**RESEARCH PAPER**

# Characterization of High-Level Aminoglycoside Resistance and Vancomycin Resistance among Enterococcus Species Isolates in Various Clinical Samples

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**ABSTRACT**

**Background:** The increasing incidence of multidrug-resistant Enterococcus species, particularly those exhibiting high-level aminoglycoside resistance (HLAR) and vancomycin resistance (VRE), poses a significant challenge to effective clinical management and infection control. The present study aim to characterization of high level aminoglycoside resistance and vancomycin resistance among enterococci species isolates in various clinical samples.

**Method:**The present cross-sectional study was carried in the Department of Microbiology, Santosh Medical College and Hospital, Ghaziabad, Uttar Pradesh, India. The study was carried out for a period of 1 year. Total 366 samples including urine, blood, pus, BAL fluid, ET samples were collected. The samples were processed for enterococcal species identification by Microscopy, Culture, biochemical test identification by conventional and Vitek-2 both and antimicrobial susceptibility test were performed by Kirby Bauer disc diffusion test and Vitek -2.

**Results:** Out of 366 samples, 51 isolates of Enterococcus species were isolated. 49% were E. faecalis and E. faecium each and 1% were E. gallinarum isolates. Maximum number of isolates were from 0-20 years and above 60 years age. 53% isolates from female and 47% were from male cases. 56.86% urine, 27.45%, blood, 11.7% others and 3.92% pus were enterococcal isolates. Maximum number of isolates were from ICU 41.17% followed by 15.69% NICU. Urinary isolates were found sensitive to linezolid, daptomycin, Teicoplanin and Vancomycin by both methods. Blood isolates were sensitive to linezolid, teicoplanin, daptomycin, tigecycline. HLGM resistance and VRE in urinary enterococcal isolates were 68% and 24% respectively by vitek-2. HLGM resistance and VRE in blood isolates were 93% and 50% by vitek-2 respectively

**Conclusion:** The present study showed high percentage of HLGM and VRE among Enterococcus isolates. So its need of hour now a days to provide correct sensitivity with MIC so correct treatment can be given to patient in both resources limited and as well as equipped laboratories also aiding in the formulation of appropriate antimicrobial stewardship strategies and infection control policy.

**Keywords**: *Antibiogram, HLGM, VRE, Enterococcus, Antimicrobial Stewardship, Antibiotic susceptibility test*

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## INTRODUCTION

Enterococci are ubiquitously found Gram-positive bacteria which are widely distributed in nature, residing in varied environments. They prefer to inhabit the mucosal surface – gastrointestinal tract, the genitourinary system, and the oral cavity of the human and animals as a part of their normal microbial flora. They are not restricted to this host organism associated environments. Environmental reservoirs such as soil, freshwater bodies, marine ecosystems, and even the surfaces of plants are a few of the non-host habitats where they have been identified.

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Agricultural products, dairy products and fermented food items are some of the food-related sources.[1]

Generally, they are harmless in healthy people, but they can become infectious and cause certain disease in humans in certain situations like compromised immune system. Therefore, they are classified as opportunistic pathogens. Depending upon the tissue effecting Enterococcus can be responsible for a diverse array of infections in humans. One of the most prevalent infections caused by these bacteria is mild to complicated forms of urinary tract infection (UTI). In health care setting they are frequently implicated in surgical or traumatic wounds infections. In hospitalized or immunocompromised patients these organisms can also invade the bloodstream, leading to bacteraemia, a serious condition commonly observed. They can also cause catheter related infections. [2]

Enterococci can also cause several other severe and life-threatening conditions. They can infect the inner lining of the heart chambers and valves, leading to infective endocarditis. They can also lead to meningitis. They may contribute to the development of intra-abdominal abscesses and pelvic infections. These more invasive infections are typically seen following medical or surgical procedures that compromise the natural barriers of the body, allowing the bacteria to penetrate deeper tissues and establish infection.[2]

