

Occurrence of Vancomycin Resistant Enterococci in Various Clinical Samples**Rajesh Kumar¹, Umar Farooq^{2*}, Sudhir Singh³**¹JR3; Department of Microbiology, Teerthanker Mahaveer Medical College & Research Center, Moradabad, (TMMC & RC), Uttar Pradesh, India²Prof & H.O.D. of Microbiology, Teerthanker Mahaveer Medical College & Research Center, Moradabad, (TMMC & RC), Uttar Pradesh, India³Assistant Prof. Department of Microbiology, Teerthanker Mahaveer Medical College & Research Center, Moradabad, (TMMC & RC), Uttar Pradesh, India**Original Research Article*****Corresponding author**

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Article History

Received: 22.01.2018

Accepted: 05.02.2018

Published: 15.02.2018

DOI:

10.36347/sjams.2018.v06i02.004



Abstract: Enterococci are an indigenous flora of the intestinal tract, oral cavity and the genitourinary tract of the human beings and animals, are known to be relatively a virulent in healthy individuals but have become opportunistic pathogens especially in hospitalized patients. The most common nosocomial infections produced by these organisms are urinary tract infection, pelvic infection. Etc to isolate the enterococci from different clinical samples. To identify the AST pattern of enterococci. To determine the occurrence of vancomycin resistance enterococci. The present study was conducted in the Department of Microbiology of Teerthanker Mahaveer Medical College, Moradabad from January 2016 to June 2017 by performing sample collection, gram staining, culture and different biochemical tests. Out of 1257 samples, there are 355 gram positive cocci, 602 gram negative bacilli, 52 yeast and 248 samples showed no growth. 355 gram positive cocci were further processed and 100 isolates of enterococci were isolated. Maximum numbers of enterococci were obtained from patients between 31-40 years. Maximum isolates were obtained from urine (77%) followed by pus and wound swab (09%) samples.

Keywords: Urinary tract infection, enterococci and Vancomycin.

INTRODUCTION

Enterococci are an indigenous flora of the intestinal tract, oral cavity and the genitourinary tract of the human beings and animals, are known to be relatively a virulent in healthy individuals but have become opportunistic pathogens especially in hospitalized patients.

They belong to group D Streptococci as characterized by Lancefield in 1938[1]. Although enterococci were not previously considered as nosocomial pathogens but recent studies have confirmed this route of transmission [2, 3].

An increase in the number of isolates of *E. faecium* or especially increase in the rarely encountered species would be an epidemiological clue to the presence of an outbreak [4, 5]. The most common nosocomial infections produced by these organisms are urinary tract infection associated with instrumentation and antimicrobial administration, followed by intra-abdominal and pelvic infections. They also cause surgical wound infection, bacteraemia, endocarditis, neonatal sepsis and rarely meningitis. *E. faecalis* is the most common cause of infections (80-90%) and followed by *E. faecium*, (10-15%)[6].

Emergence of enterococci with multi drug resistance particularly to the vancomycin is particularly seen in *E. faecium* followed by increase in frequency of its recovery from infection. Since vancomycin resistant enterococci (VRE) also have ampicillin resistance and high level amino glycoside resistance (HLAR) thus they are very difficult to treat. The acquisition of high level amino glycoside resistance and vancomycin resistance has limited the therapeutic options for clinicians [7].

For most enterococci, they usually show bacteriostatic activity for penicillin, ampicillin, vancomycin, and teicoplanin at clinically achievable concentrations with MBCs (minimum bacterial concentration) many times higher than MICs. Bactericidal activity is achieved by the addition of streptomycin or gentamicin to one of these cell wall agents. Antimicrobial agents that provide bacteriostatic activity are usually appropriate for infection that is not deep seated or intravascular like UTI [8-10].

Bactericidal activity is generally required for deep-seated infection. For enterococci endocarditis and meningitis, therapy with a cell wall antibiotic and an amino glycoside are considered as standard therapy, bacteriostatic agents are frequently inadequate [11]. Numerous studies of enterococci endocarditis documented cure rates of >50% following treatment with penicillin or ampicillin alone, but >80% when combined with an amino glycoside. Unfortunately, enterococci resistant to first-line agents severely hamper the ability to bactericidal activity [12-15]. This merits a complete description of antimicrobial resistance, current possibilities for treatment and variety of measures that may limit the proliferation of resistance within a health care environment [6].

