ORIGINAL RESEARCH

Detection of vancomycin resistant enterococci in patients suffering from urinary tract infection in tertiary care hospital

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ABSTRACT

Purpose: Urinary tract infection patients are more likely to have vancomycin-resistant enterococci in a hospital setting. The present study was undertaken to isolate and identify enterococci from urine sample by standard technique, to detect prevalence of Vancomycin Resistant Enterococci, to detect antibiotic profile with Urinary Tract Infection caused by Vancomycin Resistant Enterococciand to identify risk factors associated with Urinary Tract Infection caused by Vancomycin Resistant *Enterococci*. **Materials and Methods:** The study comprised of 50 isolates of *Enterococcus*spp isolated from patient's urine suffering from Urinary Tract Infection using standard microbiological procedures. Antibiotic susceptibility testing by Kirby Bauer Disc Diffusion Method was performed using antibiotics as per CLSI guidelines. MIC of Vancomycin was determined by E-Test. **Results:** Out of 50 *Enterococcus* isolates the highest prevalence of Enterococcus species from urine sample was shown by *Enterococcus faecalis* (76%) than *Enterococcus faecium* (24%). The prevalence of Vancomycin Resistant Enterococci observed by E- test was greater in *E. faecium* (33.33%) in compared to *E. faecalis* (21.04%). The major risk factor for VRE colonization in UTI patient was found to be comorbidities i.e.33.33% followed by advanced age (25%), exposure to ICU (16.66%), patient with catheter (16.66) and patient on HD (8.33%) respectively. **Conclusion:** *E. faecalis* and *E. faecium* were the major enterococcal strain which are major pathogen of urinary tract infection. The prevalence of Vancomycin Resistant Observed by E. test in *E. faecium* (33.33%) was greater in compared to *E. faecalis* (21.04%).

Keywords: Urinary tract infections, Vancomycin Resistant Enterococcus, Epsilometer test.

INTRODUCTION

The genus *Enterococcus* were previously classified as faecal streptococci [1]. They are Gram-positive cocci occurs pairs or short chains,catalase-negative, facultative anaerobic commensal microorganisms of the gastrointestinal tract that are known uropathogens[1,2]. *Enterococci* are most common cause ofUTIin hospitalized patients [3].*Enterococci* are tough bacteria resistant to antibiotics, often infecting those with extensive antibiotic use or hospitalization[3,4]. They have caused conditions like endocarditis and urinary tract infections since the 1900s, with *Enterococcusfaecalis* becoming more common by the 1980s. Vancomycin-resistant strains, especially *E. faecium*, are increasing [4].Over time, there was a notable increase in the frequency of VRE infections in India, with the prevalence rising from 4.8% between 2000 and 2010 to 14.1% between 2011 and 2020[5].Due to their intrinsic and acquired resistance to widely used broad spectrum antibiotics, there are fewer options left for clinician to treat VRE infections, especially in weakened and critically ill patients. The 2 frightening thing is the increasing evidence of potential risk of transfer of Vancomycin resistance gene from VRE to various Gram-positive microorganisms especially *Staphylococcus aureus* through conjugative plasmids, which worsens the scenario further [6].

That is why it is very important for each and every hospital setup to continuously monitor such VRE infections and to assess the antibiotic susceptibility pattern of VRE isolates. Assessment of the prevalence and changing trends of VRE infections helps in planning of infection control measures which should be implemented in hospital in order to reduce Mortality and Morbidity caused by these VRE infections.

Therefore, we took this study to detect Vancomycin resistant*Enterococci* (VRE) from urine samples of patient suffering from urinary tract infection (UTI) received in Microbiology Laboratory.

MATERIAL AND METHODS

We performed a descriptive and prospective study during July 2019 to February 2021 in department of Microbiology, MGM Medical College and Hospital, Kamothe, Navi Mumbai, India. 50 isolates of *Enterococcus*spp were collected from patient's urine suffering from nosocomial Urinary Tract infection were included in the present study.