The pathogenicity of enterococci is largely attributed to their cell surface components. These include a protective polysaccharide capsule, various surface-associated adhesins, filamentous pili, and an aggregation substance that facilitates cell-to-cell contact. One of the most important virulence mechanisms is their ability to form robust biofilms. Biofilms enable the bacteria to adhere firmly to medical devices such as urinary catheters, prosthetic heart valves, and dental prostheses. Within these biofilm structures, bacteria are shielded from host immune defences and display significant resistance to antibiotic treatment. This biofilm-associated lifestyle contributes to chronic and often polymicrobial infections that are difficult to eradicate. [1,3]

Enterococci produce several virulence factors that contribute to their pathogenicity and provide them the advantageous feature in terms of environmental survival. Some of the secreted virulence factors are Cytolysin (bactericidal and cytolytic), antigen A (SagA) (interferes with cell wall metabolism and helps in binding to the extracellular matrix proteins) and Gelatinase (responsible for infective endocarditis). Some of the cell surface virulence factors are Aggregation substances (Ace, Asc10 and Scm), MSCRAMMs - microbial surface components recognizing adhesive matrix molecules (binds to extracellular matrix protein) and Pili (colonization of kidney and bladder). [4]

There are two major reasons because of which enterococci are globally posing as a significant clinical challenge: First, especially in hospitalized and immunocompromised patients there is a increasing incidence of infections caused by them and second is their increasing resistance to the commonly prescribed antibiotics, which is the most concerning problem. This includes resistance to β-lactam antibiotics, as well as high-level resistance to aminoglycosides, which are often used in combination therapy. One of the most important concerns is the emergence of glycopeptide-resistant strains, especially Enterococcus faecium, which are difficult to treat and associated with high morbidity and mortality rates.[5]

The current study aims to evaluate the patterns of antimicrobial resistance in Enterococcus species, with a specific focus on vancomycin resistance and on high-level aminoglycoside resistance (HLAR). Precise decision and selection of the most effective antimicrobial combination is of paramount importance during the early stages of treatment and it totally depends on the understanding of these resistance profiles. Improving the outcome of the patient's illness as well as curbing the inappropriate and excessive use of antibiotics should be the goal.

**MATERIALS AND METHODS:**

The present cross-sectional study was carried out in the Department of Microbiology, Santosh Medical College and Hospital, Ghaziabad, Uttar Pradesh, India after taking approval from institutional ethical committee by F. No. SU/R.2024/1350[94] Dated 17.05.2024. The sample size calculated by following formula $n= z^2 * P^* (1-P) / E^2$ where n =required sample size, Z=1.96 at 95% confidence level, P=39% prevalence rate [6], 1- P=0.61 at 61% non-prevalence rate and E=0.05 at 5% margin of error. The sample size was 366. All samples including urine, blood, bronchoalveolar lavage fluid, endotracheal tube aspirate and pus collected and processed in microbiology laboratory. All enterococcus isolates from clinical samples, all age groups, all genders, nonduplicates isolates were included and samples not collected and processed by standard protocol and open leaking container were excluded. Enterococcal species was isolated by culture on Blood agar and MacConkey agar and identification for species done by colony morphology, gram stain, catalase test, bile esculin test, growth on Potassium tellurite media 10 degree and 45degree, sugar fermentation test and Vitek-2 Compact. Antimicrobial susceptibility testing done by Kirby Bauer disc diffusion method and MIC determined by Vitek-2 Compact. The data was collected and analysed.

**RESULTS:**

**Table-1:** Demographic characteristics of study group

| S.NO | Demographic characteristics | | Number | Percentage |
|---|---|---|---|---|
| **1** | Age Group | 0-20 year | 25 | 29.41% |
| **2** | | 21-40 year | 11 | 21.57% |
| **3** | | 41-60 year | 09 | 17.65% |
| **4** | | Above 61 years | 14 | 31.37% |
| **1** | Sex wise | Male | 24 | 47.05% |
| **2** | | Female | 27 | 52.94% |

Table -1 showed out of 51 isolates maximum percentage of isolates from extreme of age group above 61 year 31.37% and 0-20 year 29.41%, 52.94% (27) isolates from female patients and 47.03% (24) were from male patients.

