1 Prevalence of High-Level Aminoglycoside Resistance (HLAR) and Vancomycin Resistance (VR) among Enterococcal Isolates

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Abstract

Enterocoocci are ubiquitous in nature and being formidable nosocomial pathogen it acquires high level resistance to multiple antibiotics either by mutation or by receipt of foreign genetic material through the transfer of plasmids and transposons. With rising prevalence of High-level aminoglycoside resistance (HLAR) and Vancomycin resistance enterococci (VRE) regularly screening can limits the spread of multidrug resistant infection. The present study was carried out at Fortis Escorts Hospital from various clinical samples, and urine, blood and pus specimen was found to be highly infected. Phenotypic screening was determined via Kirby-Bauer disk diffusion method on Mueller Hinton agar or Agar screen method following clinical laboratory standards. Among 4458 samples, a total of 59 enterococcal species were isolated which include 49% E.faecium, 42% E.faecalis, 7% E.gallinarum and 2% E.avium. Maximum 20.3% of enterococcal isolates were observed in patient of age group 51-60 years and more in females than males. Total HLAR among E.faecium isolates (59%) was higher than E.faecalis (28%). Vancomycin resistance of 20.3% was observed among the Enterococcus species. E.faecium was most commonly isolated from hospitalized patients and E.faecalis was most commonly isolated from outpatients. Linezolid was found the most sensitive antibiotic against the Enterococcus strain followed by Nitrofurantoin (88.8% sensitivity) and Teicoplan (79.6%). Combinedly Ampicillin, Vancomycin and HLA (high level aminoglycoside) noted in 28% of Enterococcs strain.

Keywords: Nosocomial, Aminoglycoside, Vancomycin, Linezolid, Nitrofurantoin, Teicoplanin, Ampicillin.

Introduction

The transition of **Enterococcus** from a sub-category of *Streptococcus* to its own genus in the 1980s was more than just a name change; it was a necessary response to the discovery of one of the most resilient and adaptable pathogens in modern medicine. While these Gram-positive cocci are natural, helpful inhabitants of our gut, their "ubiquity"—being found everywhere from soil to hospital sinks—makes them a formidable foe when they enter the bloodstream or urinary tract. Before 1984, these organisms were known as **Group D Streptococci**, but genetic sequencing revealed they were distinct enough to warrant their own genus, with *E. faecalis* and *E. faecium* emerging as the primary clinical culprits. While *E. faecalis* is more common, *E. faecium* is significantly more difficult to treat due to its aggressive resistance profile.

What truly makes Enterococcus a "superbug" is its two-pronged approach to surviving antibiotics. First, they possess **intrinsic (natural) resistance**, meaning they are born with a "built-in" shield against many common drugs, including most Cephalosporins and low doses of Aminoglycosides. Most impressively, they can bypass **folate synthesis inhibitors** by simply absorbing folic acid from their environment—essentially "eating" the very thing the drug is trying to prevent them from making. Second, they excel at **acquired resistance**, acting as the "thieves" of the microbial world by picking up DNA from other bacteria. This has led to the rise of **Vancomycin-Resistant Enterococci (VRE)**, where the bacteria alter their cell wall structure so Vancomycin can no longer latch on, rendering a formerly powerful drug useless.

For severe infections like endocarditis or meningitis, doctors cannot rely on a single antibiotic because Enterococci are often "bacteriostatic," meaning the drug only pauses their growth rather than killing them. To achieve a kill, clinicians traditionally use a "one-two punch" known as **synergy**, where a cell-wall inhibitor break open the bacterial defenses so an Aminoglycoside can slip through to attack the internal machinery. However, this strategy is failing due to the emergence of **High-Level Aminoglycoside Resistance (HLAR)**, where bacteria produce enzymes that neutralize the drug. When a strain is both HLAR and VRE, therapeutic options become dangerously limited. Consequently, local surveillance and screening, such as those conducted at Fortis Escorts Hospital, are critical. These studies allow doctors to identify which resistant strains are circulating in specific wards, enabling them to bypass failing standard treatments and move immediately to "last-resort" drugs like Linezolid or Daptomycin.

