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Vancomycin Resistant Enterococci Isolated from Urinary Tract Infections in Tertiary Care Hospital

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Abstract

Background: Vancomycin resistant enterococcus (VRE) is an important emerging pathogen responsible for causing urinary tract infection (UTI). Hence precise and complete knowledge of its prevalence and antimicrobial resistance profile is necessary in particular area for their adequate treatment. **Material and Methods:** This study was carried out in bacteriology section, Department of Microbiology of Teerthanker Mahaveer hospital, Moradabad from July 2021 to June 2022 by performing urine sample collection, culture and different biochemical tests. **Results:** Out of 898 samples, there were 252 gram positive cocci, 430 gram negative bacilli, 37 yeast and 179 samples showed no growth. 252 gram positive cocci were further processed and 72 isolates of enterococci were isolated. Maximum numbers of enterococci were obtained from patients between 31-40 years. **Conclusion:** Patient should be empirically treated for Enterococcal diseases according to the local patterns of antibiotic resistance. VRE are generally treated with fosfomycin, nitrofurantoin or linezolid.

Keywords: Enterococci, Urinary tract infection and Vancomycin.

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Introduction

Enterococcus genus contains gram positive bacteria with more than 29 species present as angulated pairs in human gastro intestinal tract as normal flora as well noscomial pathogen.[1] Recent studies have shown Enterococcus faecalis isolated as the commonest species (>.80%) afterwards followed by E. faecium (10-15%). Some rare species isolated in india being E. durans, E.avium, E. casseliflavus, E. gallinarum, etc.[1,2] Association of Enterococcus have been found with several infections like complicated urinary tract infection (UTI), peritoneal and abdominal abscesses, septicaemia, wound infection and catheter related infection.[3] Enterococcus has been listed as the 2nd common cause of healthcare associated UTI by CDC (Center for Disease Control) and NNSS (National Nosocomial Surveillance Survey),[4] with limited antibiotic options and emergence of vancomycin resistance from every part of the world, raises the concern of availability of antibiotic with bactericidal activity against enterococcus (VRE).[5]

It is necessary to deliver precise antimicrobial resistance pattern and latest protocols for management of Enterococcal urinary tract infections. Researches in this field are necessary to understand the epidemiological pattern of VRE infection in these areas by investigating VRE cases.[6]

Material and Methods

The present study was conducted in the bacteriology section of Department of Microbiology, Teerthanker Mahaveer hospital, Moradabad. The samples were obtained from patients admitted or attending all outpatient departments. 898 clinical samples were processed over

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the period of 12 months from July 2021 to June 2022. This is a prospective study satisfying inclusion and exclusion criteria. Urine samples were collected in clean, wide mouthed, air tight and sterile universal container. All samples were immediately transported to the laboratory. culture were carried out of total 898 samples and they were categorized into gram positive cocci (252), gram negative bacilli (430), yeast (37) and no micro-organism seen (179).

Following mentioned Kass criteria was used for result interpretation, especially for gram negative bacteria.

10^5 cfu/ml	Significant
10^4 to 10^3 cfu/ml	Doubtful significant
10^2 cfu/ml	Insignificant

Note: 10^2 cfu/ml is significant for urine sample in case of staphylococcus aureus.
Presumptive identification of Enterococci was done on the following basis.

- Culture on CLED agar
- Catalase test
- Growth and bile esculin agar blackening
- Growth at 10^0 and 45^0 C for Enterococci
- Growth in 6.5% NaCl for Enterococci
- Motility test
- PYRase test
- H2S production
- Ammonia from arginine deamination

Antibiotic sensitivity testing (AST):[7]

AST was performed on all isolates by Kirby Bauer technique (Disc diffusion method). Media used for AST was Muller-Hinton Casein hydrolysate agar (MHA) with 5% sheep blood.

Inoculum preparation:

Bacterial colonies were emulsified in BHI broth and incubated for 4 hours at 37^0 C. The density of the resulting suspension was prepared by comparing its turbidity to Mac Farland 0.5 standard. Further adjustment to the inoculums was performed after comparison as required.

Result interpretation:

MIC values of all vancomycin resistance enterococci were obtained by using HIMEDIA MIC strips.

