# SPECIATION AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF *ENTEROCOCCI* FROM A RURAL TERTIARY HEALTH CARE CENTER- A TWO YEARS STUDY.

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## ABSTRACT

***Aims:*** *Enterococcus species are major nosocomial pathogen and are exhibiting vancomycin resistance with increasing frequency. Continouous monitoring and determination of antimicrobial susceptibility pattern is a necessity. The present study aims to determine the prevalence and susceptibility pattern of Enterococci in tertiary care hospital.* ***Methods and Material****: Total of 200 enterococcal strains isolated from various samples were identified and speciated by miniAPI(BioMeriux, France). Antibiotic susceptibility was determined for various drugs by Kirby bauer disc diffusion method. Results were interpreted as per CLSI guidelines and vancomycin MIC wasdetermined by Agar dilution method.*

***Results****: 138 strains were E.faecalis, 52 were E.faeciumand 6 were E.avium and 4 were E.durans. High level resistance to penicillin, ampicillin, gentamicin and streptomycin were observed. Most (95%)of the strains were sensitive to linezolid.12% strains showed vancomycinresistance.****Conclusions****: High rate of resistance to penicillin and amino glycosides is observed in our tertiary care hospital and emergence of VRE has further worsened this situation. So, there is an urgent need for more rational and restricted use of antimicrobials.*

Key words: Antimicrobial susceptibility, HLAR, VRE

 

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## Introduction

Speciation and antimicrobial susceptibility pattern of *Enterococci* from a rural tertiary health care center-A two years study.

Enterococci have attracted much attention in recent years due to their increased recognition as a cause of nosocomial infection in patients receiving antimicrobial agents.Serious enterococcal infections are often refractory to treatment and mortality is high.[1] Infections by *Enterococci* have traditionally been treated with cell-wall active agents in combination with an aminoglycosides however emergence of high level resistance to aminoglycosides, β lactam antibiotics and to vancomycin by some strains together with association of HLAR with multidrug resistance has led to failure of synergistic effects of combination therapy.[1, 2, 3]

Since the advent of VRE by Utley et al [5] in 1988, enterococcal infections have been a cause of great concern among the health professionals. Therefore, VRE along with HLAR is making the treatment of such infections extremely difficult and pose a great challenge to clinicians.

Although 12 species in genus *Enterococcus* have been recognized, most common species is *E.faecalis*followed by *E.faecium*. *E.faecium*predominantly is more resistant species than *E.faecalis*and emergence of vancomycin resistance in it has caused an increase in frequency of its isolation.[6]

The present study was undertaken considering the paucity of data on high level aminoglycoside resistance (HLAR) and Vancomycin resistance in enterococci, especially from a rural set –up and due the fact that enterococci are second leading cause of hospital acquired infection.

## Material and method

Thepresent study was conducted fromOctober 2010 to October 2012, in the Department of Microbiology, UP Rural Institute of Medical Sciences and Research. Our hospital primarily caters to the rural population of western UP.Ethical clearance for the study was taken from Ethical Committee of the Institute.

A total of 200 enterococcal strains were isolated from various clinical samples (urine, blood, pus, high vaginal swab, ascetic fluid, bile) andidentified and speciated by biochemical tests [rapid ID 32 STREP (Mini API®BioMérieux SA, France)]. All isolates were stocked in duplicate for further testing.

Antibiotic susceptibility testing was done by Modified Kirby Bauer disc diffusion method using discs and Mueller Hinton agar as per CLSI guidelines.Various antibiotics tested were: Penicillin (10U/disc), Ampicillin (10µg), Tetracycline(30 µg), High

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Strength gentamicin (120µg), High Strength Streptomycin (300µg), Ciprofloxacin (5µg), Vancomycin (30µg), Teicoplanin (30µg), Dalfopristin-Quinopristin (15 µg) and Linezolid (30µg). The minimum inhibitory concentrations of Vancomycin were determined by Agar dilution method (range from 2µg /ml to 1024 µg /ml).

The source of media, antibiotic discs and Vancomycin powder were Hi- media ltd. Standard strains *E.faecalis*ATCC 29212 was used as control.

