Original Article

PHENOTYPIC CHARACTERISATION OF *ENTEROCOCCI* FROM URINE SAMPLES

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ABSTRACT:

Introduction: *Enterococci* are one of the leading causes of health care associated infection. The clinical significance of *Enterococci* is directly linked to its resistance traits which contribute to the risk of infection and colonization.

Aims & Objectives: The present study aim to detect the phenotypic traits of *Enterococci* and its antimicrobial resistance pattern.

Materials & Methods: 708 isolates of *Enterococci* from urine samples were identified into species level by sugar utilization test using brain heart infusion broth containing 1% sugars. The Minimum Inhibitory Concentration (MIC) of the isolates to various antibiotics was determined by Vitek 2 automated system.

Results: *Enterococcus faecalis* (93.64%) was the predominant isolate obtained followed by *E. Faecium* (4.24%), *E. avium* (1.70%) and *E. Durans* (0.42%). 13 (1.84%), out of 708 isolates were identified as VRE. Van a phenotype was identified in two isolates of *E. faecalis* with Vancomycin MIC of 8 and 16µg/ml. Ten isolates of *E. faecalis* exhibited glycopeptides MIC in the susceptibility range (1-4µg/ml) and showed the presence of Van a phenotype by vitek. Van a phenotype was observed in a single isolate of *E. faecium* with Vancomycin MIC (≥ 32µg/ml) and Teicoplanin MIC (32µg/ml).

Isolate with Van a phenotype was found to be resistant to Penicillin, High Level Gentamicin, Fluoroquinolones, and Macrolides.

Conclusion: Identification to species level is important for appropriate therapy of Enterococcal infection. Phenotypic detection of VRE helps to monitor the resistance pattern of clinical isolates, thereby limiting the spread of bacterial resistance.

Key Words: *Enterococcus* species, Van Phenotypes, High Level Gentamicin Resistance, Penicillin Resistance.

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INTRODUCTION:

Enterococcus is part of normal gut flora of humans and often colonizes oral cavity, genitourinary tract and skin. Nosocomial urinary tract infections (UTI's) are the most frequent infection caused by these organisms. The predisposing conditions for urinary colonisation and infection by *Enterococci* rise with patients who have structural abnormalities, recurrent urinary tract infections, frequent instrumentation and prior therapy with antibiotics.[1]

The antimicrobial therapy of *Enterococci* infection is complicated due to its innate mechanism of resistance and the ability to develop resistance to broad spectrum antibiotics. Glycopeptide antibiotics are the first choice alternative drug to penicillin- aminoglycoside combination to treat severe *Enterococci* infection.[2]

Vancomycin Resistant *Enterococci* (VRE) are being reported from various parts of the world. High level resistance to glycopeptides is conferred by the modification in the terminal peptidoglycan precursors from D-Ala-D-Ala to D-Ala-D-Lac. Low level resistance is due to peptidoglycan precursors ending in D-Ala-D-Ser, which decreases its binding ability to glycopeptides.[2,3]

The epidemiology of *Enterococci* varies with geographical location and *Enterococci* species varies in their susceptibility pattern to glycopeptides. Hence phenotypic characterization of *Enterococci* is mandatory for the management of infection.

AIMS & OBJECTIVES:

To isolate and identify *Enterococci* from urine samples

To determine phenotypic characteristics of *Enterococci* and their antimicrobial susceptibility pattern.

MATERIALS & METHODS:

TYPE OF STUDY: Cross sectional study

INCLUSION CRITERIA: Patients of all age group isolates of *Enterococci* in pure culture from urine samples.

EXCLUSION CRITERIA: Non enterococcal infection clinically insignificant isolates of *Enterococci.* The present study was done at Department of Microbiology from January 2015 to March 2019. A total of 708 isolates collected from inpatients and outpatients at VIMS hospital and VMKV Medical College, Salem were included in this study. A loopful of the sample was inoculated onto Blood agar and MacConkey agar. The plates were incubated at 37^0C overnight for 24-48 hours.[4,5]A colony count of more than 10^5 CFU / ml was considered as significant bacteriuria. Antimicrobial susceptibility testing of *Enterococci* was determined by Vitek 2 automated system according to manufacturer's instructions. Detection of Minimum inhibitory concentrations was performed for Penicillin, Erythromycin, Vancomycin, Teicoplanin, Ciprofloxacin, Tigecycline, Tetracycline, Nitrofurantoin, Linezolid and Daptomycin. [6]Isolates were identified as genus *Enterococcus* based on gram staining, colony morphology, catalase test, growth in 6.5% Nacl and bile esculin agar, heat tolerance test etc.

