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# Species prevalence, antimicrobial susceptibility of enterococci isolated from various clinical samples in tertiary care hospital

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***Abstract***---Introduction: Emergence of multidrug resistant nosocomial enterococcus strains emphasizes the need for further investigating enterococci. Objectives: To characterize enterococci from various clinical specimens, to determine the antimicrobial susceptibility pattern and to explore the association between virulence factors and antimicrobial resistance. Material and Methods: Two hundred and eighty three clinical isolates of enterococcus were speciated and subjected to antimicrobial susceptibility testing using Kirby Bauer disc diffusion method. They were screened for vancomycin resistance by vancomycin screening agar method as recommended by Clinical Laboratory Standards Institute 2020, and confirmed by determination of minimum inhibitory concentration using agar dilution and E test. Genotypic confirmation was done by polymerase chain reaction. Results: Of the 283 enterococci isolated, 12 species were identified; predominant species was Enterococcus faecalis (82.33%). High level gentamicin resistance (HLGR) and vancomycin resistance were observed among 55.57% and 6.01% of Enterococcus isolates respectively. All vancomycin resistant enterococci (VRE) were Enterococcus faecalis and had vanA phenotype and genotype. Sensitivity to linezolid was 100 per cent among enterococci. Conclusion: Our study reveals the occurrence of sizable number of HLGR isolates and emergence of VRE.

***Keywords***---enterococcus, vancomycin resistant, HLGR

## Introduction

Enterococci have traditionally regarded as low grade pathogens, have emerged as an increasingly important cause of nosocomial infections in the last decade.

Although about a dozen enterococcus species have been identified, only two are responsible for the majority of human infections, i.e., Enterococcus faecalis and E. faecium [1]. Nevertheless, incidence of other species of enterococci from clinical sources shows an alarming increase with properties of intrinsic resistance to several antibiotics [2]. The species identified causing human infections includes E. avium, E. casseliflavus, E. durans, E. faecalis, E. faecium, E. gallinarum, E. hirae, E. malodoratus, E. mundtii, E. pseudoavium, E. raffinosus, E. solitarius, E. cecorum, E. columbae, E. saccharolyticus, E. dispar, E. sulfureus, E. seriolicida and E. flavescents [2,3].

The most common nosocomial infections produced by these organisms are urinary tract infections (associated with instrumentation and antimicrobial resistance), followed by intra-abdominal and pelvic infections. They also cause surgical wound infections, bacteraemia, endocarditis, neonatal sepsis and rarely meningitis [1]. One of the major reasons why these organisms have survived in the hospital environment is their intrinsic resistance to several commonly used antibiotics and, perhaps more important, their ability to acquire resistance to all currently available antibiotics, either by mutation or by receipt of foreign genetic material through the transfer of plasmids and transposons [4]. Because most enterococci are tolerant to the bactericidal activity of b-lactam and glycopeptide antibiotics, bactericidal synergy between one of these antibiotics and an aminoglycoside is needed to treat most serious enterococcal infections such as endocarditis and meningitis [4]. However, emergence of high level resistance to aminoglycosides (HLAR), β lactam antibiotics and to vancomycin by some strains, together with association of HLAR with multi drug resistance has led to failure of synergistic effects of combination therapy [5].

Until recently, vancomycin was virtually the only drug that could be consistently relied on for the treatment of infections caused by multidrug-resistant enterococci [4]. However, emergence of VRE and their increasing prevalence worldwide has made it difficult to treat serious enterococcal infections [1]. Epidemiological data also suggest that enterococci are important reservoirs for transmission of antibiotic resistance genes among different species of bacteria [4]. Thus, the occurrence of antimicrobial resistant enterococci, especially VRE is a persisting clinical problem in health care facilities in all geographical areas. Along with emergence of multidrug resistance, presence of several virulence factors in enterococci is an emerging concept [2]. Knowledge of the profile of enterococcal species and their antimicrobial resistance is quintessential for management and prevention of these bacteria in any healthcare facility and to formulate treatment guidelines for infections caused by enterococci. Knowing the paucity of data on enteroccous from Kumaon region this study was undertaken.

