



Original Research Article

Identification of Species, Their Prevalence and Antimicrobial Susceptibility of Enterococci Isolated From Urine Samples in a Tertiary Care Hospital in Bengaluru

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ABSTRACT

Background: Enterococci, though considered as normal commensal of the intestinal tract, have emerged as medically important nosocomial pathogens. The emergence of Vancomycin Resistant Enterococci (VRE) in addition to the increasing incidence of High Level Aminoglycoside Resistance (HLAR), presents a serious challenge for clinicians.

Materials and Methods: This is a hospital based prospective study carried out in the Department of Microbiology, VIMS & RC, Bengaluru, during a period of one year from Jan 2013 to Dec 2013. Identification and speciation of the isolates were done by the standard conventional methods and antibiotic sensitivity pattern was determined according to CLSI guidelines. All isolates suspicious of resistance to Vancomycin by disc diffusion method were further put up for E-test to determine their Minimum Inhibitory Concentration (MIC).

Results: A total of 105 strains of Enterococci were isolated, of which 102 (97.2%) were *Enterococcus faecalis*, 02 (01.9%) were *E. faecium*, and 01 (00.9%) was *E. dispar*. Enterococcal isolates showed good sensitivity to Linezolid and Nitrofurantoin. A total of 33 isolates showed high level resistance to Gentamicin and 39 to Streptomycin by high content disc diffusion. Vancomycin resistance (MIC \geq 32 μ g/ml) was seen in 4 (3.8%) isolates.

Conclusion: Among the enterococcal isolates from urine samples *E. faecalis* was the commonest. Antibiotic sensitivity pattern revealed presence of multidrug resistance in *E. faecalis* and also in *E. faecium*, HLAR and Vancomycin resistance. Disparity in detection of VRE by disc diffusion method was observed when compared with the E-test, highlighting the importance of accurate determination of MIC for Vancomycin.

Key Words: Enterococcus species, HLAR, VRE, MIC.

INTRODUCTION

Urinary tract infection (UTI) is one of the most common bacterial infections encountered in clinical practice. [1] Urinary tract infections are a major public health

problem in terms of morbidity and financial costs, and incur the highest total health care cost among urological diseases, exceeding that of chronic renal failure even when renal dialysis and renal transplantations are

included. UTI represents one of the most common diseases encountered in medical practice today with an estimated 150 million UTIs per annum worldwide. [2]

Young, otherwise healthy, women are commonly affected with an estimated incidence of 0.5-0.7 infections per year. Of the women affected, 25%–30% go on to develop recurrent infections not related to any functional or anatomical urinary tract abnormality. Although uncomplicated infections do not result in long term sequelae, for example renal scarring, they cause significant morbidity, particularly when recurrent. [3]

UTIs are commonly caused by *Enterococci*, particularly among hospitalised patients; enterococcal prostatitis and perinephric abscess have also been reported. Among young healthy women who have not undergone instrumentation, do not have recurrent infections, and do not have structural abnormalities, *Enterococci* cause <5% of UTIs. In persons who have been instrumented, received antibiotics, have structural abnormalities, and/or have recurrent UTIs, the rate of urinary colonisation and infection by *Enterococci* rises. [4]

The genus *Enterococcus* consists of Gram-positive, facultative anaerobic organisms that are ovoid in shape and may appear on smears in short chains, in pairs or as single cells. [4]

Enterococci, though commensal in adult faeces are important nosocomial pathogens. Prior to the 1990s, *Enterococci* have been recognised as an important cause of bacterial endocarditis for almost a century. However, more recently they have been recognised as a cause of nosocomial infections and "superinfection" in patients receiving antimicrobial agents. The most common *Enterococci*-associated nosocomial infections are infections of the urinary tract,

followed by surgical wound infections and bacteremia. [5]

Among enterococcal species, *E. faecalis* and *E. faecium* have been reported as the two major human pathogens accounting for 85-89% and 10-15% of all enterococcal infections, respectively. [6]