IDENTIFICATION OF GENUS *ENTEROCOCCUS*:

GRAM STAIN: From the solid media, small portion of colonies were picked up with a loop and mixed on a slide with loopful of sterile normal saline to form a light suspension. Stain was applied to heat fixed smear. Presence of gram positive oval cocci in pairs and short chains indicated the presence of *Enterococci.*

CATALASE TEST: A part of colony was emulsified in saline on a clean slide and a drop of 3 % hydrogen peroxide was added to the suspension. A positive test is indicated by the appearance of effervescence. No effervescence confirmed the absence of enzyme

TOLERANCE WITH 6.5% NACL: The colonies were inoculated in nutrient broth with 6.5% NaCl, and incubated at 37^0C for 3 days. The tubes were checked for any increase in turbidity and further confirmed by sub-culturing onto blood agar plate. Turbidity confirmed the presence of organism, indicated the ability of *Enterococci* to grow in this concentration.[4,5]

HEAT TOLERANCE TEST: *Enterococci* were inoculated in nutrient broth and incubated in water bath set at 10^0C and 45^0C for half an hour, and sub-cultured on blood agar plate. *Enterococci* survived in both the temperature showed positive reaction.[4,7]

BILE ESCULIN TEST: Colonies from overnight culture was inoculated in media containing 40% bile. Blackening of the medium confirmed the positive reaction.[7]

IDENTIFICATION OF *ENTEROCOCCUS* SPECIES:

MOTILITY TEST: Stab culture of the colony was done in a semi-solid media and motility was indicated by the spread of the organism into the media. The strains which did not diffuse into semisolid media were considered non-motile. Tubes were incubated for upto 7 days for confirming motility.[8]

ARGININE TEST: A well isolated colony of *Enterococcus* from the culture was inoculated in Moeller's arginine decarboxylase media overlaid with mineral oil and incubated at 37^0C for 48 hours. The change in colour of the tube to purple indicated positive reaction. Yellow colour indicated only acid production and no deamination.[8]

POTASSIUM TELLURITE REDUCTION TEST: Colonies were inoculated onto media containing 0.04% potassium tellurite and incubated for 48 hours. *E. faecalis* formed black colonies with the reduction of tellurite to tellurium.[5]

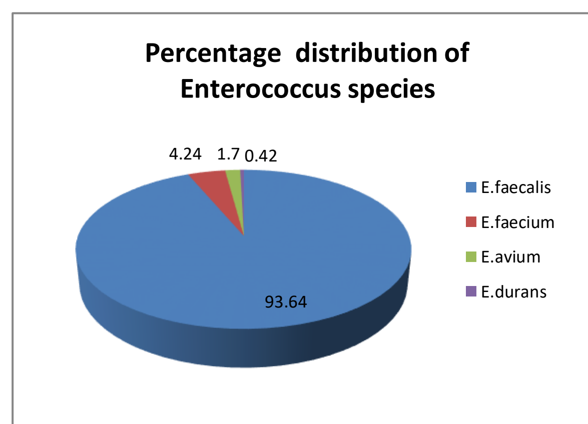
PIGMENT PRODUCTION: Ability to produce pigment was tested by sub culturing colonies in nutrient agar and any colour produced was further confirmed by touching the colony with a sterile swab.[8]

SUGAR FERMENTATION TEST: Species identification was further done by sugar utilization by inoculating the organism in brain heart infusion broth containing 1% sugars with Bromothymol blue indicator. The sugars used were Mannitol, Sorbitol, Sorbose, Arabinose, Raffinose, Lactose and Sucrose. Any change in colour from green to yellow indicated positive for sugar fermentation. Absence of colour change was taken as negative.[8]

RESULTS:

Among the *Enterococci* isolates from urine 663 were *Enterococcus faecalis*, 30 were *E. faecium*, 12 were *E. avium* and 3 were *E. durans*. (**Fig: 1**)

Fig 1: Distribution of *Enterococcus* species from urine sample



69 (10.41%) isolates of *E. faecalis* and 6 (20.00%) isolates of *E. faecium* were resistant to Penicillin with MIC of 16µg/ml.399 (60.18%) isolates of *E. faecalis* and 21(70.00%) isolates of *E. faecium*, were resistant to Ciprofloxacin with MIC ≥ 8µg/ml. 6 (0.90%) isolates of *E. faecalis* from urine were resistant to Linezolid with MIC ≥ 8µg/ml. [**Table: 1**]

Among the isolates resistant to Nitrofurantoin, 111 (16.74%) were *E. faecalis* with MIC of >128µg/ml and 12 (1.81%) isolates of *E. faecalis* showed MIC of 256µg/ml. 6 (20.00%) isolates of *E. faecium* were resistant with MIC of 128µg/ml.

Table 1: Detection of antimicrobial resistance among *Enterococci* isolates by MIC method.