## Results

A total 200 enterococcal isolateswere recovered from various clinical samples during the study period, of which, 138 strains were *E.faecalis*, 52 were *E.faecium*and 6 were *E.avium*and 4 were *E.durans*.The maximum numbers of isolates [91(45.5%)] were obtained from patients admitted to the intensive care units (ICUs), followed by surgical wards [69 (34.5%)] and medical wards [40(20%)]. Antibiotic susceptibility tests showed high level resistance to various antibiotics tested. [Table 1] Most of the strains (95%) the strains were sensitive to linezolid. High-level aminoglycoside resistance (HLAR) to Gentamycin was shown by 102 (51%) and to Streptomycin by 94 (47%) enterococcal isolates respectively. Among glycopeptides, 92% Enterococci were sensitive to teicoplanin and 12% strains showed vancomycin resistance which were confirmed by Agar Dilution Test. The MIC of VRE ranged from 16-256µg/ml.

## Discussion

Enterococci have become important nosocomial pathogens worldwide and are associated with a high mortality[1-3]. Further their infections poses a great challenge due to the inherent resistance of Enterococci to many antibiotics.

In the present study, *E.faecalis*(68%) was the predominant isolate followed by *E.faecium*(26%). Various studies done on Enterococci support the same findings.[9]

Beta-lactams along with aminoglycosides are considered as treatment of choice. Therefore resistance of Enterococci against these antibiotics has important clinical implications. Present study showed 69% and 66% resistance to Penicillin and Ampicillin respectively. Resistance to Penicillin may be due to low affinity penicillin binding proteins or due to production of β-lactamases.

In our study, HLAR was seen in 51% of the strains for Gentamicin (High Level) and 47% for Streptomycin (High level). [Table 2] HLAR was more in *E.faecium*than*E.faecalis*. These finding also reported in some study.[10,12] HLAR in these strains can well nullify the efficacy of combination therapy. Therefore, distinguishing HLAR from simple intrinsic resistance is important and should be adopted as a part of routine microbiology laboratory.

We, in this rural set up, found that the prevalence of HLAR among Enterococci to be lower than reported from urban

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hospitals.[13, 16] Thereason for higher prevalence in urban hospitals could be because of the set up where chronic cases are prevalent and there is wider usage of broad spectrum antibiotics.

Present study showed 12% vancomycin resistance. 7.5% strains were *E.faecalis*and 4.5% were *E.faecium*.Results were also compared with Agar dilution method, which is based on MIC values.VanA (66.67%) and VanB(33.33%) phenotype were found to be predominant with MIC value 16-256µg/ml. Most (58.3%)of the VRE strains were isolated from urine, followed 16.67% each from blood and pus. Previously from India, there are few reports of emergence of VRE strains with increased MIC values.[12,13,14,15,17] [Table 5]. Most Isolates (95%) were sensitive to Linezolid. Amongst 24 VRE, 5isolates were resistant to Linezolid. Linezolid can be considered as drug of choice to treat infections with VRE, however resistance to it has been reported in many studies.[18,19,20]Hence judicious use of Linezolid is highly recommended.

## CONCLUSION

High rate of resistance to penicillin and amino glycosides along with increased MIC values is observed in our tertiary care hospital and emergence of VRE strains has further worsened this situation. Prompt diagnosis and efficient infection control measures can restrict its spread. There is a need to study the antibiogram of enterococcal strains in order to minimize the selection and spread of such strains.

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**TABLE 1:** Antimicrobial susceptibility pattern of Enterococci by modified Kirby Bauer Disc Diffusion Test

| Antibiotic tested | % Sensitive | % Resistant |
|---|---|---|
| Penicillin | 31 | 69 |
| Ampicillin | 34 | 66 |
| Tetracycline | 40 | 60 |
| High Strength Gentamycin | 49 | 51 |
| High Strength Streptomycin | 53 | 47 |
| Erythromycin | 19 | 81 |
| Vancomycin | 88 | 12 |
| Teicoplanin | 92 | 8 |
| Linezolid | 95 | 5 |
| Dalfopristin-Quinopristin | 85 | 15 |
| Ciprofloxacin | 13 | 87 |

**Table 2: Frequency of HLAR among Enterococcus isolates**

| Specimen | Species | Total no. of isolates | High level resistance to Gentamycin | High level resistance to Streptomycin |
|---|---|---|---|---|
| Urine (n=136) | E.faecalis | 100 | 33 | 31 |
| | E.faecium | 30 | 29 | 27 |
| | Other spp | 06 | 06 | 04 |
| Blood (n=14) | E.faecalis | 07 | 03 | 04 |
| | E.faecium | 07 | 06 | 05 |
| | Other spp | 00 | 00 | 00 |