Inclusion criteria: Isolates of *Enterococcus* species from urine with significant bacteriuria.

Exclusion criteria: Urine sample without bacteriuria.

Collection of specimens

Specimens like midstream urine, urine obtained by supra-pubic needle aspiration were accepted for urine culture [7]. Collection of urine sample were done as per standard method. All samples were collected in a sterile leak proof container labelled with patient details.

Urine Sample Processing and Identification

The urine samples obtained were immediately processed in the microbiology laboratory by semi-quantitative method as per the standard protocols. Gram staining was done from the isolated colony on the blood agar and MacConkey agar[8]. Direct microscopic examination of urine sample was also done to look for the presence of pus cells, red blood cells, casts, crystals or any bacterial or fungal element.

Identification

Presumptive identification of *Enterococcus* was done in following basis.

- Catalase test [9].
- Growth and Bile Esculin agar blackening [1].
- Pyrrolidonyl –Acrylamides (PYR) Test [1].
- Resistance to Optochin[1].
- Resistance to Bacitracin [1].
- Growth at 37ºC and 45ºC [1].
- Hippurate hydrolysis test [1].
- Sugar fermentation test [1].
- Antibiotic Susceptibility Testing was done by Kirby Baur disk diffusion method according to Clinical and Laboratory Standard institute (CLSI) guidelines [11].
- Vancomycin Resistance -Disc diffusion method [11], MIC Method [12].

RESULT

Total 50 isolates of *Enterococcus*spp were obtained from patients with urinary tract infection in tertiary care hospital.

Table 1: Distribution of *Enterococcus* species.

Sample	Total no. of Enterococci	Total no. of Enterococcus faecalis	Total no. of Enterococcus Faecium
Urine (n= 1452)	50	38(76%)	12(24%)

Table 2: First line & Second line Antibiotic susceptibility pattern of 38 *E. faecalis* isolates.

Antibiotics	Sensitive	Intermediate	Resistant
Ciprofloxacin	8(21.05%)	3(7.9%)	27(71.05%)
Levofloxacin	15(39%)	3(7.9%)	3(7.9%)
Nitrofurantoin	18(47.36%)	1(2.63%)	19(50%)
Penicillin	7(18.42%)	0(0.00%)	31(81.6%)
Ampicillin	11(28.94%)	1(2.63%)	26(68.42%)
Tetracycline	23(60.52%)	3(7.9%)	12(31.57%)
Gentamycin	12(31.6%)	1(2.63%)	25(65.8%)
Linezolid	28(73.8%)	5(13.1%)	5(13.1%)
Vancomycin	26(68.42%)	3(7.9%)	9(23.68%)

Table 3: First line & Second line Antibiotic susceptibility pattern of 12 *E. faecium* isolates.

Antibiotics	Sensitive	Intermediate	Resistant
Ciprofloxacin	3(25%)	1(8.33%)	8(66.66%)
Levofloxacin	5(41.66%)	1(8.33%)	6(50%)
Nitrofurantoin	5(41.66%)	1(8.33%)	6(50%)
Penicillin	1(8.33%)	0(0.00%)	11(91.66%)
Ampicillin	2(16.66%)	0(0.00%)	10(83%)
Tetracycline	7(58.33%)	0(0.00%)	5(41.66%)

Gentamycin	4(33.33%)	0(0.00%)	8(66.66%)
Linezolid	9(75%)	1(8.33%)	2(16.66%)
Vancomycin	4(33.33%)	3(25%)	5(41.66%)

Table 4: Vancomycin resistant observed in *Enterococci* by E-Test.

Enterococcal Species	Isolated no.	Sensitive	Resistant
E. faecalis	38	30(78.94%)	8(21.05%)
E. faecium	12	8(66.66%)	4(33.33%)
Total	50	38	12

Table 5: Comparison of antibiotic susceptibility testing of *Enterococcus*spp with Vancomycin by Disk diffusion and E-test.