Review of Literature

Data from Mathur et al. (2003) and Karmarker et al. (2004) demonstrate a high prevalence of High-Level Aminoglycoside Resistance (HLAR) and the emergence of Vancomycin-Resistant Enterococci (VRE) with diverse phenotypes. These findings underscore how resistance to standard treatments like Ampicillin and Vancomycin severely restricts therapeutic options, necessitating enhanced clinical screening and strict infection control to manage these escalating bacterial threats.

In their 2004 study, Taneja et al. screened 144 Enterococci strains isolated from urine specimens for Vancomycin resistance. Using agar screen methods, E-tests, and agar dilution, they identified eight Vancomycin-resistant Enterococci (VRE) isolates, primarily E. faecium and E. faecalis. The study noted that while VRE frequency was relatively low compared to Western findings, the clinical impact was significant; two patients with nosocomial urinary tract infections (UTIs) developed sepsis and died despite treatment. Based on these results, the authors recommended routine screening for Vancomycin resistance in patients with significant bacterial counts in their urine.

The 2005 study by Marothi, Agnihotri, and Dubey explores the antimicrobial resistance of Enterococcus, identifying it as a critical nosocomial pathogen due to its ability to survive in high-antibiotic environments. The authors detail both intrinsic and acquired resistance mechanisms, noting that while these bacteria are generally inhibited by β-lactams, they are not typically killed by them. The study concludes that using Agar Screen methods and high-content aminoglycoside discs is essential for accurately differentiating resistant isolates and guiding the management of severe Enterococcal infections.

In their 2007 retrospective study, Chaudhary, Shamma, and Yadav analyzed 260 Enterococcus strains collected from clinical specimens between 2004 and 2005. While E. faecalis (72.3%) was the most prevalent species overall, E. faecium was identified as the most frequent isolate specifically in blood cultures. The study reported high susceptibility rates for Vancomycin (98.1%), Teicoplanin (88.57%), and Linezolid (79.44%). However, the researchers also identified five Vancomycin-resistant Enterococci (VRE) isolates with a Minimum Inhibitory Concentration (MIC) of 32ug/ml. With an overall prevalence rate of 18%, the authors concluded that managing these multidrug-resistant pathogens requires prudent antibiotic use and strict infection control measures.

The 2008 review by Sood, Malhotra, Das, and Kapil highlights the transition of Enterococci from low-grade pathogens to significant causes of nosocomial infections, particularly in the last decade. They identify E. faecalis and E. faecium as the primary species responsible for most human infections, including UTIs, neonatal sepsis, and endocarditis. The authors emphasize that these organisms survive in hospitals due to their ability to acquire resistance to nearly all available antibiotics through plasmids and transposons. Notably, they report high and rising resistance levels in E. faecium isolates—60% for Vancomycin and 80% for Ampicillin—and warn that the transfer of resistance genes to Staphylococcus aureus poses a major threat to patient safety.

In his 2010 study, Adhikari identified Enterococcus faecalis (72.22%) as the most prevalent species among 180 clinical isolates, followed by E. casseliflavus and E. faecium. The research highlighted a high occurrence of high-level aminoglycoside resistance (HLAR), which was significantly more common in E. faecium strains. While no isolates showed full resistance to vancomycin, several strains of E. faecalis and E. faecium exhibited intermediate resistance (MIC = 8μg/mL). Ultimately, the study concluded that the agar-screen method is superior to disk diffusion for detecting these resistant strains, emphasizing the need for routine clinical testing to prevent the spread of VRE in hospitals.