The intrinsic antibiotic resistance of *Enterococci*, coupled with their promiscuity in acquisition and dissemination of genetically mobile antibiotic resistance elements, presents serious challenges to the treatment of enterococcal infections. Infections by *Enterococci* have traditionally been treated with cell wall active agents (e.g. Penicillin or Ampicillin) in combination with an aminoglycoside (Streptomycin/Gentamicin); however, emergence of High Level Aminoglycoside Resistance (HLAR), β lactam antibiotics and to Vancomycin by some strains has led to failure of synergistic effects of combination therapy. [5]

Species identification of *Enterococci* may be useful both as an epidemiologic tool in the investigation of outbreaks of nosocomial infections and for clinical decisions about therapy because antimicrobial susceptibility may vary with species; especially *E. faecium* and other species tend to be more resistant than *E. faecalis* to several commonly used antimicrobial agents. [5]

There is thus a need in tertiary care hospitals to identify, isolate and speciate *Enterococci*, for better understanding of their role in infections. Hence, the present study was undertaken to isolate, identify and speciate *Enterococci* and analyse their antibiotic susceptibility pattern.

MATERIALS AND METHODS

The present study was carried out in the Department of Microbiology, Vydehi Institute of Medical Sciences and Research Centre, Bengaluru, to investigate the

prevalence of enterococcal isolates and different species from urine specimens and to determine antimicrobial susceptibility patterns of the isolated *Enterococcus* species as per Clinical and Laboratory Standards Institute (CLSI) guidelines.

Inclusion Criteria: All urine samples received in the diagnostic section of the Microbiology Laboratory, from patients, both males and females (> 18 years), clinically diagnosed with urinary tract infection, from various wards and OPDs.

Exclusion Criteria: All paediatric (<18 years) urine samples and urine samples from patients in the intensive care units and catheterised patients.

Sample Collection, Transportation and Processing: A total of 105 *Enterococci* were isolated from all urine samples received in the diagnostic section of the Microbiology Laboratory, from patients clinically diagnosed with urinary tract infection.

All 105 samples were obtained from adult patients by the “clean catch midstream urine technique”. Urine was collected from the patients in a wide mouthed sterile container. The specimens were transported to the laboratory within 2 hours after collection and processed without any delay.

Urine wet mount examination was done for all the urine samples. Routine microscopic examination was done for the presence of leucocytes (≥ 10 WBC/mm³), leucocyte casts, and other cellular elements.

Simultaneously, the samples were plated onto HIMEDIA Urichrome Agar by using semi-quantitative loop technique. A calibrated loop which delivers a known volume of 0.01 ml of urine was used. Once inoculated, the plates were streaked to obtain isolated colonies and incubated overnight at 35°C for 24 hours.

Initial identification was based on the character of colonies on hiMedia Urichrome agar; *Enterococci* produce small

turquoise blue colonies after 24 hours of incubation. [6]

Enterococci isolated in significant number $\geq 10^3$ CFU/ml were included in the study. The colonies of *Enterococci* were subjected to biochemical reactions for further identification. [7]

Isolation, identification and speciation of *Enterococci* were done by the following:

- Colony morphology
- Morphology on Gram staining.
- Catalase test.
- Bile Esculin test.
- Growth in the presence of 6.5% Sodium Chloride.
- Growth at 45°C and 60°C.
- Fermentation of sugars – 1% Glucose, Lactose, Sucrose, Mannitol, Sorbitol, Arabinose, Raffinose.
- Arginine hydrolysis
- Tellurite reduction

Antibiotics Susceptibility Testing:

Antibiotic susceptibility testing of the isolated strains of *Enterococci* was carried out by Kirby-Bauer disc diffusion technique using Mueller Hinton Agar, according to CLSI guidelines. [9]

The antibiotics tested were Penicillin (10 U), Ampicillin (10 µg), Gentamicin (10 µg), High level Gentamicin (120 µg), High level Streptomycin (300 µg), Vancomycin (30 µg), Teicoplanin (30 µg), Ciprofloxacin (5 µg), and Nitrofurantoin (30 µg).