**Table-2:** Percentage of isolates from various samples

| S.NO | Sample type | E. faecalis 25(49%) | E. faecium 25(49%) | E. gallinarum 01(1.9%) | Total n (%) |
|---|---|---|---|---|---|
| 1 | Urine | 18(72%) | 10(40%) | 01(100%) | 29(56.86%) |
| 2 | Pus | 01(4%) | 01(4%) | 0 | 02(3.92%) |
| 3 | Blood | 03(12%) | 11(44%) | 0 | 14(27.45%) |
| 4 | Others | 03(12%) | 03(12%) | 0 | 04(11.74%) |
| **Total** | | 25(100%) | 25(100%) | 01(100%) | 51(100%) |

Table -2 showed maximum isolates of enterococcus isolated from urine samples (56.86%) followed by blood (27.45%), others samples including BAL fluid and ET aspirate etc (11.74%) and pus (3.92%). 72% E faecalis isolated from urine, 12% from blood and others samples, 4% from pus. 44% E. faecium isolated from blood, 40% from urine,12% from others samples and 4% from pus. E. gallinarum only one isolates from urine sample. Out of 366 samples ,51 enterococcus isolates were isolated. The Prevalence of E. faecalis and E. faecium was 49% (25 isolates) each and E. gallinarum 1.9%.

**Table-3:** Distribution of isolates IPD ward wise

| S.NO | IPD Ward | E. faecalis 25 (49%) | E. faecium 25 (49%) | E.gallinarum 01(1.9%) | Total n (%) |
|---|---|---|---|---|---|
| 1 | ICU | 3 | 18 | 0 | 21 (41.17) |
| 2 | NICU | 5 | 3 | 0 | 8 (15.69) |
| 3 | PICU | 1 | 1 | 1 | 3 (5.88) |
| 4 | Paediatrics | 3 | 0 | 0 | 3 (5.88) |
| 5 | Surgery | 4 | 1 | 0 | 5 (9.88) |
| 6 | Urology | 3 | 0 | 0 | 3 (5.88) |
| 7 | Medicine | 3 | 0 | 0 | 3 (5.88) |
| 8 | Emergency | 3 | 2 | 0 | 5 (9.80) |
| **Total** | | 25 (100%) | 25 (100%) | 01 (100%) | 51(100) |

Table-3 showed enterococcal isolates from ICU 41.1%, NICU 15.69%, Surgery and Emergency department 9.80% each department. 5.88% from PICU, paediatrics, urology and medicine each department. 18 isolates of E faecium isolated from ICU patients and E. faecalis isolated from all of the wards and maximum 5 isolates were from NICU. E. gallinarum single isolate isolated from PICU ward.

**Table-4:** Antimicrobial susceptibility of Enterococcus isolates from urine by Kirby Bauer disc diffusion and MIC by Vitek-2 compact

| S. No | Name of Antibiotic | Isolates from Urine (n=29) | | | Isolates from Urine(n=29) | | |
|---|---|---|---|---|---|---|---|
| | | Disc diffusion method | | | Vitek-2 | | |
| | | S | I | R | S | I | R |
| 1 | HLGM | 8 (27.59%) | 0 (0%) | 21 (72.41%) | 9 (32%) | 0(0%) | 20 (68%) |
| 2 | Vancomycin | 20 (69%) | 0 (0%) | 9 (33%) | 22 (76%) | 0 (0%) | 7 (24%) |
| 3 | Penicillin G | 17 (58%) | 0 (0%) | 12 (42%) | 17 (58%) | 0 (0%) | 12 (42%) |
| 4 | Erythromycin | 2 (6%) | 0 (0%) | 27 (93%) | 2 (6%) | 0 (0%) | 27 (94%) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 5 | Tetracycline | 5 (17%) | 0 (0%) | 24 (83%) | 5 (17%) | 0 (0%) | 24 (83%) |
| 6 | Teicoplanin | 20 (69%) | 0 (0%) | 9 (31%) | 20 (69%) | 0 (0%) | 9 (31%) |
| 7 | Linezolid | 24 (83%) | 0 (0%) | 5 (17%) | 25 (86%) | 0 (0%) | 4 (16%) |
| 8 | Nitrofurantoin | 14 (48%) | 8 (27%) | 7 (24%) | 14 (49%) | 8 (27%) | 7 (24%) |
| 9 | Daptomycin | 22 (79%) | 6 (17%) | 2 (3%) | 23 (79%) | 5 (17%) | 1 (3%) |
| 10 | Ciprofloxacin | 0 (0%) | 0 (0%) | 29 (100%) | 0 (0%) | 0 (0%) | 29 (100%) |
| 11 | Levofloxacin | 5 (17%) | 2 (^%) | 22 (75%) | 5 (17%) | 2 (6%) | 22 (76%) |