In their 2012 study, Ajay Kumar Oli et al. examined the antimicrobial susceptibility of 122 Enterococcus strains isolated in South India, identifying E. faecalis (62.29%) as the predominant species. The research revealed an alarming resistance profile, particularly among E. faecalis strains, which showed high resistance to Vancomycin (77.63%), Gentamicin (64.47%), and Oxacillin (55.26%). While E. faecium demonstrated lower overall resistance compared to E. faecalis, it still showed significant resistance to Gentamicin (44.4%) and Streptomycin (40.8%). The study utilized both disk diffusion and Broth Dilution methods to determine Minimum Inhibitory Concentrations (MICs), finding high-level resistance in several isolates. Ultimately, the researchers concluded that Enterococci are exhibiting a dangerous trend of multi-drug resistance to standard clinical therapies.

Material and Methods

The methodology employed at the Microbiology Laboratory of Fortis Escorts Hospital in Jaipur represents a meticulous, multi-staged approach to isolating and identifying *Enterococcus* species, a process that is as much about biochemical precision as it is about clinical vigilance. Over a focused six-month period from January to June 2012, researchers collected 59 distinct strains from a wide array of clinical specimens, a distribution that highlights the pathogen's ability to infect diverse biological systems, with the majority of samples derived from urine (28), blood (13), and pus or wound swabs (10), while less frequent but equally critical isolates were gathered from sputum, respiratory tract secretions like endotracheal suction tips, peritoneal fluid, and miscellaneous sites such as popliteal artery tips. The journey of identification began with the inoculation of these specimens onto a carefully curated selection of culture media designed to promote the growth of Gram-positive cocci while suppressing competitors; this included the use of Sheep Blood Agar to observe characteristic colony morphology, MacConkey agar to screen for enteric organisms, and Cysteine Lactose Electrolyte Deficient (CLED) agar specifically for urine samples, where a standardized 10 ul volume was applied using a flexi loop to ensure quantitative accuracy. Following an incubation period of 24 to 48 hours at a controlled temperature of 37°C, the laboratory staff transitioned from primary culture to morphological assessment using Gram’s stain, confirming the presence of the signature pair-and-chain arrangements that define the genus. To move beyond simple visual observation, a battery of classic biochemical "gatekeeper" tests was implemented to distinguish these hardy organisms from their close relatives in the

Streptococcus family. Central to this was the Bile Esculin Hydrolysis test, which leverages the ability of Enterococci to grow in the presence of 40% bile and hydrolyze esculin to esculetin; when this esculetin reacts with ferric ions in the medium, it produces a distinct blackening of the agar slant, a definitive indicator of a positive result. This was further corroborated by the Salt Tolerance test, an essential diagnostic maneuver where the organism is challenged to survive and multiply in a high-salinity environment of 6.5% sodium chloride (NaCl) broth. While non-enterococcal Group D streptococci typically find such saline concentrations inhibitory, Enterococcus species flourish, resulting in visible turbidity or a pH-driven color change in the medium. These traditional phenotypic markers provided the foundational evidence for genus-level identification, but the study reached its zenith of precision through the utilization of the MICROSCAN autoSCAN-4 automated system. By employing the Positive Breakpoint Combo panel type 20, the researchers were able to achieve definitive speciation and precise identification of the isolates, moving past manual interpretation to an automated, standardized readout that is vital for modern clinical diagnostics. This rigorous combination of manual biochemical testing and automated technology ensures that the specific species—whether the more common *E. faecalis* or the more resistant *E. faecium*—are correctly identified, thereby providing a clear roadmap for understanding the local prevalence of these pathogens and guiding the subsequent antimicrobial susceptibility testing that is so critical in an era of rising hospital-acquired resistance.

The laboratory investigation into the antibiotic susceptibility profiles of Enterococcus isolates was conducted using standardized phenotypic methods to identify critical resistance patterns, specifically High-Level Aminoglycoside Resistance (HLAR) and Vancomycin Resistance. All culture media utilized for these procedures, including Mueller Hinton Agar (MHA) and Brain Heart Infusion (BHI) agar, were sourced from Hi-Media Ltd. (Mumbai, India) in dehydrated form and prepared according to the manufacturer's specifications. Antibiotic disks and powders were obtained from BD (Becton Dickinson India Pvt. Ltd.). To ensure the accuracy and reproducibility of the results, E. faecalis ATCC 29212 was employed as a quality control strain throughout the testing process.