Species	Disk Diffusion Method			E Test	
	Sensitive	Intermediate	Resistant	Sensitive	Resistant
Enterococcus Faecalis	26(68.42%)	3 (25%)	9(23.68%)	30(78.94%)	8(21.05%)
Enterococcus Faecium	4(33.33%)	3(25%)	5(13.157%)	8(66.67%)	4(33.33%)

Risk factor associated with Vancomycin Resistant *Enterococci* colonization in UTI patients.

Table 6: Showing the risk factor associated with VRE colonization in urinary tract Patients:

Risk Factors	No. of VRE in UTI patients
Exposure to ICU	2(16.66%)
Patients on HD	1(8.33%)
Patients with catheter	2((16.66%)
Comorbidities	4(33.33%)
Advanced age	3(25%)
Total	12 (100%)

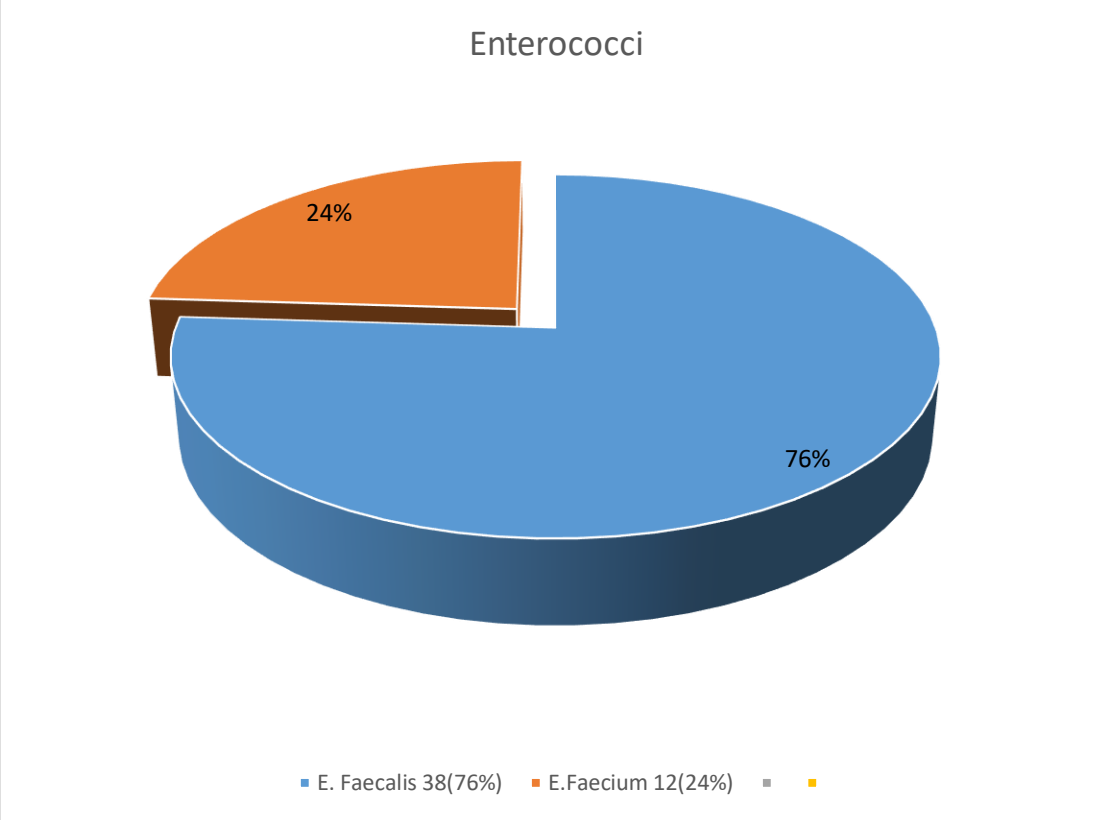

Figure 1. Showing Percentage of different species of Enterococcus

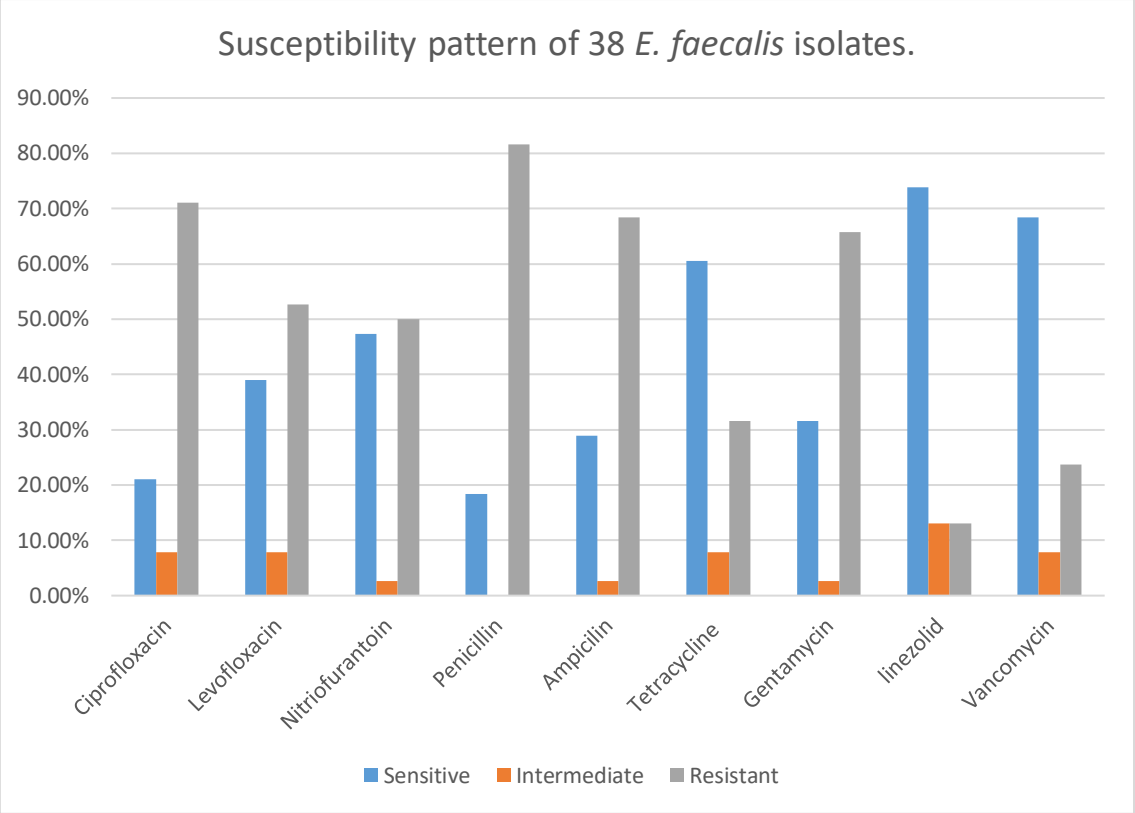

Figure 2. Bar diagram showing First line & Second line antibiotic sensitivity pattern of *Enterococcus faecalis*