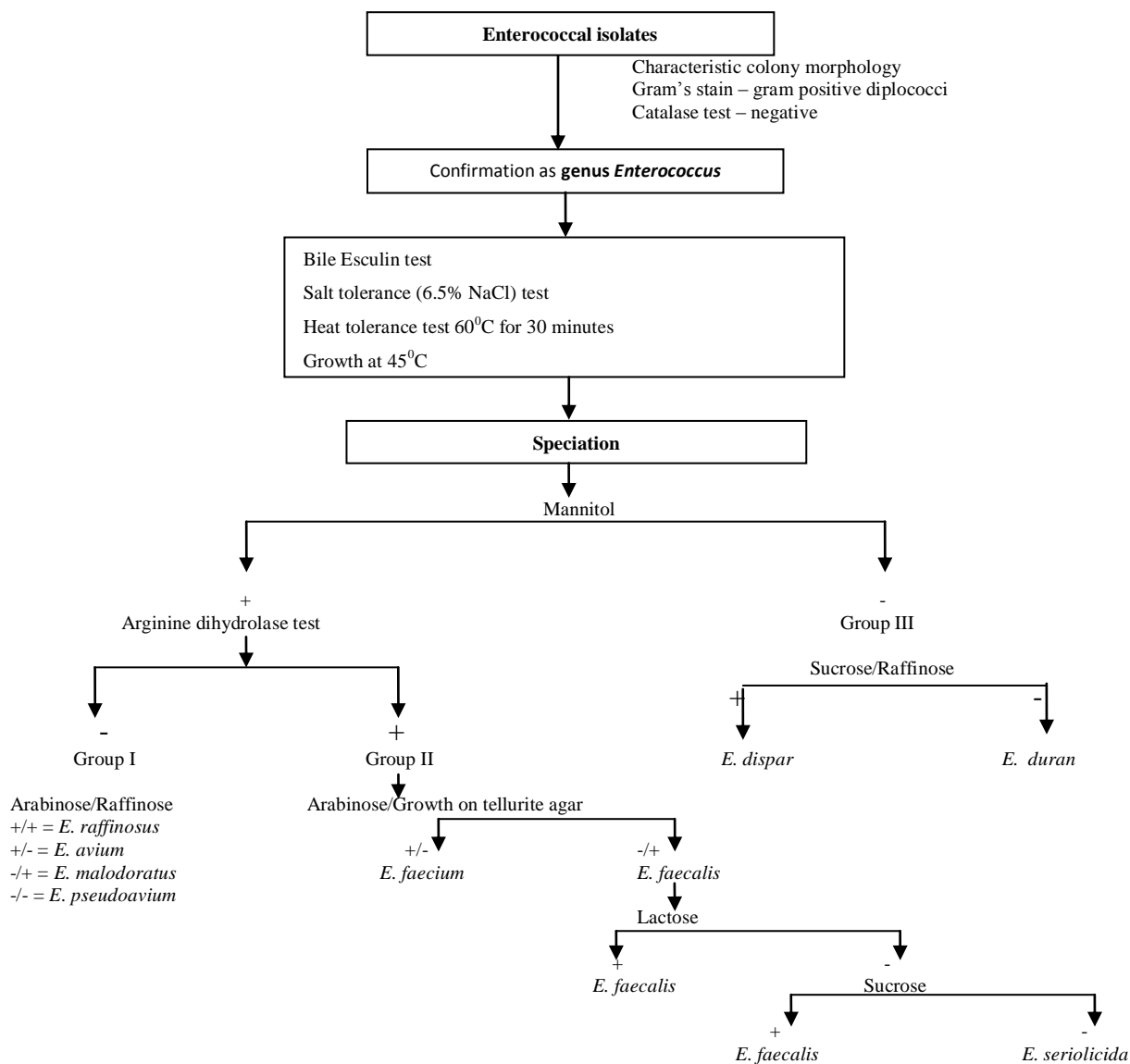
These antibiotics were obtained as commercial discs from HIMEDIA LABORATORY, Mumbai

Detection of VRE by E Test: All samples suspicious to be resistant to Vancomycin by disc diffusion method were retested by E-test for determination of MIC. E-test strips were obtained from bio Merieux.