RESULTS

The samples were obtained from patients admitted or attending all OPD of Teerthanker Mahaveer Hospital, Moradabad. 898 clinical samples were processed over the period of 12 months from July 2021 to June 2022

Table 1: Results of catalase negative gram positive cocci in various identification tests (n=187)

Identification test	Positive	Negative
Aesculin Hydrolysis test	72	115
Bacitracin resistance	72	115
Salt Tolerance Test	72	115
VP test	00	187

ISSN: 0975-3583,0976-2833 VOL14, ISSUE 08, 2023

[Table 1] shows that out of 187 catalase negative cocci, 72 were identified as enterococcus. All 72 were found to be positive for aesculin hydrolysis, salt tolerance test and negative for VP test. They were also resistant to bacitracin.

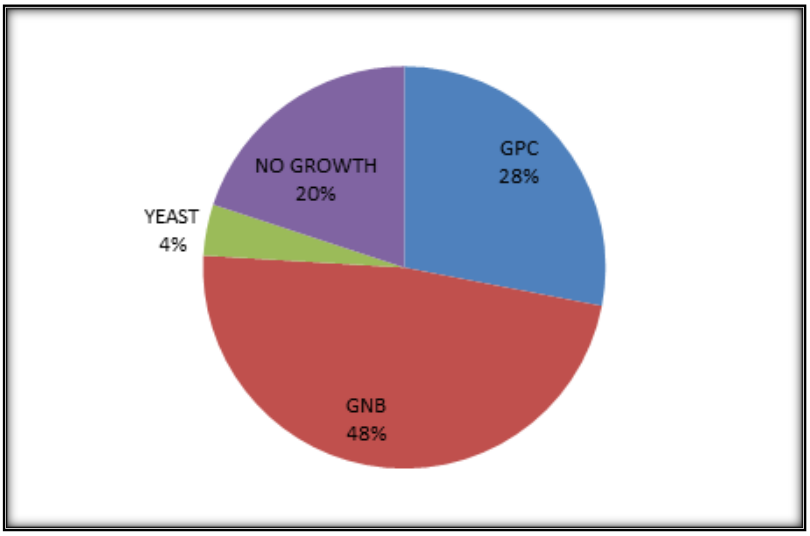

Figure 1: Showing percentage distribution of micro-organism isolated from sample

[Figure 1] showing percentage distribution of organisms like gram negative bacilli (48%), gram positive cocci (28%), no micro-organism seen (20%) and yeast (4%).

Table 2: Age and Sex wise distribution of patients (n=72)

Age (years)	Male (%)	IPD(%)	OPD(%)	Female (%)	IPD(%)	OPD(%)
<10	00	00	00	08 (15.68%)	06 (26.08%)	02 (7.14%)
11 to 20	02 (9.5%)	01 (8.33%)	01 (11.11%)	03 (5.88%)	01 (4.34%)	03 (10.71%)
21 to 30	03 (14.28%)	02 (16.66%)	01 (11.11%)	10 (19.60%)	02 (8.68%)	08 (28.57%)
31 to 40	05 (23.80%)	01 (8.33%)	04 (44.44%)	10 (19.60%)	04 (17.36%)	06 (21.42%)
41 to 50	03 (14.28%)	01 (8.33%)	02 (22.22%)	11 (21.56%)	04 (17.36%)	07 (25.00%)
>50	08 (38.09%)	07 (58.33%)	01 (11.11%)	09 (17.64%)	06 (26.04%)	03 (10.71%)
Total	21	12	09	51	23	28

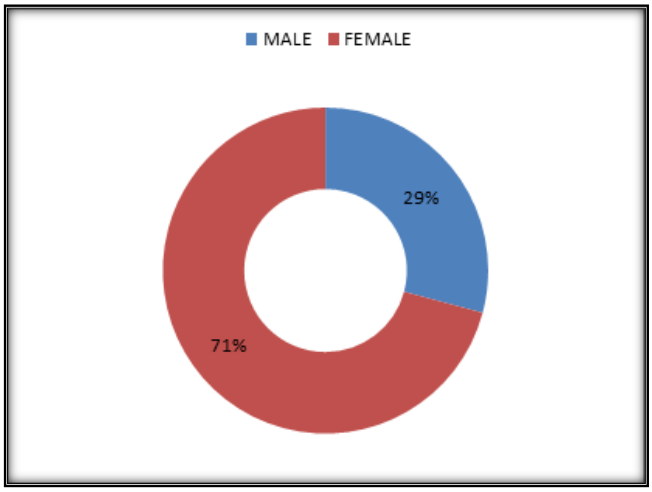

Figure 2: Sex wise distribution of patients suffering with Enterococcal infection (n=72)