**S-sensitive, I- intermediate and R- Resistant**

Table-4 showed most of urinary isolates were found sensitive to linezolid, daptomycin, teicoplanin and vancomycin by both methods. The maximum resistance found for ciprofloxacin, tetracycline and levofloxacin by both methods. HLGM resistance found 72% by disc diffusion method and 68% by vitek-2. Vancomycin resistance was found 31% by disc diffusion method and 24% by vitek-2.

**Table-5:** Antimicrobial susceptibility of Enterococcus isolates from Blood by Kirby Bauer disc diffusion and MIC by Vitek-2 compact

| S. No. | Name of Antibiotic | Isolates from Blood (n=14) | | | Isolates from Blood (n=14) | | |
|---|---|---|---|---|---|---|---|
| | | Disc diffusion method | | | Vitek-2 | | |
| | | S | I | R | S | I | R |
| 1 | HLGM | 0 (0%) | 0 (0%) | 14 (100%) | 1 (7%) | 0 (0%) | 13 (93%) |
| 2 | Vancomycin | 4 (28%) | 3 (32%) | 7 (50%) | 5 (36%) | 2 (14%) | 7 (50%) |
| 3 | Penicillin G | 5 (36%) | 0 (0%) | 9 (64%) | 6 (43%) | 0 (0%) | 8 (57%) |
| 4 | Erythromycin | 4 (28%) | 2 (14%) | 8 (58%) | 5 (36%) | 1 (7%) | 8 (57%) |
| 5 | Tetracycline | 3 (28%) | 0 (0%) | 11 (72%) | 4 (28%) | 0 (0%) | 10 (72%) |
| 6 | Teicoplanin | 9 (65%) | 0 (0%) | 5 (35%) | 9 (64%) | 0 (0%) | 5 (36%) |
| 7 | Linezolid | 11 (79%) | 2 (14%) | 1 (7%) | 12 (86%) | 1 (7%) | 1 (7%) |
| 8 | Daptomycin | 5 (35%) | 3 (22%) | 6 (43%) | 8 (57%) | 0 (0%) | 6 (43%) |
| 9 | Ciprofloxacin | 2 (22%) | 2 (14%) | 10 (72%) | 1 (7%) | 0 (0%) | 13 (93%) |
| 10 | Levofloxacin | 3 (22%) | 2 (14%) | 9 (64%) | 3 (21%) | 2 (14%) | 9 (65%) |
| 11 | Tigecycline | 5 (36%) | 2 (14%) | 7 (50%) | 7 (50%) | 0 (0%) | 7 (50%) |

**S-sensitive, I- intermediate and R- Resistant**

Table-5 showed most of blood isolates were found sensitive to linezolid and teicoplanin by both methods. The maximum resistance found for HLGM, ciprofloxacin, erythromycin, levofloxacin, penicillin G, tetracycline and levofloxacin by both methods. HLGM resistance found 100% by disc diffusion method and 93% by vitek-2. Vancomycin resistance was found 50% by disc diffusion method and 50% by vitek-2.

**DISSCUSSION**

In present study a total of 366 samples including urine, blood, pus, BAL fluid, ET culture etc were collected from various sites of Santosh Hospital. Out of 366 samples, 51 isolates of Enterococcus species were isolated and subjected to phenotypic identification and automated vitek-2 identification with antimicrobial susceptibility testing by Kirby Bauer disc diffusion test and vitek-2. Enterococcus has emerged as a nosocomial pathogen capable of producing life threating infections in humans. Enterococcus ability to play two roles is enhanced by their intrinsic and acquired resistance to essentially all the antibiotics now in uses. The emergence of multidog resistant enterococcus to commonly used antibiotics, for example aminoglycosides and cephalosporins, is because of their ability to attain and transfer the drug resistance gene, giving rise to enterococci with high level aminoglycosides (HLAR) and glycopeptide resistance.[6] HLAR and VRE have becoming a major health concern for health care settings.