E-test Interpretation was according to CLSI guidelines [9] as:

CLSI MIC criteria (µg/ml)	Susceptible	Intermediate	Resistant
	≤ 4	8-16	≥ 32

Identification and speciation flow chart [8]



Antibiotic Susceptibility Testing Scheme:

Kirby Bauer disc diffusion method

Ampicillin (10 µg), Penicillin (10 U),
Ciprofloxacin (5 µg), Nitrofurantoin (300 µg),
Vancomycin (300 µg), Teicoplanin (30 µg),
Linezolid (30 µg)

HLAR:

Gentamicin (120 µg), Streptomycin (300 µg)

MIC for Vancomycin by E-test, for all samples suspected to be resistant to

Vancomycin by Kirby Bauer disc diffusion method.

RESULTS

During the study period for a period of one year, from January 2013 to December 2013, conducted in the Department of Microbiology, Vydehi Institute of Medical Sciences and Research Centre, Bengaluru. A total number of 105 *Enterococcus* species were isolated from urine samples of patients

clinically diagnosed to be suffering from urinary tract infections.

Out of the 105 enterococcal isolates, *E. faecalis* was the predominant species 102 (97.11%). There were 2 (1.9%) isolates of *E. faecium* followed by a single isolate of *E. dispar* 1(0.95%). 61 (58.09%) were female patients with symptoms of UTI and 44 (41.9%) were male with symptoms of UTI. Out of the 44 male patients, maximum age group was found to be in the 31-40 years age group (29.6%); followed by 18-30 years age group (25%); and of the 61 female patients, was seen in the 18-30 years age group, comprising (44.3%) of all cases.

Out of the 102 *E. faecalis* isolates, 85.2% were sensitive to Vancomycin; 85.2% were sensitive to Teicoplanin; 77.5% were sensitive to Linezolid; 92.1% were sensitive to Nitrofurantoin; 50.1% were sensitive to Ampicillin; and 48.03% were sensitive to Penicillin. *E. faecium* was resistant to all antibiotics except one isolate which was sensitive to Nitrofurantoin. *E. faecalis* was seen to be resistant in 31.4% of cases for HLGR, and 41.2% for HLSR. *E. faecium* showed 50% HLGR, but no resistance to HLSR. *E. dispar* showed no resistance to either of the high level aminoglycosides.

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Out of the 15 Vancomycin resistant isolates of *E. faecalis* by disc diffusion, 3 isolates showed Vancomycin resistance by E-test. One out of two isolates of *E. faecium* which was resistant by disc diffusion to Vancomycin was resistant by E-test also.

Table 1: Different *Enterococcus* species isolated (n = 105)

<i>Enterococcus</i> species	No of isolates	Percentage (%)
<i>E. faecalis</i>	102	97.14%
<i>E. faecium</i>	2	1.9%
<i>E. dispar</i>	1	0.95%
Total	105	

Table 2 Distribution of isolates in relation to Patient's Sex (n= 105)

Sex	Number	Percentage (%)
Females	61	58.09
Males	44	41.90
Total	105	

Table 3: Distribution of male patients' age (n= 44)

Age group (years)	Number	Percentage
18-30	11	25.0
31-40	13	29.6
41-50	7	15.9
51-60	7	15.9
61-70	6	13.6
Total	44	100

Table 4: Distribution of female patients' age (n= 61)

Age group (years)	Number	Percentage
18-30	27	44.3
31-40	12	19.7
41-50	10	16.4
51-60	6	9.8
61-70	4	6.6
71-80	02	3.2

Table 5: Antibiotic susceptibility of all enterococcal isolates by Kirby Bauer disc diffusion