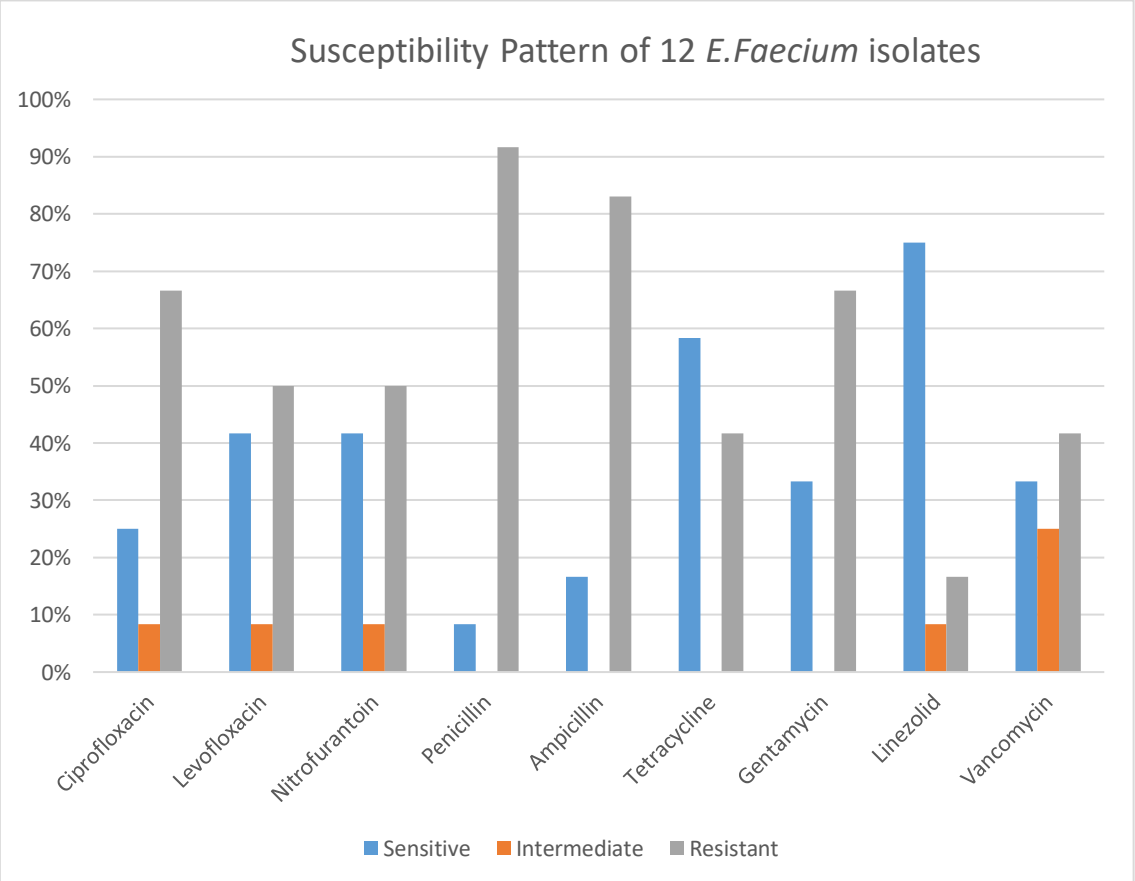

Figure 3. Bar diagram showing First line & Second line antibiotic sensitivity pattern of *Enterococcus faecium*

