that this gene was the predominant HLGR determinant in both species.

Species-Wise Distribution of Virulence Genes: Marked species-related differences were observed for several virulence genes. asa1 was detected in 63.6% of E. faecalis compared with 34.0% of E. faecium isolates (p=0.001). Similarly, gelE was significantly more frequent among E. faecalis than E. faecium (69.3% vs 37.7%; p<0.001), and cylA was detected in 38.6% and 15.1%, respectively (p=0.006).

In contrast, hyl was significantly more frequent among E. faecium (41.5%) than E. faecalis (15.9%; p=0.001). The frequency of esp was comparable between E. faecalis and E. faecium (59.1% vs 67.9%), with no statistically significant difference (p=0.385).

Table 6: Species-wise distribution of resistance and virulence genes among HLGR isolates

Gene	E. faecalis n=88, n (%)	E. faecium n=53, n (%)	χ^2	p value
aac(6′)-Ie-aph(2″)-Ia	83 (94.3)	49 (92.5)	0.01	0.934
asa1	56 (63.6)	18 (34.0)	10.52	0.001
gelE	61 (69.3)	20 (37.7)	12.24	<0.001
cylA	34 (38.6)	8 (15.1)	7.68	0.006
esp	52 (59.1)	36 (67.9)	0.76	0.385
hyl	14 (15.9)	22 (41.5)	10.10	0.001

Principal Molecular Findings: The molecular analysis showed three important patterns. First, aac(6′)-Ie-aph(2″)-Ia was detected in more than 90% of HLGR isolates of both species, with no significant species-wise difference. Second, E. faecalis showed significantly greater carriage of asa1, gelE, and cylA. Third, hyl was significantly associated with E. faecium, while esp was common in both species without a significant difference.

Overall, these findings demonstrate that although the predominant molecular mechanism of HLGR was similar in E. faecalis and E. faecium, the distribution of virulence-associated genes differed substantially between the two species. This version is stronger because it avoids unnecessary demographic and antibiotic tables that distract from the molecular title, and it makes the statistically significant molecular findings the focus of the Results section.

Discussion

The present study provides molecular characterization of phenotypically high-level gentamicin-resistant (HLGR) Enterococcus faecalis and Enterococcus faecium recovered from clinical specimens. Among 370 enterococcal isolates, HLGR was detected in 141 (38.1%). The prevalence was significantly higher among E. faecium than E. faecalis (56.4% vs 31.9%; p<0.001). Banerjee et al. [12] reported HLGR in 47.41% of clinical enterococcal isolates, whereas Hasani et al. [9] reported a higher frequency of approximately 60%. Differences between studies may be related to geographical variation, patient population, antimicrobial exposure, hospital setting, and local resistance epidemiology.

The major molecular finding of the present study was detection of aac(6′)-Ie-aph(2″)-Ia in 132/141 (93.6%) phenotypically HLGR isolates. Hasani et al. [9] detected aac(6′)-Ie-aph(2″)-Ia in all HLGR isolates included in their molecular analysis, while Rosvoll et al. [13] reported this determinant in nearly all HLGR E. faecium isolates studied. These observations, together with the present findings, support aac(6′)-Ie-aph(2″)-Ia as one of the predominant molecular mechanisms underlying HLGR in clinical enterococci.

Nine phenotypically HLGR isolates in the present study did not carry the targeted aac(6′)-Ie-aph(2″)-Ia gene. Vakulenko et al. [5] demonstrated that additional resistance determinants, including aph(2″)-Ib, aph(2″)-Ic, and aph(2″)-Id, can also mediate high-level gentamicin resistance. Therefore, absence of aac(6′)-Ie-aph(2″)-Ia in these isolates does not exclude a genetic basis of HLGR. Broader molecular testing or sequencing would be required to identify alternative mechanisms. The aac(6′)-Ie-aph(2″)-Ia gene was detected at comparable frequencies in HLGR E. faecalis and E. faecium (94.3% vs 92.5%; p=0.934). This indicates that although phenotypic HLGR was more frequent among E. faecium, the predominant molecular determinant of gentamicin resistance was similar in both species. Rosvoll et al. [13] also demonstrated dissemination of aac(6′)-Ie-aph(2″)-Ia through transferable genetic elements among hospital-associated E. faecium, emphasizing its epidemiological importance.

Virulence-associated genes were frequently detected among HLGR isolates. The most common gene was esp (62.4%), followed by gelE (57.4%), asa1 (52.5%), cylA (29.8%), and hyl (25.5%). Hasani et al. [9] reported gelE, asa1, and esp in substantial proportions of clinical enterococcal isolates. The frequency of asa1 reported by them was similar to the present study, while variation in esp and gelE frequencies may reflect differences in species composition and epidemiological characteristics of the study populations.

The predominance of esp in the present study is notable because enterococcal surface protein has been associated with adherence, colonization, persistence, and biofilm formation. Tendolkar et al. [14] demonstrated that Esp enhances biofilm formation by E. faecalis. Similarly, Shankar et al. [15] showed that Esp contributes to persistence in an experimental model of ascending urinary tract infection. Nevertheless, detection of esp alone should not be interpreted as proof of increased virulence or biofilm production because expression of the corresponding phenotype depends on multiple bacterial and environmental factors.