Antibiotics	Susceptible	Resistant
Penicillin	**577(81.50%)**	**131(18.50%)**
High Level Gentamicin	**312(44.07%)**	**396(55.93%)**
Ciprofloxacin	**252(35.59%)**	**456(64.41%)**
Erythromycin	**126(17.80%)**	**582(82.20%)**
Linezolid	**702(99.15%)**	**6(0.85%)**
Nitrofurantoin	**480(67.80%)**	**228(32.20%)**
Vancomycin	**705(99.58%)**	**3(0.42%)**
Teicoplanin	**707(99.86%)**	**1(0.14%)**

537 (81.00%) isolates of *E. faecalis* showed MIC of 0.5µg/ml for Teicoplanin. The Teicoplanin MIC for 29/30 (96.67%) isolates of *E. faecium* were 0.5µg/ml and one isolate showed MIC of 32µg/ml.

13 (1.84%), out of 708 isolates were identified as VRE. Van A phenotype was identified in two isolates of *E. faecalis* with Vancomycin MIC of 8 and 16µg/ml. Ten isolates of *E. faecalis* exhibited glycopeptide MIC in the susceptibility range (1-4µg/ml) and showed the presence of Van A phenotype by vitek**. [Fig: 2]** Van A phenotype was observed in a single isolate of *E. faecium* with Vancomycin MIC (≥ 32µg/ml) and Teicoplanin MIC (32µg/ml). **[Fig: 3] (1-32µg/ml) by Vitek**

Fig 2: Detection of *E. faecalis* phenotypes

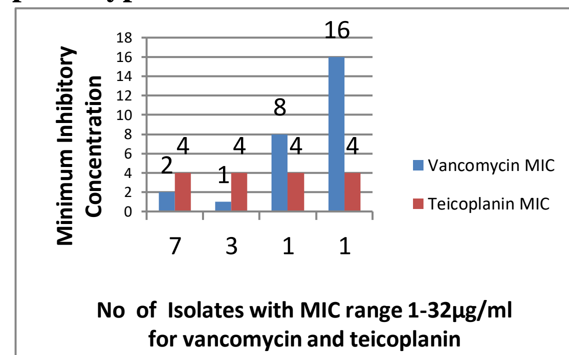
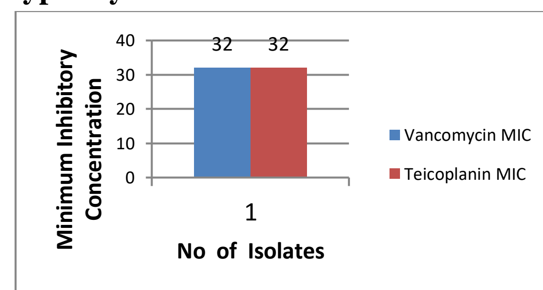


Fig 3: Detection of *E. faecium* pheno types by Vitek



DISCUSSION:

The identification of *Enterococci*to species level is essential as each species varies in their resistance to antibiotics and species level identification may be useful for epidemiologic surveillance within hospitals. In the present study the most common species isolated was *E. faecalis* (93.64%) followed by *E. faecium* (4.24%) and *E. avium* (1.70%). A study finding by PJ Desai et al., have showed 49.50% of *E. faecalis* and 35.64% *E. faecium* isolates from urinary tract infection.[9] A study conducted by N Taneja et al. , have reported *Enterococcus faecalis* (55%) as predominant isolate from urine sample.[10]

The penicillin MIC for 10.41% *E. faecalis* and 20.00% isolates of *E. faecium* were 16µg/ml. In a similar study, M R Maradia

et al., have reported 97.44% isolates from urine resistant to Penicillin.[11] In a North Indian study by N Gangurde et al, *E. faecium* isolates exhibited significantly high resistance to penicillin as compared to *E. faecalis*.[12] In the present study no statistically significant difference in resistance to Penicillin was found between *E. faecalis* and *E. faecium* isolates (p value >0.05).

In the present study, 14.93% *E. faecalis* were resistant to Nitrofurantoin with MIC of 128µg/ml. In a study from South India by K Suresh et al., 100% isolates showed susceptibility to Nitrofurantoin.[13] A similar study by D A tray et al., have reported 80% urinary isolates susceptible to Nitrofurantoin.[14]

The present study reports two isolates of *Enterococcus faecalis* showing intermediate resistance to Vancomycin. Van A phenotype was identified in two isolates of *E. faecalis* with Vancomycin MIC of 8 and 16µg/ml. Presence of Van A phenotype among *E. faecalis* have been reported from various Indian studies.[15,16,17,18]. *Enterococcus faecium* isolate with Van a phenotype was found to be resistant to Penicillin, High Level Gentamicin, Fluoroquinolones, and Macrolides.

CONCLUSION: There has been a rapid increase in the incidence of VRE infection due to extended use of Vancomycin and extended spectrum Cephalosporins in health care settings. The ability of these organisms to transfer Vancomycin-resistance genes to other gram positive organisms is a cause of clinical concern. Phenotypic characterisation of *Enterococci* is essential to detect low-level glycopeptide resistance and to distinguish between the different Van phenotypes.

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