The detection of high-level resistance to Gentamicin (120 µg) and Streptomycin (300 µg) was performed using the Kirby-Bauer disk diffusion method. This procedure followed the standardized guidelines recommended by the National Committee for Clinical Laboratory Standards (NCCLS/CLSI). The process began with the preparation of a bacterial inoculum; isolated colonies of Enterococci were carefully picked from a fresh culture and inoculated into sterile peptone water. The turbidity of this suspension was adjusted to match the 0.5 McFarland standards, ensuring a consistent concentration of approximately 1.5×10^8 CFU/mL Once the inoculum was standardized, a "lawn culture" was created by swabbing the bacterial suspension evenly across the surface of a Mueller Hinton Agar plate. High-level Gentamicin (120 μg) and Streptomycin (300 μg) disks were then placed onto the agar surface. The plates were inverted and incubated at a controlled temperature of 37°C for a full 24 hours. Following incubation, the diameter of the zone of inhibition surrounding each disk was measured using a millimeter ruler. According to the interpretative criteria, the absence of any zone of inhibition (no zone) indicated resistance, while susceptibility was defined by a zone diameter of ≥ 10 mm. This method is critical for identifying isolates that have lost the synergistic effect typically seen when Aminoglycosides are combined with cell-wall active agents like penicillin.

Vancomycin resistance in the Enterococci isolates was determined using the Agar Screen method, a highly sensitive technique for detecting low-level resistance that might be missed by standard disk diffusion. The screening medium consisted of Brain Heart Infusion (BHI) agar supplemented with a specific concentration of 6 μg/mL of Vancomycin powder. The preparation for this test involved growing the isolates on agar plates for 24 hours. A bacterial suspension was then prepared and adjusted to a 0.5 McFarland standard. From this suspension, a 10 μL aliquot was "spotted" onto the surface of the Vancomycin-supplemented BHI agar. This volume provided a final inoculum of approximately 10^6 CFU per spot. The plates were then incubated at 37°C for an initial 24-hour period.

The results were interpreted based on the presence or absence of growth. The appearance of more than one colony or a visible haze of growth at the site of inoculation was recorded as resistance to vancomycin. Conversely, plates showing no bacterial growth after the initial 24 hours were not immediately ruled negative; they were returned to the incubator for an additional 24 hours (for a total of 48 hours) to ensure that slow-growing resistant strains were not overlooked. This rigorous 48-hour incubation period is essential for the reliable detection of various vancomycin resistance phenotypes, such as vanA or vanB.

Observation and Results

During the six-month study period from January to June 2012, a total of 4,458 clinical samples were processed, leading to the isolation of 59 distinct Enterococcal strains. Taxonomic identification revealed that Enterococcus faecium was the most prevalent species, accounting for 49% (29 strains) of the total isolates. This was followed by Enterococcus faecalis at 42% (25 strains), while less common species such as E. gallinarum (7%) and a single isolate of E. avium (2%) were also identified. Together, E. faecium and E. faecalis constituted over 90% of the clinical Enterococcal burden. The analysis of clinical specimens showed that Urine (28 isolates) was the most frequent source of Enterococci, followed by Blood (13 isolates) and Pus/Wound swabs (10 isolates). Other sources included respiratory tract specimens, sputum, body fluids (peritoneal fluid), and miscellaneous samples such as cervical swabs.

A significant distinction was observed based on patient status:

- **Hospitalized Patients:** This group contributed the vast majority of isolates (49 out of 59). Within this setting, E. faecium was the dominant species, particularly in urine and blood samples, highlighting its role as a major nosocomial pathogen.
- **Outpatients:** A smaller subset of 10 isolates was recovered from outpatients. In contrast to the hospitalized group, E. faecalis was the predominant species here, notably appearing in 8 out of the 10 outpatient urine samples.