[Table 2] shows that maximum numbers of Enterococcus isolates were obtained from male patients>50 years (38.09%) followed by 31-40 year's age group (23.80%). In all age groups female were more in numbers then males except in age group of > 50 years. Ratio of male to

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female was 1:2.4. Among males, clinical samples obtained were more from IPD patients (57.14%) and in females more clinical samples were obtained from OPD patients (54.90%). Out of 13 patients under the age of 20 years 11 were females and 2 was male.
[Figure 2] shows distribution of patients on the basis of sex. Males were 21 while female were 51 in number.

Table 3: distribution of other bacterial isolates

Organism	No. of isolate
E.Coli	10
Klebsiella	03
Enterobacter	03
S.Aureus	01

[Table 3] show highest isolation of E.coli(10) followed by Klebsiella(03),Enterobacter(03) and S.aureus(01).

Antibiotic susceptibility test:

The antibiotic susceptibility test were performed as per the CLSI guidelines on isolated organism with Drugs such as penicillin, Ampicillin, vancomycin, erythromycin, tetracycline, doxycycline, ciprofloxacin, high level gentamicin and linezolid, nitrofurantoin and norfloxacin for urine samples by Kirby Bauer Method.

Table 4: Antibiotic susceptibility

Drugs	Sensitive(%)	Resistant(%)
Penicillin(P)	37	35
Ampicillin(A)	23	49
Vancomycin(Va)	64	02+06*
Teicoplanin(TEI)	72	00
Erythromycin(E)	28	44
Tetracycline(TE)	43	29
Doxycycline(DO)	35	37
Ciprofloxacin(CIP)	27	45
High level gentamycin(HLG)	43	29
Linezolid(LZ)	72	00
Nitrofurantoin(NIT)	65	07
Norfloxacin(NX)	49	23

*- Indicates number of VRE whose inhibition zone were in between 14 mm to 17 mm

[Table 4] shows the sensitivity pattern of 72 Enterococcal isolates in which maximum sensitivity was seen to Lenozolid (100%) and teicoplanin (100%) followed by vancomycin (99%). Maximum resistant was seen to ampicillin (68%) followed by ciprofloxacin (62%) and erythromycin (61%).Out of the additional 2 drugs used for urinary isolates, Maximum sensitivity was seen for nitrofurantoin (90%).

Table 5: Showing interpretive criteria for susceptibility provided by HIMEDIA

Drugs	Sensitivity (µg/ml)	Intermediate sensitivity (µg/ml)	Resistant (µg/ml)
Vancomycin	<_ 4	8-16	>_32

There were 02 Vancomycin Resistant Enterococci (VRE) obtained by the Kirby Bauer Method. These 02 were confirmed by MIC E-Test. Out of 72 isolates of enterococci, 06 were found that their minimum inhibitory zone in between 14 mm to 17 mm by Kirby Bauer

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Method. They were proven by MIC E – Test for the conformation of VRE. 04 were resistant and 02 were sensitive out of 06 intermediate resistant Enterococci. Thus total number of VRE detected in our study is 06.

DISCUSSION

Genus Enterococci genus was initially regarded as a bacteria of little clinical importance, however they are being increasingly isolated from various clinical samples, and have rapidly emerged as an significant community and nosocomial acquired pathogen, As per CDC, enterococcus has emerged as an important HAI organism.[8] Vancomycin resistant enterococci have been reported worldwide. VRE may also have ampicillin resistance and HLAR, making it difficult to treat such infections.[9] The following study has identified enterococcus and their antibiotics susceptibility profile.