MATERIALS AND METHODS

The present study was conducted in the Department of Microbiology, of TMMC & RC, Moradabad. The samples were obtained from patients admitted or attending all outpatient departments. 1257 clinical samples were processed over the period of 18 months from January 2016 to June 2017. This is a

Following mentioned Kass criteria is 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: 10² cfu/ml is significant for urine sample in case of staphylococcus aureus.

Presumptive identification of *Enterococci* was done on the following basis.

- Gram staining
- Catalase reaction
 - Growth and blackening of bile esculin agar
 - Growth at 10⁰ and 45⁰ C for *enterococci*
 - Growth in 6.5% NaCl for *enterococci*
 - Motility test
 - PYRase test
 - H₂S production
 - Ammonia from arginine deamination

Antibiotic sensitivity testing (ast) [16]

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.-

prospective study which satisfying inclusion and exclusion criteria.

Samples were collected in blood culture bottles and pus swabs were collected with sterile swabs moistened with physiological saline where as other samples like urine and CSF were collected in clean, wide mouthed, air tight and sterile universal container. All samples were immediately transported to the laboratory.

Gram's staining were carried out of total 1257 samples and categorized them into gram positive cocci (355), gram negative bacilli (602), yeast (52) and no micro-organism seen (248).

Blood culture was done by using BHI Broth that was generally dispensed in a round blood culture bottle of about 100 ml capacity and fitted with a screw cap. An adequate space above the broth which was filled with air, available for strict aerobes. Smaller bottle are used for neonates and young children. Apart from them urine culture & pus culture were also done.

Inoculum preparation

Bacterial colonies were emulsified in BHI broth and incubated for 4 hours at 37⁰ C. The density of the resulting suspension was adjusted to approximately 10⁸ colony forming unit (cfu) per millimeter by comparing its turbidity to Mac Farland 0.5 standard. The standard was prepared by adding 0.5 ml to 0.048 M BaCl₂ (1.175% W/V BaCl₂ H₂O) to 99.5ml of 0.36 H₂SO₄ 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.

OBSERVATIONS AND RESULTS

The samples were obtained from patients admitted or attending all outpatient department of Teerthanker Mahaveer Medical College & Research Center, Moradabad. 1257 clinical samples were processed over the period of 18 months from January 2016 to june 2017.

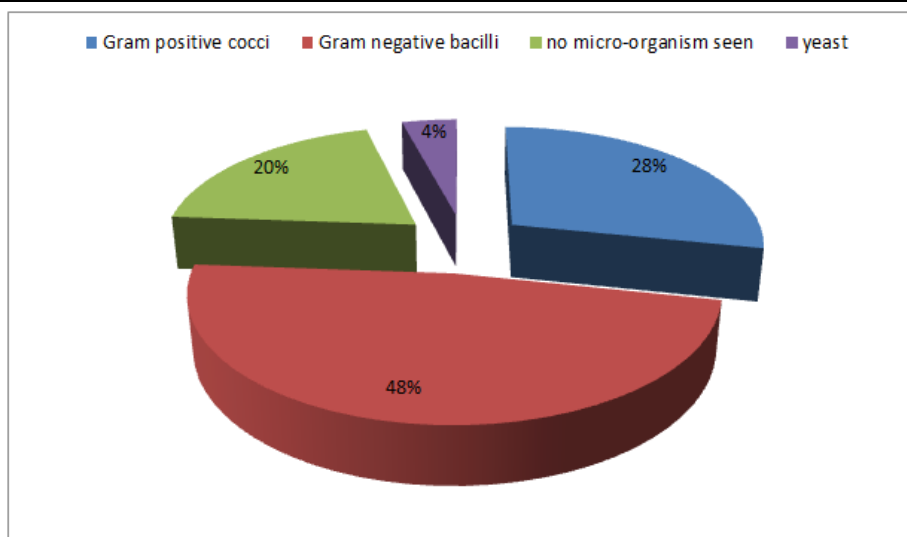


Fig-1: Showing percentage distribution of micro-organisms isolated from sample

Pie chart showing percentage distribution of organisms like gram negative bacilli (48%), gram

positive cocci (28%), no micro-organism seen (20%) and yeast (4%).