Marked species-wise differences were observed in the distribution of several virulence genes. asa1 was significantly more frequent among E. faecalis than E. faecium (63.6% vs 34.0%; p=0.001). Similarly, gelE occurred significantly more frequently in E. faecalis (69.3% vs 37.7%; p<0.001), while cylA was detected in 38.6% of E. faecalis compared with 15.1% of E. faecium (p=0.006). Kiruthiga et al. [10] also reported significantly higher frequencies of asa1, gelE, and cylA among E. faecalis than E. faecium, supporting the species-related pattern observed in the present study.

The greater frequency of these classical virulence-associated determinants among E. faecalis is also consistent with the observations of Hasani et al. [9], who found several virulence genes to be more common in E. faecalis. Comerlato et al. [16] similarly demonstrated species-related differences in virulence determinants and reported a greater representation of certain virulence characteristics among E. faecalis. These findings suggest that E. faecalis may possess a broader repertoire of some classical virulence-associated genes despite the

greater HLGR prevalence observed among E. faecium in the present study.

In contrast, hyl was significantly more frequent in E. faecium than E. faecalis (41.5% vs 15.9%; p=0.001). Vankerckhoven et al. [6] also reported hyl particularly among hospital-associated E. faecium isolates, supporting the species-specific pattern observed in the present study. However, the biological significance of hyl should be interpreted cautiously, and detection of the gene alone does not establish increased pathogenicity.

The distribution of esp differed from that of the other virulence genes. It was detected in 59.1% of HLGR E. faecalis and 67.9% of HLGR E. faecium, with no statistically significant difference (p=0.385). Vankerckhoven et al. [6] also reported a high occurrence of esp among hospital-associated E. faecium. The present finding therefore indicates that esp was common in both species rather than being restricted predominantly to E. faecalis.

The overall molecular pattern is also comparable with Banerjee et al. [12], who evaluated multiple virulence determinants among clinical enterococci and observed that several were more commonly associated with E. faecalis. Importantly, their study also demonstrated that the presence of a virulence gene does not necessarily correspond to phenotypic expression, particularly for gelE. This supports cautious interpretation of molecular virulence findings. Similarly, Fernandes et al. [17] demonstrated frequent occurrence of virulence-associated characteristics together with antimicrobial resistance among clinical enterococci. Although their work included phenotypic virulence testing rather than precisely the same molecular panel used in the present study, their observations further support the coexistence of antimicrobial resistance and virulence-associated traits in clinical Enterococcus isolates.

The coexistence of aac(6′)-Ie-aph(2″)-Ia with multiple virulence-associated genes is epidemiologically important. However, the simultaneous detection of resistance and virulence determinants does not establish a direct causal relationship between them. Furthermore, the presence of asa1, gelE, cylA, esp, or hyl represents genetic potential and does not necessarily indicate gene expression or greater clinical virulence. Banerjee et al. [12] demonstrated genotype-phenotype discordance for some virulence determinants, emphasizing the importance of correlating molecular findings with phenotypic and clinical data.

Overall, the present study demonstrates two important molecular patterns. First, aac(6′)-Ie-aph(2″)-Ia was the predominant HLGR-associated determinant in both E. faecalis and E. faecium. Second, virulence-gene distribution differed considerably between the two species, with asa1, gelE, and cylA significantly more frequent among E. faecalis, while hyl was significantly more frequent among E. faecium. In contrast, esp was common in both species without a significant difference. These findings demonstrate the usefulness of combining phenotypic HLGR detection with targeted molecular characterization to better understand the molecular epidemiology of clinically significant enterococci.

Limitations

This study has several limitations. First, it was conducted at a single tertiary-care centre, which may limit the generalizability of the findings to other hospitals or geographic regions. Second, molecular testing was restricted to the major HLGR-associated gene aac(6′)-Ie-aph(2″)-Ia and five selected virulence-associated genes (asa1, gelE, cylA, esp, and hyl); therefore, other aminoglycoside-resistance genes and additional virulence determinants were not evaluated. Third, gene expression was not assessed, so the presence of a virulence gene does not necessarily indicate phenotypic expression or increased clinical virulence. Clonal relatedness and the location of resistance determinants on plasmids, transposons, or chromosomes were also not investigated. Thus, the findings should primarily be interpreted as institution-specific molecular epidemiological data.

Conclusion

The present study demonstrated that high-level gentamicin resistance was common among clinical Enterococcus isolates and was significantly more frequent in E. faecium than in E. faecalis. The aac(6′)-Ie-aph(2″)-Ia gene was detected in the large majority of phenotypically HLGR isolates, confirming it as the predominant molecular determinant of HLGR in both species.

Virulence-associated genes were also frequently detected, with esp, gelE, and asa1 being the most common. Species-specific differences were evident: asa1, gelE, and cylA were significantly more frequent in E. faecalis, whereas hyl was significantly more frequent in E. faecium. The distribution of esp was comparable between the two species. These findings highlight the value of combining phenotypic high-level aminoglycoside-resistance testing with targeted molecular characterization for surveillance of clinically significant enterococci. Further multicentre studies incorporating broader resistance-gene panels, gene-expression analysis, and whole-genome sequencing are needed to better understand the molecular epidemiology, transmission, and clinical significance of HLGR enterococci.

Declarations

Informed consent: Written informed consent was obtained according to the approved study protocol.

Data availability: Data supporting the findings are available from the corresponding author on reasonable request, subject to institutional and ethical requirements.

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