<i>Enterococcus</i> sp.	No of isolates	Ampicillin (10 µg)	Penicillin (10 U)	Ciprofloxacin (5 µg)	Vancomycin (30 g)	Teicoplanin (30 µg)	Linezolid (30 µg)	Nitrofurantoin (300 µg)
<i>E. faecalis</i>	102	52(50.1%)	49(48.03%)	23(22.5%)	87(85.2%)	87(85.2%)	79(77.5%)	94(92.1%)
<i>E. faecium</i>	02	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	1(50%)
<i>E. dispar</i>	01	1(100%)	0(0%)	0(0%)	1(100%)	1(100%)	1(100%)	1(100%)

Table 6: High Level Aminoglycoside Resistance (HLAR) pattern of enterococcal isolates

Sl. no	Isolate	Number of isolates	HLGR (120 µg) (%)	HLSR (300 µg) (%)	HLGR + HLSR (HLAR)
1	<i>E. faecalis</i>	102	32 (31.3%)	42 (41.2%)	20 (19.6%)
2	<i>E. faecium</i>	2	1 (50%)	0 (0%)	0 (0%)
3	<i>E. dispar</i>	1	0 (0%)	0 (0%)	0 (0%)
	Total	105	33 (31.4%)	42 (40%)	20 (19.04%)

Table 7: Minimum Inhibitory Concentration (MIC) of Vancomycin Resistant *Enterococci* by E-test

Sl. no	<i>Enterococcus</i> species	Total number of VRE by disc diffusion	VRE by E-test MIC
1	<i>E. faecalis</i>	15	3 (≥32µg/ml)
2	<i>E. faecium</i>	2	1 (≥32µg/ml)

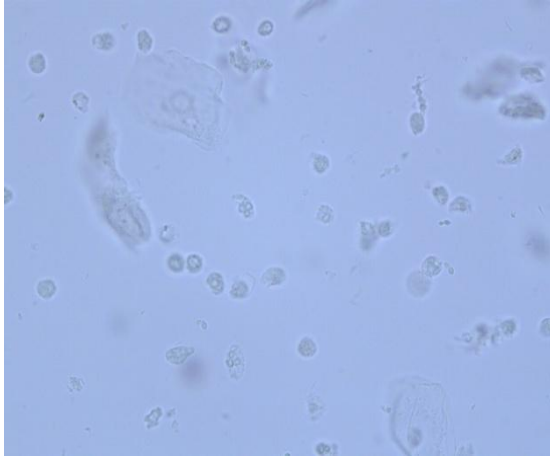


Figure 1. Wet mount of urine showing pus cells

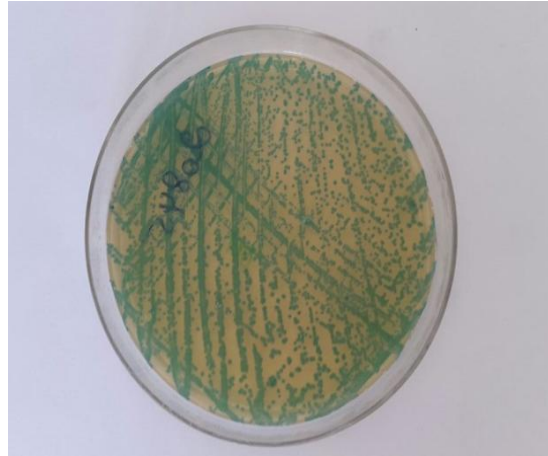


Figure 2. Urichrome agar showing turquoise blue coloured colonies of *Enterococci*

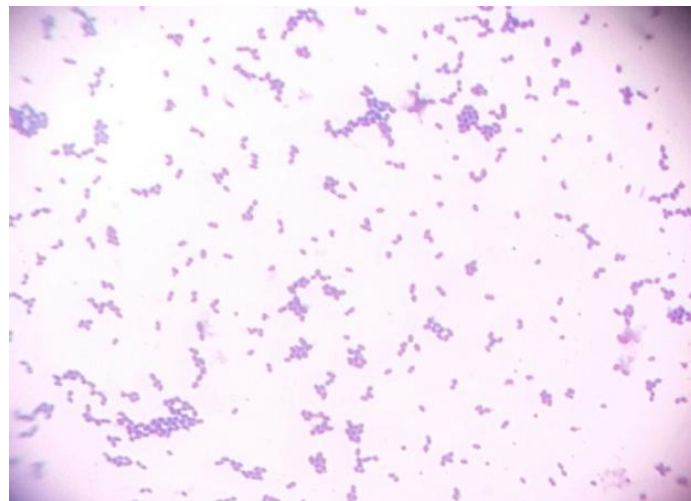


Figure 3. Gram stain showing gram positive cocci in pairs



Figure 4. Bile esculin test
(Positive control, Test sample, Negative control)