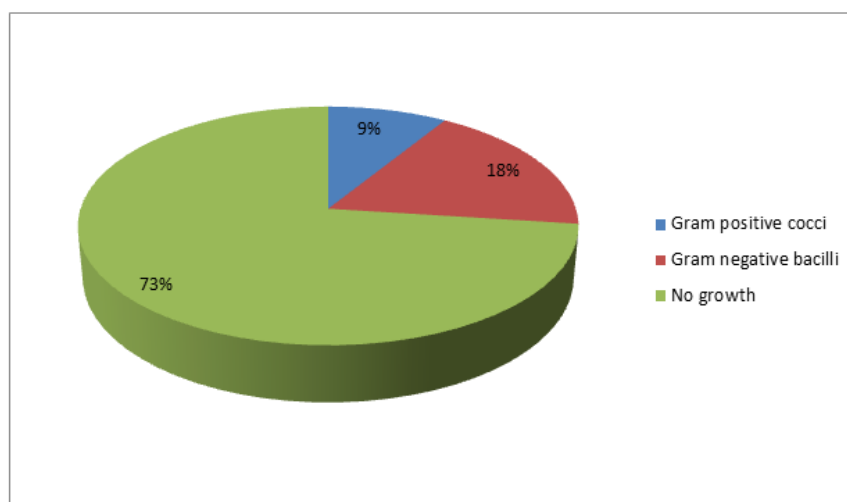


Fig-2: Showing distribution of organisms on the basis of culture (n = 248)

Fig showing percentage distribution of organisms in those 248 samples that were negative on

gram staining like grampositive cocci (9%), gram negative bacilli (18%) and no growth (73%).

Table-5: Results of catalase negative gram positive cocci in various identification tests (n=260)

Identification test	Positive	Negative
Aesculin Hydrolysis	100	160
Salt Tolerance Test	100	160
Resistance to Bacitracin (0.05 units)	100	160
VP test	00	260

Table shows that out of 260 catalase negative cocci, 100 were identified as *enterococcus*. All 100 were found to be positive for aesculin hydrolysis, salt

tolerance test and negative for VP test. They were also resistant to bacitracin.

Table-6: Age and sex wise distribution of patients (n=100)

Age group (years)	No. of Male (%)	IPD (%)	OPD (%)	No. of Female (%)	IPD (%)	OPD (%)
0-10	00	00	00	11(15.94)	09(27.27)	02(05.55)
11-20	01(3.22)	01(5.88)	00	05(07.24)	02(06.06)	03(08.33)
21-30	05(16.12)	03(17.64)	02(14.28)	13(18.84)	03(09.09)	10(27.77)
31-40	08(25.80)	01(5.88)	07(0.5)	13(18.84)	06(18.18)	07(19.44)
41-50	05(16.12)	02(11.76)	03(21.42)	15(21.73)	06(18.18)	09(25.00)
> 50	12(38.70)	10(58.82)	02(14.28)	12(17.39)	07(21.21)	05(13.88)
Total	31	17	14	69	33	36

Table shows that maximum numbers of *enterococcus* isolates were obtained from male patients >50 years (38.70%) followed by 31-40 year's age group (25.80%). In all age groups female were more in numbers than males except in age group of > 50 years. Ratio of male to female was 1:2.2. Among males, clinical samples obtained were more from IPD

patients (54.83%) and in females more clinical samples were obtained from OPD patients (52.17%). Out of 17 patients under the age of 20 years 16 were females and only one was male and all 4 neonates admitted were females. Of the total 50 IPD patients 33 were females (31/50; 62%).

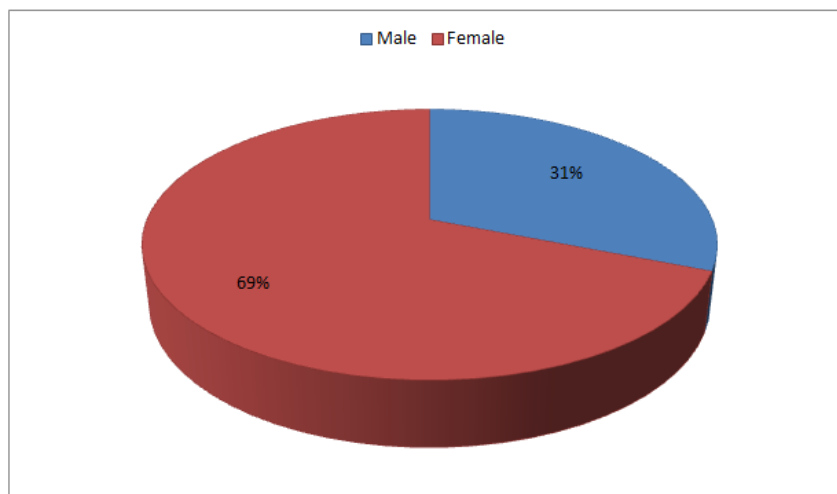


Fig-5: Sex wise distribution of patients suffering with Enterococcal infection (n=100)

Fig 5 shows distribution of patients on the basis of sex. Males were 31 while female were 69 in number.