The demographic analysis revealed a nearly balanced gender distribution, with 30 (51%) isolates from females and 29 (49%) from males. However, age-wise distribution showed a distinct concentration in older populations; the 51–60 years age group had the highest frequency at 20.3% (12 isolates), followed by the 61–70 years bracket at 18.6% (11 isolates). Collectively, patients over the age of 50 accounted for approximately 47% of all isolates. Interestingly, gender distribution shifted dramatically across age categories. Younger and middle-age brackets (11–40 years) showed a marked female predominance, peaking at 83% in the 31–40 age group. Conversely, the geriatric population aged 71 and above consisted exclusively of male patients (100%). These combined findings suggest that while Enterococcal infections affect a wide demographic, they are most frequently encountered as E. faecium in hospitalized older adults, whereas E. faecalis remains a common cause of outpatient urinary tract infections. While the majority of isolates remained sensitive, 3 strains (5.1%) were identified as vancomycin-resistant. Notably, all three resistant strains were E. faecium isolates recovered from hospitalized patients, specifically from urine and blood samples. No resistance was detected in E. faecalis, E. gallinarum, or E. avium during the 48-hour agar screening process. This finding correlates with the species distribution data, suggesting that E. faecium is not only the most prevalent species in the hospital setting but also the most likely to harbor advanced resistance mechanisms. The concentration of these resistant strains in urine and blood specimens underscores the high risk of treatment failure in systemic infections. Collectively, these results highlight a critical need for rigorous antibiotic stewardship and continuous surveillance, as the dominance of highly resistant E. faecium in the inpatient environment poses a formidable challenge to standard empirical treatment protocols.

The most striking observation is the 100% sensitivity to Linezolid, which stands as the only agent with total efficacy across all isolates, reinforcing its status as the definitive "reserve" antibiotic for these pathogens. This is followed by high susceptibility rates for Nitrofurantoin (approx. 90%) and Teicoplanin (approx. 85%), both of which remain highly effective, particularly for managing urinary tract infections

ISBN: 978-93-49468-00-9

where Enterococci were most frequently recovered. In sharp contrast, the isolates displayed a profound resistance profile against widely used classes; Ciprofloxacin and Levofloxacin faced massive resistance rates of approximately 80–85%, while Tetracycline showed a similarly high resistance of 75–80%. Traditional cell-wall active agents like Ampicillin and Penicillin also proved largely ineffective, with resistance levels hovering between 65% and 70%, a finding that aligns with the high prevalence of E. faecium identified in the hospital setting. Furthermore, moderate resistance was noted for Synercid and Rifampin, both exceeding 50% resistance. These overall susceptibility patterns, when viewed alongside the high rates of High-Level Aminoglycoside Resistance (HLAR) and the presence of Vancomycin-Resistant Enterococci (VRE), underscore a critical shift toward a more resistant clinical population. The data highlights a reliance on specialized antimicrobials and emphasizes that standard treatment like fluoroquinolones and penicillin are increasingly obsolete for Enterococcal management in this specific clinical environment, necessitating a strict adherence to culture-guided therapy and robust antibiotic stewardship.

Discussion

The study highlights a significant epidemiological shift, with E. faecium (49%) emerging as the dominant clinical isolate over E. faecalis (42%), primarily within hospital settings. This trend is alarming because 83% of all isolates originated from hospitalized patients, where high antimicrobial pressure drives resistance. Specifically, E. faecium exhibited a staggering 79% high-level Gentamicin resistance (HLGR) and 96.5% resistance to Ampicillin, compared to the significantly lower resistance rates found in E. faecalis.