**Material and Methods**

Out of 283 enterococci, 279 enterococci have been speciated. Out of 279 enterococci, predominant species was Enterococcus faecalis 233 (82.33%), followed by E. hirae 10 (3.53%), E. dispar 09 (3.18%), E. durans 07 (2.47%), E. asini 04 (1.41%) and E. faecium 03 (1.06%). Enterococcus isolated from clinical sample over a period of two years in department of microbiology, at GMC Haldwani. Strains were isolated from Urine, Pus, Blood Culture and various

sterile body fluids. The isolates were identified to the genus and species level by the cultural characteristics, Gram's stain, motility testing and conventional biochemical tests using standard microbiological techniques (Facklam and Collins, 1989; Facklam and Teixeira, 1998). These included catalase negativity, growth on and blackening of bile-esculin agar, growth in the presence of 6.5% sodium chloride, tellurite reduction, pigment production, arginine dihydrolase reaction and generation of acid from mannitol, arabinose, sorbitol and raffinose. The carbohydrate fermentation reactions were performed in brain heart infusion broth containing 1% carbohydrate with bromocresol purple as indicator (Facklam and Collins, 1989; Facklam and Teixeira, 1998)

For studying the antimicrobial susceptibility pattern in enterococcal isolates, four methods were used (a) Kirby–Bauer disc diffusion technique,[5] (b) vancomycin screening agar method,[5] (c) minimum inhibitory concentration (MIC) testing by E strips, and (d) agar dilution method[5] for vancomycin. For disc diffusion testing, ampicillin (10 µg), high-level gentamicin (HLG) (120 µg), erythromycin (15 µg), vancomycin (30 µg), teicoplanin (30 µg), and linezolid (15 µg) discs were used. For urine isolates, additional discs of levofloxacin (5 µg), norfloxacin (10 µg), and nitrofurantoin (300 µg) were used. Antimicrobial resistance pattern of enteroccal isolate from various clinical specimens is depicted in Table 2. On the basis of MIC of vancomycin (>64 µg/ml) and teicoplanin (>16 µg/ml), all vancomycin-resistant isolates were categorized as Van A phenotype. A total of 17 VREs were detected phenotypically as VanA. Polymerase chain reaction (PCR) was performed for detection of VanA gene among VRE isolates. Briefly, the 25 µl of PCR contained 2–4 well-isolated colonies, 2.5 µl, ×10 PCR buffer, 2 µl, 25 Mm MgCl2, 1 µl, 10 Mm dNTPs, 1 µl, 10 pm forward primer (5'GCGATATTCAAAGCTCAGCAA3') 1 µl, 10 pm reverse primer(5'TGCCGATTCAATTGCGTAGTC3'), 0.5 µl Taq DNA, and 17 µl nuclease-free water. Reaction was performed in thermocycler, and initial denaturation was done at 94°C for 4 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 51.70°C for 1 min, extension at 72°C for 1 min, and final extension done at 72°C for 7 min. Amplified PCR products were detected by 1.5% agarose gel electrophoresis. All 17 VREs were detected phenotypically as VanA and were further confirmed by PCR as VanA genotype.

**Result**

Out of 283 enterococci, 279 enterococci have been speciated. Out of 279 enterococci, predominant species was Enterococcus faecalis 233 (82.33%), followed by E. hirae 10 (3.53%), E. dispar 09 (3.18%), E. durans 07 (2.47%), E. asini 04 (1.41%) and E. faecium 03 (1.06%) (Table 1). Of the 283 isolates, 55.57% had high level gentamicin, 82% were resistant to erythromycin, 54.77% to ampicillin, 49.13% to levofloxacin, 6.01% to vancomycin and teicoplanin. Among urinary isolates maximum resistance was seen against norfloxacin (87.93%), followed by minocycline (76.70%), high level gentamicin (55.47%), ampicillin (54.77%), levofloxacin (49.13%), nitrofurantoin (26.72) No resistance was seen against linezolid (TABLE 2).

**Antimicrobial resistance pattern of enterococci isolates from various clinical specimens**

Highest numbers of ampicillin resistance was observed in enterococci isolated from blood (67.27%) and lowest in pus (47.05%). High level gentamicin resistance was maximum in urine (63.79%) and minimum in body fluids (40%). Erythromycin resistance was highest in body fluids (80%) and lowest in blood (12.72%). Vancomycin and teicoplanin resistance were highest in body fluids (20%) and lowest in urine (0.86%) (TABLE 3).

**Antimicrobial resistance pattern of various species of enterococci**

Among various species of enterococcus highest resistance was seen in *E. faecalis* as shown in table 4.