Table 7 shows that out of 100 isolates, maximum were obtained from urine (77%) and pus/wound swabs (09%) followed by blood (04%), sputum (3%), HVS & tracheal secretion (2%

each), ET tube, Foley's catheter and ET secretion (1% each). Fifty six isolates were from IPD patients and 44 were from OPD patients. Urine was the main clinical sample to be collected from IPD patients (40/56; 71.42%) Whereas urine (37/44, 84.09%), Pus (5/44, 11.36%) blood and sputum (01/44; 2.27%), were the main clinical samples to be collected from OPD patients.

Table-7: Distribution of *Enterococci* as per the clinical samples (n=100)

Sample	Total No.	IPD (%)	OPD (%)
Urine	77	40 (51.94)	37 (48.05)
Pus/Wound swabs	09	04 (44.44)	05 (55.55)
Blood	04	03(75)	01 (25)
Sputum	03	02 (66.66)	01 (33.33)
HVS	02	02 (100)	00
Tracheal secretion	02	02 (100)	00
Foley's catheter	01	01 (100)	00
ET Tube	01	01(100)	00
ET secretion	01	01 (100)	00
Total	100	56	44

HVS-- High Vaginal Swab, ET Tube—Endo Tracheal Tube

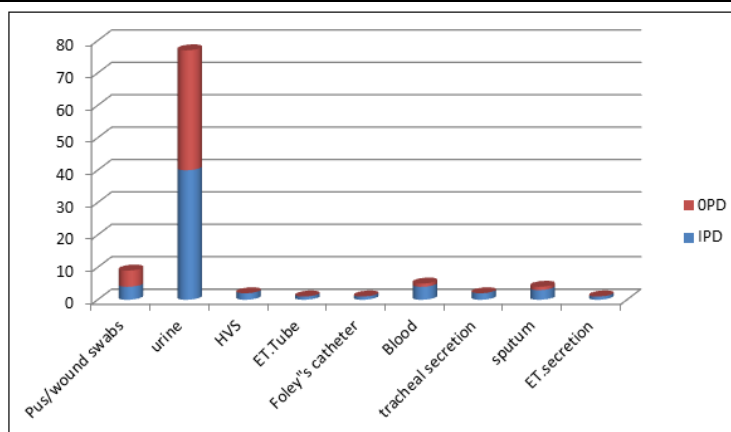


Fig-7: Distribution of enterococci as per the clinical samples (n=100)

HVS – High vaginal swab. ET tube – Endotracheal tube
ET secretion – Endotracheal secretion

Bar chart shows number wise distribution of enterococci among different types of samples like urine (77), Pus (09), blood (04), sputum (03) HVS (02), ET. Secretion (02), ET. Tube (01), foley’s catheter (01), tracheal secretion (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. Additionally two drugs were used are nitrofurantoin, norfloxacin for urine samples by Kirby Bauer Method.

Table-10: Antibiotic susceptibility Test of Enterococci

Drugs	Sensitive (%)	Resistant (%)
Penicillin (P)	49	51
Ampicillin (A)	32	68
Vancomycin (Va)	93	02 + 05*
Teicoplanin (TEI)	100	00
Erythromycin (E)	27	73
Tetracycline (TE)	49	51
Doxycycline (DO)	45	55
Ciprofloxacin (CIP)	58	42
High level gentamicin (HLG)	58	42
Linezolid (LZ)	100	00
FOR URINARY ISOLATES		
Nitrofurantoin (NIT)	91	09
Norfloxacin (NX)	73	27

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

Table shows the sensitivity pattern of 100 enterococcal isolates in which maximum sensitivity was seen to Lenozolid (100%) and teicoplanin (100%) followed by vancomycin (98%). Maximum resistant

was seen to erythromycin (73%) followed by ampicillin (68%) and doxycycline (55%). Out of the additional 2 drugs used for urinary isolates, Maximum sensitivity was seen for nitrofurantoin (91%).

Table-11: 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 100 isolates of *enterococci*, 05 were found that

their minimum inhibitory zone in between 14 mm to 17 mm by Kirby Bauer Method. They were proven by MIC E – Test for the conformation of VRE. 03 were resistant and 02 were sensitive out of 05 intermediate resistant

enterococci. Thus total number of VRE detected in our study is 05.