A critical finding is the prevalence of multi-drug resistance (MDR); 28% (8 strains) of E. faecium were found to be resistant to all three major drug classes: Vancomycin, Ampicillin, and high-level Aminoglycosides. This MDR profile effectively neutralizes standard combination therapies, as the loss of synergism between cell-wall active agents and Aminoglycosides makes treating serious infections like bacteremia or endocarditis exceptionally difficult. The high resistance rates in this study are largely attributed to its tertiary care setting, where chronic cases and the widespread use of broad-spectrum antibiotics are common.

While Linezolid remains 100% effective, the rise of Vancomycin-Resistant Enterococci (VRE) and HLAR underscores a major threat to patient safety. To mitigate this, prompt recognition through effective laboratory detection methods and active surveillance for colonization is essential. Furthermore, the rationale use of antimicrobials—particularly limiting the use of 3rd generation Cephalosporins, which exert selection pressure for resistant strains—is of paramount significance. Ultimately, managing these formidable nosocomial pathogens requires a move away from empirical treatment toward strictly culture-guided therapy and robust infection control protocols to protect vulnerable, older patient populations.

Conclusion

In conclusion, this study underscores a critical shift in the clinical landscape of Enterococcus, with E. faecium emerging as the dominant and most resistant species in hospital settings. The high prevalence of Vancomycin Resistance (20.3%) and High-Level Aminoglycoside Resistance (40.6%), particularly the "triple resistance" observed in 28% of E. faecium strains, significantly compromises traditional synergistic therapies. While Linezolid remains a 100% effective last-resort option, the widespread resistance to ampicillin and fluoroquinolones necessitates a move away from empirical treatment. Ultimately, managing these formidable nosocomial pathogens requires regular screening, robust infection control, and strictly culture-guided antimicrobial therapy to limit the spread of multidrug-resistant infections in high-risk patient populations.

References

1. Agarwal, J., Kalyan, R. and Singh, M. 2009. High-level aminoglycoside resistance and β-Lactamase production in Enterococci at a tertiary care hospital in India. Jpn J Infect Dis, 62, pp: 158-159.
2. Baragundi, M. C., Sonth, S. B., Solabannawar, S., Patil, C. S. and Yemul, V. L. 2010. The species prevalence and the antimicrobial resistance pattern of the Enterococcal isolates in a tertiary health care centre. J of Clin and Diag Res, 4: 3405-09.
3. Bhat, K. G., Paul, C. and Ananthakrishna, N. C. 1998. Drug resistant Enterococci in a south Indian hospital. Trop Doct, 28: 106-7.
4. Clinical updates in infectious diseases. April 1998. Available at nfid.org. 2005.
5. De, A., Bindlish, A., Kumar, S. and Mathur, M. 2009. Vancomycin resistant Enterococci in a tertiary care hospital in Mumbai. Indian J Med Microbial, 27: 375-76.
6. Facklam, R. R. and Teixeira, L. M. 1998 Enterococcus. In: Lollier L, Balow A, Sussman M, editors. Topley & Wilson's microbiology and microbial infections. 9th Ed. New York: Oxford University Press; p. 669-82.
7. Jett, B. D., Huycke, M. M. and Gilmore, M. S. Virulence of Enterococci. Clin Microbiol Rev 1994; 7:462-78.
8. Linden, P. K., Pasculle, A. W., Manez, R., Kramer, D. J., Fung, J. J., Pinna, A. D. & Kusne, S. 1996. Differences in outcomes for patients with bacteremia due to vancomycin-resistant Enterococcus faecium or vancomycin susceptible E. faecium. Clin Infect Dis 22, 663-670.

9. Murray, B. E. 2005. The life and times of the Enterococcus. Clin Microbiol Rev 1990; 3:46-65. Available at nih.gov.

10. Oberoi, Loveena. and Aggarwal, Aruna. 2010. Multidrug resistant Enterococci in a rural tertiary care hospital- A cause of concern. www.jkscience.org Vol. 12 No. 3, pp: 157-158.

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