Table 1
Distribution of Enterococcus species in various clinical specimens

| S.No | Species (n=283) | Urine | Pus | Blood | CSF | Bile | Peritoneal Fluid | Pleural Fluid |
|---|---|---|---|---|---|---|---|---|
| 1 | *E. faecalis* (n=233) | 95 | 86 | 44 | 04 | 01 | 02 | 01 |
| 2 | *E. hirae* (n=10) | 06 | 01 | 02 | | 01 | | |
| 3 | *E. dispar* (n=09) | 02 | 03 | 03 | 01 | | | |
| 4 | *E. durans* (n=07) | 03 | 03 | 01 | | | | |
| 5 | *E. asini* (n=04) | 02 | 01 | 01 | | | | |
| 6 | *E. cecorum* (n=04) | 01 | 01 | 01 | | | | |
| 7 | *E. caccae* (n=03) | 01 | 02 | | | | | |
| 8 | *E. faecium* (n=03) | 03 | | | | | | |
| 9 | *E. phoeniculicola* (n=02) | 02 | | | | | | |
| 10 | *E. avium* (n=02) | | 02 | | | | | |
| 11 | *E. italicus* (n=02) | | | 02 | | | | |
| 12 | *E. hermanniensis* (n=01) | | 01 | | | | | |
| 13 | Unidentified (n=04) | 01 | 02 | 01 | | | | |
| | Total | 116 | 102 | 55 | 05 | 02 | 02 | 01 |

Table 2
Antimicrobial susceptibility patterns of enterococci by Kirby- Bauer disc diffusion method

| Antibiotic | No of sensitive isolates (%) | No of resistant isolates (%) |
|---|---|---|
| Ampicillin | 128 (45.22) | 155 (54.77) |
| HLG | 126 (44.52) | 157 (55.47) |
| Erythromycin | 29 (17.36) | 138 (82.63) |
| Nitrofurantoin | 85 (73.27) | 31 (26.72 ) |
| Norfloxacin | 14 (12.06) | 102 (87.93) |
| Levofloxacin | 59 (50.86) | 57 (49.13) |

| | | |
|---|---|---|
| Minocycline | 27 (23.27) | 89 (76.70) |
| Vancomycin | 266 (93.99) | 17 (6.01) |
| Teicoplanin | 266 (93.99) | 17 (6.01) |
| Linezolid | 283 (100) | 0 (0) |

Table 3
Antimicrobial resistance pattern of enterococci isolates from various clinical specimens

| Specimens (n=283) | Amp No (%) | HLG No (%) | E No (%) | Nf No (%) | Nx No (%) | Lx No (%) | Mi No (%) | Va No (%) | Tei No (%) | Lz No (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| Urine (n=116) | 64 (55.17) | 74 (63.79) | - | 31 (26.72) | 102 (87.93) | 57 (49.13) | 89 (76.7) | 1 (0.86) | 1 (0.86) | 0 (0) |
| Pus (n=102) | 48 (47.05) | 43 (42.16) | 81 (79.41) | - | - | - | - | 6 (5.88) | 6 (5.88) | 0 (0) |
| Blood (n=55) | 37 (67.27) | 35 (63.63) | 48 (87.27) | - | - | - | - | 8 (14.55) | 8 (14.55) | 0 (0) |
| Body fluid (n=10) | 5 (50) | 4 (40) | 8 (80) | - | - | - | - | 2 (20) | 2 (20) | 0 (0) |

Amp- Ampicillin, HLG- High level gentamicin, E- Erythromycin, Nf- Nitrofurantoin, Nx- Norfloxacin, Lx- Levofloxacin, Mi-Minocycline, Va- Vancomycin, Tei- Teicoplanin, Lz- Linezolid

Table 4
Antimicrobial resistance (%) pattern of various species of enterococci

| Enterococcus Species | Amp | HLG | E | Nf | Nx | Lx | Mi | Va | Tei | Lz |
|---|---|---|---|---|---|---|---|---|---|---|
| *E. faecalis* (n=233) | 62.23 | 62.66 | 89.13 | 28.42 | 90.05 | 41.05 | 75.79) | 6.01 | 6.01 | 0 |
| *E. hirae* (n=10) | 50 | 30 | 50 | 33.33 | 66.67 | 66.67 | 66.67 | 0 | 0 | 0 |
| *E. dispar* (n=09) | 22.22 | 11.11 | 57.14 | 0 | 100 | 100 | 100 | 0 | 0 | 0 |
| *E. durans* (n=07) | 0 | 14.29 | 75 | 33.33 | 33.33 | 66.67 | 100 | 0 | 0 | 0 |
| *E. asini* (n=04) | 25 | 25 | 50 | 0 | 50 | 100 | 100 | 0 | 0 | 0 |
| *E. cecorum* (n=04) | 0 | 0 | 0 | 0 | 100 | 100 | 100 | 0 | 0 | 0 |
| *E. caccae* (n=03) | 0 | 33.33 | 50 | 0 | 100 | 100 | 100 | 0 | 0 | 0 |
| *E. faecium* (n=03) | 0 | 66.67 | 0 | 0 | 0 | 66.67 | 66.67 | 0 | 0 | 0 |
| *E. phoeniculicola* (n=02) | 0 | 0 | 0 | 0 | 100 | 100 | 50 | 0 | 0 | 0 |
| *E. avium* | 50 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| (n=02) | | | | | | | | | | |
| *E. italicus* (n=02) | 0 | 50 | 50 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *E. hermanniensis* (n=01) | 0 | 0 | 50 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Unidentified (n=04) | 25 | 25 | 33.33 | 100 | 100 | 100 | 100 | 0 | 0 | 0 |