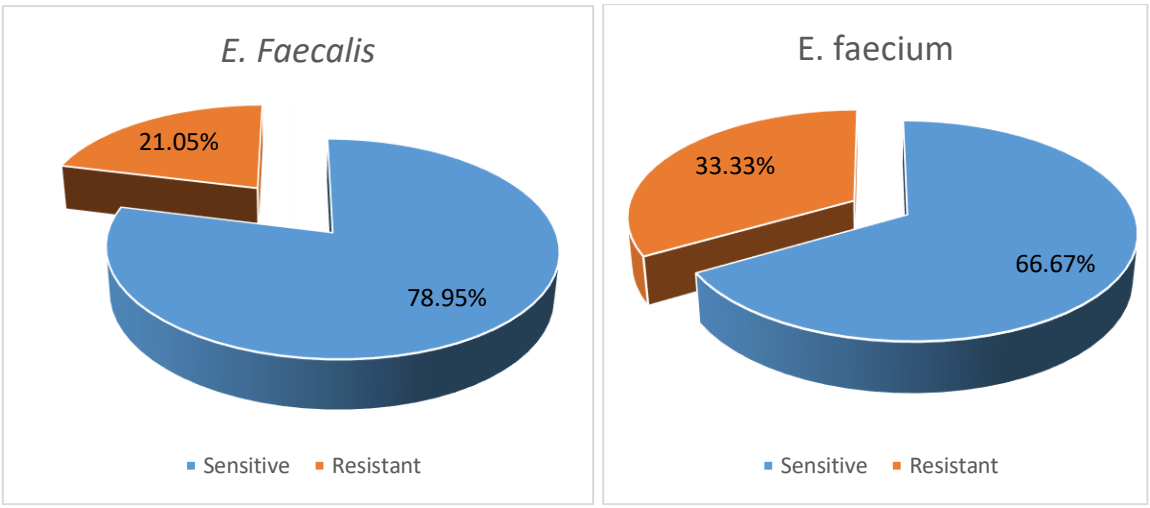

Figure 4.showing Vancomycin resistant observed in *Enterococcusfaecalis&Enterococcusfaecium* by E-test.

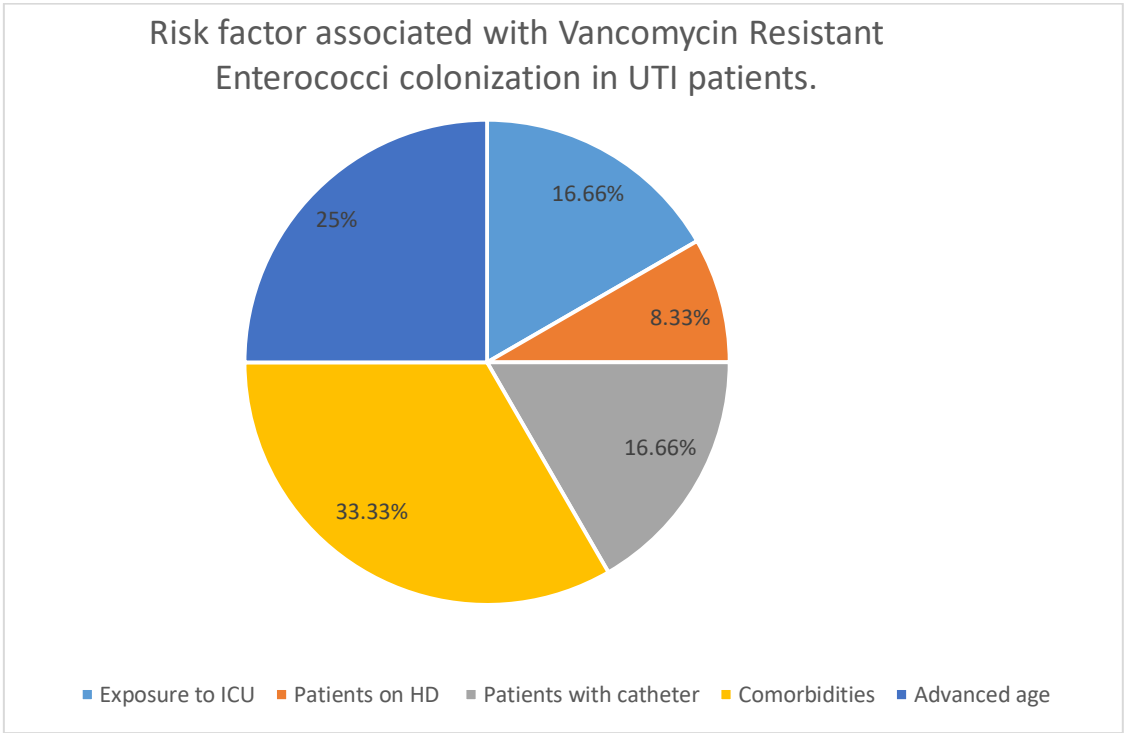

Figure 5. showing Risk factor associated with Vancomycin Resistant Enterococci colonization in UTI patients.