The spectrum of diseases caused by the various Enterococcus species ranges from urinary tract infections bacteraemia, particularly in immune-compromised patients, diabetic and decubitus ulcers, surgical site infections peritonitis, gingival infections and even root canal failure. [7,8] Enterococcus have acquired prominence as a result of recent occurrence in conjunction with serious symptoms infections and development of resistance.

Out of 51 isolates the maximum numbers of enterococcus isolates were found in two extreme of age group i.e. 15 isolates were from 0-20 year age group and 16 isolates were from above 61 years age group. (Table-1) Similar demographic observations were made by Sangita Chanda et al (2022) in their study from Mysore.[9] Mansi Mahajan et al (2024) also reported similar type of results in their study.[10] This may be done to greater exposure of patients to infection due to lower immunity in extreme of ages and sex dependent genetic factors.

Our study showed more isolates were from females (52.9%) in comparison to male (47.05%). The higher rate in females may be due to their increased risk of urinary

tract infections, which are commonly caused by Enterococcus species. Mansi Mahajan et al (2024) [10] in their study found more Enterococcus isolates from male patients than females. Such variations in results could be due to variation in participants of study. (Table-1)

In our study we found maximum numbers of isolates 56.8% were from urine samples followed by 27.45% were from blood, 11.76% were from others samples such as BAL, tissue specimen, swabs and ET culture and 3.92% were from pus (Table-2) Similar results were reported by Sangita Chanda et al 2022 and Priyam Barak et al 2025. [8,9] However, in a study done by Ayan Adan Moussa et al 2019 enterococci was predominantly isolated from pus samples 38.7% followed by blood 18.6%.[11]

In our study maximum number of isolates 41% were form ICU, 15.69% from NICU, 9.8% form surgery and emergency each and 5.88% were form PICU, Paediatrics ward and urology and medicine ward each respectively [Table 3]. Priyam Barak et al 2025 [8] also found in their study that 26.4% were from ICU and 23% were form OPD. In present study prevalence of Enterococcus species was 13.93% (51 out of 366). Percentage prevalence of Enterococcus faecalis and E. faecium were 49% each and E. gallinarum was 1%. Priyam Basak et al 2025 [8] reported 52.8% were E. faecalis and 37.6% were E. faecium and 9.6% belongs to other species. Ayan Aden Moussa et al 2019 [11], Kaarthiga et al 2020 [12] and Mansi Mahajan et al 2024 [10] also reported similar results. V. Sharifzadeh Peyvasti et al 2020 [13] found E. faecalis as predominant species 31.8% and E. faecium 2.6% in their study. (Table- 3)

HLAR and VRE both have become serious problem in most health care facilities. In our study HLGM resistance was found in 72.41% and Vancomycin resistance were found in 31% enterococcal isolates in urine samples. Enterococcus species isolated from urine were found sensitive to Linezolid (83%), Daptomycin (79%) and Teicoplanin and Vancomycin (69%) by disc diffusion method (Table 4). 68% enterococcal isolates were found HLGM resistant and 24% were found VRE by Vitek-2. Most of the isolates were found sensitive to Linezolid (86%), Daptomycin (79%) and Teicoplanin (69%) and Vancomycin (76%) by Vitek -2 (Table 4)

Out of 9 isolates of E. faecium 78% HLGM and VRE were 44% in urine samples by Vitek 2. Out of 18 isolates of E. faecalis from urine 83% were found HLGM and 22% were found VRE by Vitek -2. Maximum percentage of HLGM and VRE were found from urine samples. Urinary enterococcal isolates were found sensitive to Linezolid, Daptomycin and Teicoplanin by both methods of antibiotic sensitivity test Ie Disc diffusion and Vitek -2 (Table-4). Similar results were found by Kaarthiga et al 2020. [12] Nokkhiao N et al 2025 [14] also found all VRE isolates were susceptible to Linezolid. High level of gentamicin and streptomycin have been reported in many European countries with occurrence ranging from 1% to 48% with little or no difference in geographical prevalence among these countries where the studies were conducted. [12]