Amp- Ampicillin, HLG- High level gentamicin, E- Erythromycin, Nf- Nitrofurantoin, Nx- Norfloxacin, Lx- Levofloxacin, Mi-Minocycline, Va- Vancomycin, Tei- Teicoplanin, Lz- Linezolid

**Discussion**

Enterococci, recognized as opportunistic pathogens, are natural inhabitants of the oral cavity, gut and the female genital tract in both humans and animals [1]. These are an important global cause of nosocomial infections. In the present study the maximum number of enterococci isolated from urine (40.99%), followed by pus (36.04%), blood (19.43%) and body fluids (3.53%). These findings are in concordance with other studies from India [6,7]. Ruoff et al [8] in their study also found maximum number of enterococci from urine (68.2%). Talebi et al [9] also reported 85% of enterococcal isolates from urine sample followed by pus (15.5%). Sivasankari et al [17] isolated maximum number of enterococci from urine sample followed by pus and described that urinary tract as commonest site of isolation of enterococci. Enterococci tend to be one of the leading causes of nosocomial infections, with E. faecalis and E. faecium accounting up to 90% of the clinical isolates [5,10,11,12]. Nevertheless, the incidence of other species of enterococci from clinical sources shows an alarming increase [13].

In a prospective study from North India, out of a total of 105 Enterococcus species recovered during the study period, E. faecium (42.90%) and E. faecalis (40.00%) constituted the predominant isolates. Enterococcus faecium was the commonest blood culture isolate while E. faecalis predominated pus and urine samples. In our study we isolated a total of 283 enterococci during study period from various clinical samples. The biochemical phenotyping results reveled 47 isolates belonging to ten different unusual species of enterococci (excluding E. faecalis and E. faecium) which included 10 E. hirae (3.53%), 9 E. dispar (3.18%), 7 E. durans (2.47%), 4 E. asini (1.41%) , 4 E. cecorum (1.41%) , E. caccae 03 (1.06%), E. phoeniculicola 02(1.06%), E. avium 02(0.71%), E. italicus 02(0.71%), E. hermanniensis 01(0.35%). The recent literature shows a drastic increase in the resistance pattern of the ampicillin among enterococci [14]. In our study ampicillin resistance was observed in 54.77%. Similar results were obtained by other studies from North India [15,16]. In a study among pediatric patients, 44 enterococi were isolated from blood and ampicillin resistance rate was found up to 72% [15]. However, in recent studies from South and East India reported lower level of resistance in ampicillin (22.8%) [6,17]. In the present study, isolates from blood showed higher level of resistance as compare to the isolates from other sources (urine, pus).

However, in contrast to our study, Chakraborty et al [6] in their study isolated enterococci from various clinical samples (urine, pus and blood) and found lowest resistance to each antibiotic in blood sample. In our study among urinary isolates 26.72 per cent of strains were resistant to nitrofurantoin. In our study high HLGR were observed among E. faecalis (62.66%) and E. faecium (66%), in comparison to other species isolated E. hirae (30%), E. dispar (11.11%), E. durans (14.29%). These results are in concordance with Sanal et al [18]. They also observed HLGR higher among E. faecalis (53.55%), E. faecium (53%) and16.7% in E. duran. Similar results were reported from other studies [17].

**Conclusion**

Our study reveals the problem of multiple drug resistant enterococci and emergence of glycopeptide resistant enterococci in our geographical area. Enterococci may serve as reservoir of drug resistance gene. It is also probable that frequency of infection caused by glycopeptide resistant enterococci will increase in our geographical area. To have an effective control of multidrug resistant enterococci, prudent use of antibiotic, better isolation procedures in hospitals and other patient care environments and improved and rapid surveillance measures are required.

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