Total of 50 isolates of *Enterococcus* species were obtained from patient with urinary tract infection in tertiary care hospital. The highest prevalence was shown by *Enterococcus faecalis* (76%) than *Enterococcus faecium* (24%) (Table:1). The highest incidence of *Enterococcus* species was from age group 51-70 years (48%). The maximum number of *Enterococcus* species were isolated from males 31(62%) as compared to female 19(38%). 36% of the species of *Enterococcus* were isolated from ICU wards, 52% were from IPD and the remaining 6% were from OPD respectively. The highest prevalence was observed in IPD mostly in Geriatric ward and least was isolated from OPD patients. *Enterococcus faecalis* 1st line antibiotics most susceptible to tetracycline (60.52%), least to penicillin (18.42%) Second line antibiotics most susceptible to Linezolid (73.8%), least to Gentamycin (31.6%) (Table: 2). *Enterococcus faecium* 1st line antibiotics most susceptible to tetracycline (58.33%), least to penicillin (8.33%) Second line antibiotics most susceptible to Linezolid (73.8%), least to Gentamycin (33.33%) and Vancomycin (33.33%) (Table: 3). The detection method for antimicrobial susceptibility involved using disc diffusion and E-strip methods for *Enterococcal* isolates. Out of 50 isolates, 28% were resistant, 12% intermediate, and 60% susceptible by disc diffusion. Further testing with vancomycin E-strip revealed only 24% as resistant, suggesting that some borderline resistance may not be detected by disc diffusion

(Table: 5). The major risk factor for VRE colonization in UTI patients was found to be comorbidities (33.33%) (Table: 6).

DISCUSSION

Table 1. Showed that out of 50 *Enterococcus* spp isolated in our study, 38(76%) were *E. faecalis*whereas 12(24%) were *E. faecium.* A study by Maradia MR et.al 2017, Gujarat, India showed that out of 156 *Enterococcal* isolates from urine samples 50(30.05%) were *E. faecalis* whereas 106(67.95%) were *E. faecium*[13].

A study by Ashish Karna et al 2018. Dharan Nepal showed that out of 56 *Enterococcal* isolates from urine sample 29(51.78%) were *E. faecalis* whereas 27 (48.21%) were isolated from *E.faecium*[14].One more similar study by Arif D et.al 2019.UP India showed that out of 56 *Enterococcal*species isolated .32(60.3%) were *E. faecalis* and 21 (39.6%) were *E. faecium*[15].

Table 2. Showed that out of 38 *E. faecalis* the maximum susceptibility of *E. faecalis* to first line antibiotics is for tetracycline (60.52%) and least susceptibility is for Penicillin (18.42%). In a study by DilshadArif et.al. 2019, UP, India, Out of 32 *E. faecalis* isolates maximum sensitivity of E. faecalis to first line antibiotics was shown by Ampicillin 13(40.625%) whereas lowest sensitivity to first line antibiotics was shown by ciprofloxacin 5(15.625%) [15]. The maximum susceptibility of *E. faecalis* to second line antibiotics is for Linezolid (73.8%) and least susceptibility is for Gentamycin (31.6%). In a study by DilshadArif et.al. 2019, UP, India, maximum sensitivity to second line antibiotics was shown by Linezolid 32(100%) in compare to Vancomycin 28(87.5%) and Gentamycin 9 (28.125%)[15].

Table 3. Showed that the maximum susceptibility of *E. faecium* to first line antibiotics is for tetracycline (58.33%) and least susceptibility is for Penicillin (8.33%). In a study by DilshadArif et.al. 2019, UP, India, maximum sensitivity of *E. faecium* to first line antibiotics was shown by Ampicillin 9(42.825%) whereas lowest sensitivity to first line antibiotics was shown by ciprofloxacin 3(14.28%)[15].