DISCUSSION

Genus *Enterococci* genus was initially regarded as a bacteria of little clinical importance, however they are being increasingly isolated from various clinical specimens, and have rapidly emerged as an important community and nosocomial acquired pathogen. As per CDC, *enterococcus* has emerged as an important nosocomial pathogen [17]. Vancomycin resistant *enterococci* have been reported worldwide. VRE may also have ampicillin resistance and HLAR, making it difficult to treat such infections [6]. The following study has identified enterococcus and their antibiotics susceptibility profile.

GENUS IDENTIFICATION

As per standard recommendations 100 *enterococci* were identified on the basis of conventional test scheme, using gram staining, catalase test, aesculin hydrolysis, salt tolerance test, resistance to bacitracin and VP test [18].

Age and Sex

Maximum numbers of *enterococcus* isolates were obtained from male patients >50 years (38.70%). In all age groups female were more in numbers than males except in age group of > 50 years.

Clinical Samples

Among males, clinical samples obtained were more from IPD patients (54.83%) and in females more clinical samples were obtained from OPD patients (52.17%). *Enterococci* were mainly isolated from urine (77%) and pus samples (09%).

Genus Isolation of Enterococci

Among the battery of tests used to identify *enterococcus* genus. PYRase was positive in all, this test is especially helpful in identifying *enterococcus* which produces gas from glucose and needs to be differentiated from gas producing leuconostoc and lactobacillus species [19].

Other bacterial isolates

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 [20]. Thus isolation of associated pathogens with *enterococci* depends on type of clinical samples included in the study.

High level aminoglycoside resistance

Increasing prevalence of high level aminoglycosides resistance (HLAR) among clinical isolates of enterococcus species has been more frequently reported. Disc diffusion method detected high level gentamicin resistance in 33% was detected.

Only 26 out of 34 HLAR isolates screened were found to possess MIC of > 240 ug/ml, other 7 isolates had MIC < 60 ug/ml. Discrepancy in results obtained by various methods has been reported earlier by M.G. Karmarkar *et al.* and others [21].

These errors associated with susceptibility testing may result in unwarranted utilization or elimination of the antibiotics as part of possible treatment regimen. Compared to us, Mohanty and co - workers have shown a much higher prevalence of HLAR enterococci [22].

Vancomycin and teicoplanin resistance

In our study, 02 vancomycin resistance enterococci were detected whose MIC were 2 µg/ml by E-Test while 05 were the samples whose inhibitory zone were 14 to 16 mm in diameter by disc diffusion method. I mean to say that these 05 were intermediate. Its resistance pattern was confirmed by E-Test. When E-Test was applied over these 05 samples, it was seen that 03 were 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 [23]. Kapoor *et al.* from New Delhi show MIC of 0.8 µg/ml 24. Esmaeillou M *et al.* show MIC of >4 µg/ml. No resistance to teicoplanin (MIC value<0.5 µg/ml) was found in *enterococci* while resistant to multiple drugs are present. Variable reports on teicoplanin sensitivity are available from other sources [25]. Glycopeptide resistant *Enterococci* have become a problem in some hospitals.

CONCLUSION

- Maximum numbers of *enterococcus* were obtained from patients between 31-40 years (21%). 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 1:2.2.
- Among males, samples obtained were more from IPD patients (54.83%) and in females more samples were obtained from OPD patients (52.17%). Out of 17 patients under the age of 20 years, 01 was male and rest 16 was female and all neonates (4) admitted were female.
- Maximum isolates were obtained from urine (77%) and pus/wound swab (09%) specimens.
- Urine was the main clinical sample to be collected from IPD patients (40/56; 71.42%) whereas pus/swab swab (05/44; 11.36%) .
- Out of the 26 isolates obtained from polymicrobial infections along with *enterococci*, *E.coli* was the commonest (12/26, 46.15%)
- Maximum sensitivity was seen to teichoplanin & linezolid (100%) followed by vancomycin (93%). Maximum resistance was seen to erythromycin

(73%), followed by ampicillin (68%), doxycycline (55%), tetracycline (51%) and penicillin (51%).

- Out of the additional 02 drugs used for urinary isolates maximum sensitivity was for nitrofurantoin (91%)
- High level of aminoglycoside resistance was seen in 33% of *enterococcus*.
- Vancomycin resistance was found in 02% enterococcal isolates by disc diffusion 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.
- Institution 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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