In our study 14 isolates were from blood samples. Out of which 79 % showed sensitivity to Linezolid, 65% were found sensitive to Teicoplanin, 36% were found sensitive to Tigecycline and 35% were found sensitive to Daptomycin. 100% isolates were found resistant to HLGM and 50% were found resistant to Vancomycin by disc diffusion method. 86% isolates were found to sensitive to Linezolid, 64% sensitive to Teicoplanin, 57% sensitive to daptomycin and 50% sensitive to Tigecycline by Vitek 2. 93% isolates were found resistant to HLGM and 50% were VRE by Vitek-2 (Vitek 2). Out of 11 isolates of E. faecium 81% HLGM and VRE were 45.5% in blood samples by Vitek 2. Out of 3 isolates of E. faecalis from blood 100% were found HLGM and all 3 isolates were found sensitive to Vancomycin Vitek -2. All isolates of E. faecalis from blood were found 100% sensitive to linezolid, vancomycin and Daptomycin and Penicillin G. while E. faecium showed linezolid 65% sensitive, tetracycline 65% sensitive, daptomycin 54% sensitive and vancomycin 45% sensitive. 81%. E. faecium isolates were found HLGM and 45.5% were VRE from blood samples. The pattern for HLGM resistance and VRE in urine and blood samples in our study almost found similar to study by Mittal S et al. [6]

In our study 47.05% isolates from blood and 41.1% isolates from urine were VRE. This may be due to excessive inappropriate use of vancomycin particularly in hospitalized patients. Nidhi Sood et al 2024 [16] found 20% resistant, Arif D et al [17] from UP and Rahangdale VA et al [18] from Nagpur that have reported VRE isolation rate of 30%and 11.38% respectively. The reason for high VRE rate in our study may be due to study group, antibiotic use along with geographical location. The percentage of HLGM resistant were higher in urinary isolates (57.8%) in comparison to blood (31.5%). Similar results were reported by other also. [8] Seema Mittal et al 2016 [6] reported HLAR to both gentamicin and streptomycin was found in 22% isolates specifically more in blood isolates.

**Limitation:**

Small sample size of our study also limitation so results cannot be generalized. Microbroth dilution for vancomycin MIC not included in our study due to cumbersome process along with more chances of contamination but we have included MIC determination by Vitek 2 along with identification of other species of enterococcus. Molecular testing for identification of prevalent gene responsible for drug resistance also major limitation of our study.

**Conclusions:**

Deficiency of well-equipped laboratory with hospital facilities along with inappropriate antimicrobial therapy with improper infection control policy and procedures leads to increase of multidrug resistant organism including VRE and HLGM resistant organisms. This study shows a significant percentage of high-level aminoglycoside resistance while lower percentage of vancomycin

resistance for Enterococci. Thus, it becomes important to laboratories and healthcare facilities to provide right treatment to right patient at right time to get best outcome along with affordable care to every patient. MIC determination for high-risk area and intermediate susceptible isolates is also necessary. It is also needed to go for antimicrobial stewardship and success of infection control program.

**Recommendation:**
Our study establishes the prevalence data of HLGM resistance and VRE and also provide species specific resistance trends for future studies. This also helpful for antibiotic policy and infection control along with antimicrobial stewardship program by highlighting the resistance trends. The use of disc diffusion method of AST in resource limited laboratory and MIC determination by Vitek 2 for high-risk area if facility available is best strategy to stop resistant bugs to spread.

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**CONFLICT OF INTEREST** -The authors declare that there is no conflict of interest.

**AUTHORS' CONTRIBUTION** - Priyanka Kumari and Geeta Gupta conceptualized and designed the study. Priyanka Kumari performed data collection. Priyanka Kumari and Geeta Gupta performed analysis and results interpretation. Priyanka Kumari, Geeta Gupta and Gajendra Kumar Gupta wrote the manuscript. All authors read and approved the final manuscript for publication.

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**DATA AVAILABILITY:** All datasets generated or analysed during this study were included in the manuscript

**ETHICS STATEMENT:** The study was approved by the Research Advisory Committee of Santosh Deemed to be University, Ghaziabad (F. No SU/R.2024/1350[94] Dated 17.05.2024.).

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