The maximum susceptibility of *E. faecium* to second line antibiotics is for Linezolid (73.8%) and least susceptibility is for Gentamycin (33.33%) and Vancomycin (33.33%). In a study by DilshadArif et.al. 2019, UP, India, maximum sensitivity to second line antibiotics was shown by Linezolid 21(100%) in compare to Vancomycin 9(42.85%) and Gentamycin 10(47.61%)[15].

Table 4. Showed that the prevalence of Vancomycin Resistant Observed by E. test in *E. faecium* (33.33%) is greater in compared to *E. faecalis* (21.04%).

In study by Maradia MR. et al. 2017, Gujarat India: Out of 5 VRE isolates, 4(80%) were *E. faecium* and 1(20%) was *E. faecalis* this showed that prevalence of Vancomycin Resistant Enterococci by E. test in *E. faecium* is greater in compared to *E. faecalis*which is similar to our study[13].DilshadArif et.al. 2019, UP, India Out of 16 VRE isolates 12(57.1%) were *E. faecium* and 4 (12.55) were *E. faecalis*[15].

Table 5. The number of Vancomycin Resistant *Enterococci* detected by disk diffusion method is greater for both in *E. faecalis* and *E. faecium* than that detected by E. test.

Table 6. Showed that out of 12 VRE isolates the major risk factor for VRE colonization in UTI patients is Comorbidities i.e. 4(33.33%) followed by advanced age 3(25%), exposure to ICU 2(16.66%), patients with catheter 2(16.66%) and Patient on HD (8.33%) respectively.

In study by Toner L. et.al 2016, A tertiary care hospital in United Kingdom: female sex, urinary catheterization and inpatient status were identified as major risk factors for VRE- positive urine culture[16].

Most of the studies showed that the major risk factors for VRE colonization is Extendedhospitalization, comorbidities, Advanced age, catheter placement, Exposure to intensive care unit, prolonged duration of antibiotic therapyetc[6,17].

CONCLUSION

Bacteria can develop antibiotic resistance through a variety of inherited or acquired processes. Regardless of the kinds of cases that are admitted to an ICU, antibiotic resistance is an issue that affects all of them. Antimicrobial resistance in these "superbugs" has made them one of the biggest hazards to public health in the twenty-first century.[18]

An inflammation reaction to urinary tract colonization—most often by bacteria or fungi—is known as a urinary tract infection (UTI). It's important to distinguish between a UTI and the simple finding of bacteria in the urinary tract.[19]

- The prevalence of Enterococcus species from urine sample was carried out in our present study. The highest prevalence was shown by *Enterococcus faecalis* (76%) than *Enterococcus faecium* (24%).
- *Enterococcus faecalis* shows maximum susceptibility to tetracycline (60.52%) and least
- susceptibility is for Penicillin (18.42%) to first line antibiotics.
- Maximum susceptibility of *E. faecalis* to second line antibiotics was shown to Linezolid (73.8%) and least susceptibility was for Gentamycin (31.6%).
- *Enterococcus faecium* shows maximum susceptibility to tetracycline (58.33%) and least susceptibility is for Penicillin (8.33%) to first line antibiotics.
- Maximum susceptibility of *E. faecium* to second line antibiotics was shown to Linezolid (73.8%) and least susceptibility was to Gentamycin (33.33%) and Vancomycin (33.33%).
- Out of 50 *Enterococcal* isolates, 28% were resistant, 12% were intermediate and 60% were susceptible by disc diffusion method. The isolates

which showed resistant or intermediate using vancomycin disk diffusion method were further tested for MIC level by vancomycin E-strip. It was found that only 24% showed resistant to vancomycin. This is because disk diffusion method may not detect borderline resistance.

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