Figure 5. Arginine dihydrolase test
(Negative control, Test)



Figure 6. Tellurite reduction test (*E. faecalis* showing reduction of tellurite)



Figure 7. Heat Tolerance Test on Blood Agar

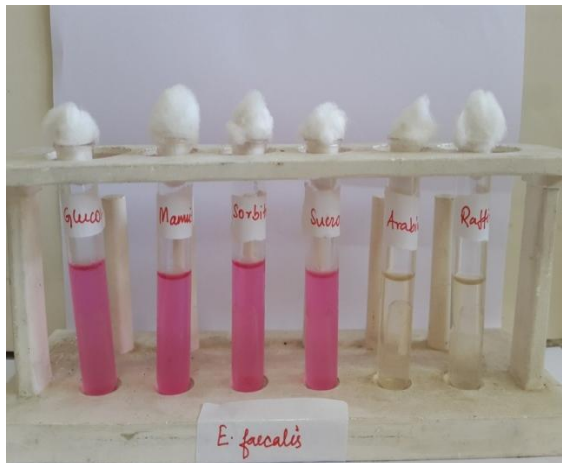


Figure 8. Sugar Fermentation Test - *E. faecalis*
Glucose, Mannitol, Sorbitol - fermented
Arabinose and Raffinose - not fermented

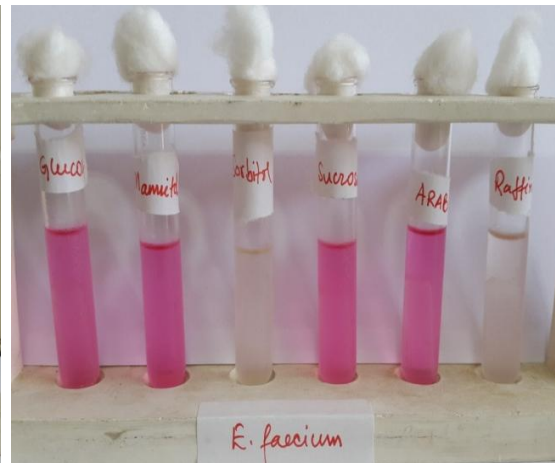


Figure 9. Sugar Fermentation Test - *E. faecium*
Glucose, Mannitol, Sucrose, Arabinose -
fermented; Sorbitol, Raffinose - not fermented



Figure 10: Sugar Fermentation Test - *E. dispar* Glucose, Sucrose, Raffinose - fermented. Mannitol, Sorbitol, Arabinose - not fermented



Figure 11. Antibiotic susceptibility pattern for *E. faecalis* showing sensitivity (S) to Van, LZ, Tei, HLS, Nit and resistance (R) to Amp, P, HLG



Figure 12. Antibiotic susceptibility pattern for *E. faecium* showing sensitivity (S) to HLG & resistance (R) to Van, Nit, Tei, LZ, P, Amp, Cip