As per standard recommendations 72 enterococci were identified on the basis of conventional test scheme, using culture, catalase test, aesculin hydrolysis, salt tolerance test, resistance to bacitracin and VP test.[10]

Maximum numbers of enterococcus isolates were obtained from male patients >50 years (38.09%). In all age groups female were more in numbers then males except in age group of > 50 years. The frequency was high in sexually active females (21-40 years). This might be due to the fact that intestinal or vaginal enterococci (normal commensals) might enter into the urinary tract after bowel movement or throughout sexual intercourse due to proximity of urethral, vaginal and anal openings. The advanced prevalence was also noted among females in various studies.[11]

The frequency (8.01%) in our study is close to that of study conducted by Miskeen et al,[12] (7.4%). it is comparatively less than that reported by PJ Desai et al. [13] (28.57%). Jaylaxmi et al,[14] has found 2.12% incidence of enterococcus in symptomless bacteriuria in pregnant women.

Among males, urine samples obtained were more from IPD patients (57.14%) and in females more clinical samples were obtained from OPD patients (54.90%).

The present study shows highest isolation of E. coli (12) followed by Klebsiella (05), Enterobacter (05) and S. aureus (04). A study done on UTI E.coli was found to be the commonest co pathogen. Thus isolation of associated pathogens with enterococci depends on type of clinical samples included in the study.[15]

Vancomycin resistance:

In our study, 02 vancomycin resistance enterococci were detected whose MIC were 2 µg/ml by E-Test while 06 were the samples whose inhibitory zone were 14 to 16 mm in diameter by disc diffusion method, which means that 06 were intermediate. Its resistance pattern was confirmed by E-Test. When E-Test was applied over these 06 samples, it was seen that 04 were vancomycin resistant and 02 were sensitive.

Our study shows that MIC of vancomycin resistant to be < 2µg/ml and 8µg/ml. which is much lower than that observed by Agarwal et al. from Nagpur who show MIC<16 µg/ml.[16]

No resistance to teicoplanin (MIC value <0.5 µg/ml) was found in enterococci while resistant resistant to multiple drugs are present. Variable reports on teicoplanin sensitivity are available from other sources.[17] Glycopeptide resistant Enterococci have become a problem in some hospitals.

In this study, 48.6% of enterococci isolate were resistant to penicillin group, which is similar to the study conducted by Parvati S et al,[18] (43%) and Bhat KG et al,[19] (55 %). But it is higher than in study conducted by Miskeen PA et al (23%).[12] The maximum level of resistance was seen against ampicillin, tetracycline and ciprofloxacin,[20] 68%, 62% and 40% isolates were resistant to erythromycin, tetracycline and ciprofloxacin respectively. Lowermost resistance was seen against linezolid and nitrofurantoin antibiotics. Nitrofurantoin

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(90.27%) was considered as the best drug for enterococcal UTI,[12] while linezolid (98.6%) is considered as an important drug to treat other enterococcal infections.

CONCLUSION

Maximum numbers of enterococcus were obtained from patients between 31-40 years (20.83%). In all age groups female were more in number than male except in age group of > 50 years where both sexes are equal in number. Ratio of male to female was 1:2.4.

- Among males, samples obtained were more from IPD patients (57.14%) and in females more samples were obtained from OPD patients (54.90%). Out of 13 patients under the age of 20 years, 02 was male and rest 11 was female. Out of the 17 isolates obtained from polymicrobial infections along with enterococci, E.coli was the commonest (10/17, 58.82%)
- Maximum sensitivity was seen to teichoplanin & linezolid (100%) followed by vancomycin (99%). Maximum resistance was seen to erythromycin (72%), followed by ampicillin (64%), doxycycline (55%), tetracycline (57%) and penicillin (51%).
- Out of the additional 02 drugs used for urinary isolates maximum sensitivity was for nitrofurantoin (91%)
- Vancomycin resistance was found in 2 enterococcal isolates by disc diffusion method and 4 isolates were found resistant by E –test method.
- Isolation mainly from IPD patients and high risk areas like ICU/NICU indicate the danger of nosocomial spread of these organisms unless preventive measures are timely initiated.
- Vancomycin and teicoplanin are drugs to be kept in reserve for outbreaks or life threatening infections, which fail to respond to other drugs. MIC of these drugs can easily be carried out E-Strip.
- Implementation of preventive measures in terms of maintaining proper aseptic precautions, use of appropriate antibiotics would go a long way in controlling the emergence of these multi drug resistant Enterococci with existence of HLAR. Vancomycin and teicoplanin should be kept as reserve drugs for life threatening infections only.

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ISSN: 0975-3583,0976-2833 VOL14, ISSUE 08, 2023

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