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| | | | | |
|---|---|---|---|---|
| Other specimen (pus, vaginal swab, bile, ascitic fluid) (n=50) | E.faecalis<br>E.faecium<br>Other spp | 31<br>15<br>04 | 08<br>13<br>04 | 06<br>13<br>02 |

**TABLE 3**

Total VRE isolation from different samples

| Sample | Vancomycin resistant *E.faecalis* | Vancomycin resistant *E.faecium* | No. of VRE |
|---|---|---|---|
| Urine (n=136) | 9 | 5 | 14 |
| Blood (n=14) | 2 | 2 | 4 |
| Pus (n=22) | 3 | 1 | 4 |
| HVS (n=18) | 0 | 0 | 0 |
| Ascitic fluid (n=5) | 1 | 1 | 2 |
| Bile (n=5) | 0 | 0 | 0 |
| Total | 15 | 9 | 24 |

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**Table 4**. Characteristics of vancomycin resistant enterococci isolated in the present study

| Isolate no. | Source | Icu/ward | Zone diameter (mm)/interpretation | | MIC(μg/ml) Agar plate test | Van phenotype |
|---|---|---|---|---|---|---|
| | | | Vancomycin | Tiecoplanin | | |
| 1 | Urine | ICU | 6 (R) | 6 (R) | 64 | Van A |
| 2 | Urine | ICU | 6 (R) | 6 (R) | 128 | Van A |
| 3 | Blood | ICU | 6 (R) | 6 (R) | 128 | Van A |
| 4 | Urine | FSW | 10 (R) | 14 (S) | 32 | Van B |
| 5 | Urine | GW | 10 (R) | 8 (R) | 64 | Van A |
| 6 | Ascitic fluid | ICU | 6 (R) | 14 (S) | 16 | Van B |
| 7 | Pus | MSW | 10 (R) | 8 (R) | 64 | Van A |
| 8 | Blood | NICU | 10 (R) | 10 (R) | 32 | Van A |
| 9 | Pus | ICU | 8 (R) | 8 (R) | 128 | Van A |
| 10 | Urine | MSW | 8 (R) | 8 (R) | 64 | Van A |
| 11 | Blood | ICU | 6 (R) | 6 (R) | 256 | Van A |
| 12 | Ascitic Fluid | ICU | 10 (R) | 16(S) | 16 | Van B |
| 13 | Urine | ICU | 10 (R) | 14(S) | 32 | Van B |
| 14 | Urine | MMW | 8 (R) | 6 (R) | 128 | Van A |
| 15 | Urine | FSW | 8 (R) | 16(S) | 64 | Van B |
| 16 | Blood | NICU | 6 (R) | 14(S) | 256 | Van B |
| 17 | Urine | FSW | 8 (R) | 8 (R) | 128 | Van A |
| 18 | Pus | MSW | 6 (R) | 8 (R) | 256 | Van A |

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| | | | | | | |
|---|---|---|---|---|---|---|
| 19 | Urine | MMW | 10(R) | 18 (S) | 16 | Van B |
| 20 | Urine | ICU | 8 (R) | 10 (R) | 64 | Van A |
| 21 | Pus | MSW | 6 (R) | 8 (R) | 128 | Van A |
| 22 | Urine | GW | 8 (R) | 14(S) | 64 | Van B |
| 23 | Urine | ICU | 6 (R) | 16 (R) | 256 | Van A |
| 24 | Urine | ICU | 6 (R) | 8 (R) | 256 | Van A |

ICU-Intensive Care Unit, NICU-Neonatal ICU, FSW-Female Surgical Ward, MSW-Male surgical Ward,MMW-Male Medical Ward, GW-Gyn&Obsward

Table 5. Comparison of VRE isolation from other studies

| | Mathur et al (2003) | Karmaker et al (2004) | Ghoshal et al (2006) | Shah et al (2011) | Present study (2013) |
|---|---|---|---|---|---|
| Total samples | 444 | 52 | 685 | 92 | 200 |
| VRE(%) | 5 (1%) | 12 (23%) | 10(1.4%) | 8 (8%) | 24(12%) |
| Positive samples | Blood(3), Urine(1), soft tissue(1) | Urine*, Blood*, Pus* | Blood*, Tissue*, Urine*, CVP tip* | Urine(5), Blood(2), CSF(1) | Urine(14) Blood(4) Pus(4) Asciticfluid(2) |
| Phenotypes | Van A,VanB | VanB | Van A | Van A, Van B | Van A, VanB |
| MIC (µg/ml) | 16-512 | >4 | 64-256 | 8-32 | 16-256 |

* Sample size not mentioned