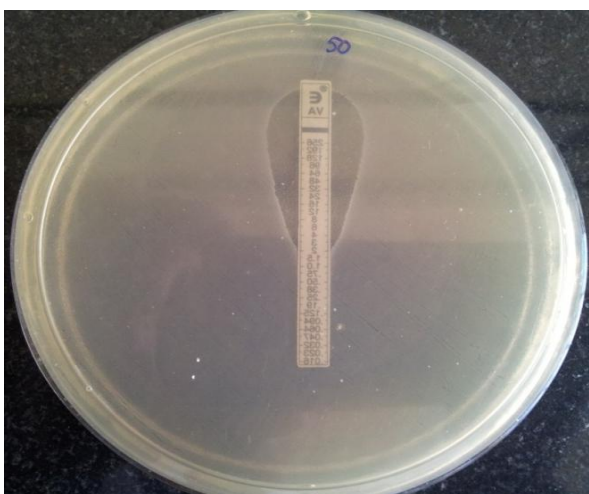


Figure 13. E-test of *E. faecalis* showing sensitivity to Vancomycin

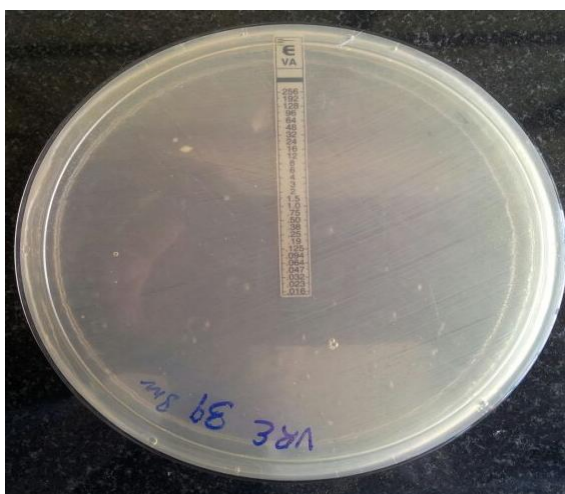


Figure 14. E-test of *E. faecalis* showing resistance to Vancomycin

DISCUSSION

Enterococci have emerged as an important nosocomial pathogen in the last few decades and the main reason for this is the trend of increasing antimicrobial resistance seen in these organisms. Enterococci have been implicated in many clinical conditions like bacteremia, urinary tract infections, peritonitis, and surgical site infections especially in the hospital setting, worldwide. Urinary tract infections are the most common cause of infectious disease produced by Enterococci, both within and outside hospital settings. [5]

Out of the 105 enterococcal isolates from urine samples, only three species of

Enterococci were isolated. Out of these, *E. faecalis* was the predominant species 102 (97.14%), with two isolates of *E. faecium* (1.9%) and a single isolate of *E. dispar* (0.9%). The findings were comparable with other studies, as done by Shrihari et al. [10] in which 96% of *E. faecalis* and one of *E. faecium* (4%) was isolated from urine samples. Palanisamy et al. [11] also isolated 91.83% of *E. faecalis* followed by 7.1% of *E. faecium* and 1.02% of *E. raffinosus*. A study done by Parameswarappa et al. [5] showed 63.3% of *E. faecalis* followed by 36.7% of *E. faecium*.

A higher prevalence was seen in females (59.1%) as compared to males

(41.9%), which is comparable to that of the study of Telkar et al. [12] wherein 63.75% were females and 36.35% were males. Bose et al. [13] also observed similar findings in their study with females accounting for 80.4% and males 14.6% of cases.

Maximum number of cases, in our study, in females was seen in the 18-30 years age group and 31-40 years for males. Telkar et al. [12] observed highest number of enterococcal isolates in the 0-20 years age group for both sexes.

Majority of the enterococcal isolates were found to be resistant to Penicillin, Ampicillin, and Ciprofloxacin which is comparable to the findings of Parameswarappa et al. [5]

Although in the present study, only two isolates of *E. faecium* were isolated, it was observed that *E. faecium* was more multidrug resistant as compared to *E. faecalis*, showing resistance to Penicillin, Ampicillin, Vancomycin, Teicoplanin, Linezolid, and High Level Gentamicin. Similar findings have been reported by Telkar et al. [12] and Parameswarappa et al. [5]

E. faecalis isolates showed 87% sensitivity to Vancomycin and Teicoplanin, 79% to Linezolid, and 97% to Nitrofurantoin. The highest sensitivity to Nitrofurantoin is a significant finding as it is a low cost antibiotic and can be of utmost utility in cases of multidrug resistant strains of *Enterococci* in urine. [13]

The single isolate of *E. dispar* was seen to be sensitive to all these four drugs.

High Level Aminoglycoside Resistant (HLAR) *Enterococci* were first reported in France in 1979 and since then have been isolated from all the continents. There is little need to test for aminoglycosides other than Streptomycin and Gentamicin, as these are the agents with the most clinical data. Till date, all strains with HLR to Gentamicin have also shown

resistance to synergism and/or HLR to Tobramycin, Sisomycin, Netilmycin, Kanamycin and Amikacin by virtue of the enzyme 2''-APH-6'-ACC. This enzyme is not active against Streptomycin, and thus Gentamicin resistant strains are not necessarily resistant to Streptomycin; in other words, a variable percentage of strains will have HLR to Gentamicin while lacking HLR to Streptomycin. [5]

Resistance to aminoglycosides is of great concern, since it eliminates the synergy of aminoglycosides with β -lactam antibiotics, which is the standard therapy of choice for enterococcal infections, thus limiting the therapeutic options. [5] In the present study, of the 105 isolates, HLAR was seen in 20 (19.04%) isolates. Telkar et al. [12] in their study observed HLAR in 55% of isolates. In our study, of the 102 *E. faecalis* isolates, 32(31.4%) showed HLGR and 42 (41.2%) showed HLSR. Telkar et al. [12] in their study reported that out of the 52 *E. faecalis* isolates, 32(61.53%) showed HLGR and 38 (73.07%) showed HLSR. [12]

Vancomycin resistance has been increasingly reported from all parts of the world. [14] The majority of Vancomycin Resistant Enterococci (VRE) encountered to date has been *E. faecium*. In our study, Vancomycin resistance by disc diffusion method was seen in 17 (16.1%) isolates. However, MIC for Vancomycin when determined by E-test for all isolates suspected to be resistant to Vancomycin by disc diffusion technique, revealed only 4(3.8%) of all the enterococcal isolates to be resistant to Vancomycin; 3 of 102 isolates of *E. faecalis* (2.94%) and only one isolate of *E. faecium*; and is in agreement with the findings of Taneja et al. [14] 8 (5.5%). Higher values have been reported by Telkar et al. [12] 11 (13.75%) and Oberoi et al. [15] 39 (20%).

Disc diffusion test when compared with E test was found to give disparate

results in the detection of VRE. Similar differences in the determination of MIC for Vancomycin by E-test versus disc diffusion method have been described by Taneja et al. [14] thus highlighting the importance of accurate determination of MIC for the identification of Vancomycin resistance.

To conclude *Enterococcus* spp. is an important pathogen causing urinary tract infections. In the present study *E. faecalis* was the predominant species isolated. Most of the enterococcal isolates were multidrug resistant. HLAR and Vancomycin resistance were increasingly observed in the enterococcal isolates from the urine samples. Good sensitivity to Linezolid and Nitrofurantoin was however still seen among the isolates. Regular screening of enterococcal isolates from urine specimen for detection of Vancomycin and high level aminoglycoside resistance is thus recommended for effective treatment of enterococcal urinary tract infections to limit the spread of multidrug resistant strains.

CONCLUSION

From the urine samples of patients suffering from symptoms of urinary tract infections in the present study, 3 species of *Enterococci* were isolated i.e. *E. faecalis*, *E. faecium* and *E. dispar*. *E. faecalis* was the predominant species. Antibiotic susceptibility pattern revealed presence of multidrug resistance in both *E. faecalis* and *E. faecium*. The prevalence of *Enterococci* in urine samples was high in the age group of 18-30 years, in females; and 31-30 years age group in males, with females having a higher rate of infection.

HLAR was detected in high percentage in both *E. faecalis* and *E. faecium*. VRE was detected in both the isolates as well. Because the therapeutic options for patients infected with multidrug resistant, HLAR and VRE *Enterococci* are limited, it is important that enterococcal

urinary tract infections in hospitals be monitored, as the organisms are very difficult to eradicate once they get established in the hospital environment.

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