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## Speciation of Enterococci to Predict Antibiotic Resistance Pattern: A Study from Tertiary Care Hospital

Authors

**P. Sreenivasulu Reddy\*, Maria Sindhura John, P. Vasundhara Devi, G. Avinash**

Department of Microbiology, Narayana Medical College, Nellore, A.P

Corresponding Author

**P. Sreenivasulu Reddy**

HOD, Department of Microbiology, Narayana Medical College, Nellore, A.P

Email: [sr.palukuru@gmail.com](mailto:sr.palukuru@gmail.com)

### ABSTRACT

**Background:** *Enterococci* has recently emerged as a medically important pathogen causing hospital as well as community acquired infections. Detection of multi drug resistance is major health concern among the enterococci species in the hospitalized patients. However at present prevalence of *E.faecium* is more common than *E.faecalis* in hospital acquired infections. So exact speciation may help to predict the antibiotics to be used.

**Objective:** To isolate and speciate *Enterococci* from the clinical specimens and to detect their antibiotic susceptibility pattern.

**Materials and methods:** This study was conducted over a period of 2 years from January 2013 to January 2015. Clinical samples were received from various departments of Narayana Super speciality Hospital in the form of pus, urine, blood and peritoneal, synovial, ascetic fluids ect. Antibiotic sensitivity patterns were determined by Kirby-Bauer's disc diffusion method after isolation and speciation of enterococci. The work is carried out at Department of Microbiology, Narayana Medical College.

**Results:** During this period total of 150 clinical samples were processed for the presence of *Enterococci* and its speciation and antibiotic sensitivity test. Among the 150 samples processed 83% were *E.faecalis* and 67% were *E.faecium*. In our study more number of *Enterococci* were isolated from Pus followed by urine and blood. Among antibiotic resistance of *Enterococci* species, *E.faecium* showed more resistance than *E.faecalis*.

**Conclusion:** Our study findings are contrary to the belief. So correct speciation can help to predict antibiotic resistant pattern and treatment. Though the study has not been included the prevalence of fecal carriage of enterococci and vancomycin resistant enterococci (VRE), microbiologists and clinicians should know the threat of these specie. Surveillance for screening tests to detect fecal VRE carriage among the hospitalized and general population must be carried out for effective therapy and infection control measure to implement.

**Key words:** *Enterococci*, Community acquired infection, Speciation, Antibiotic resistance, VRE

## INTRODUCTION

Enterococci are aerobic and facultative anaerobic, non-capsulate, nonsporing and non motile Gram positive cocci, previously considered as normal commensals of gastrointestinal tract of humans and other animals, has recently emerged as a medically important pathogen causing hospital as well as community acquired infections<sup>(1,2)</sup>. Although Enterococci is known to colonize the oral cavity and vaginal tract, recovery from these sites is as less as 20%<sup>(3)</sup>. Enterococci are multi drug resistant intrinsically. Different species of enterococci will differ in their drug susceptibility patterns. Even though combination therapy with penicillin and gentamicin was effective earlier, now this combination is ineffective due to acquisition of high level resistance to aminoglycosides. Enterococci are medically important as they far outweigh the relatively insignificant proportion of the total adult human commensals they represents. It is ranked as one of the leading organisms causing hospital associated infections<sup>(4)</sup>.

Enterococcus is wide spread in nature which can grow and persists in harsh environments and readily recovered from various environmental sources like waste and surface water and also from foods such as milk and meat products<sup>(5)</sup>. *E.faecalis* is the most common species isolated from hospital associated infections followed by *E.faecium*, which accounts for about 90-95% of infections caused by Enterococci and others like *E.gallinarum*, *E.casseliflavus*, and *E.durans* are isolated less frequently accounting for 5% of clinical infections<sup>(6)</sup>. However at present,

prevalence of *E.faecium* is more common than *E.faecalis* in hospital acquired infections<sup>(7)</sup>.

Enterococci are not fastidious organisms as they grow readily on ordinary media like Nutrient agar, Blood agar, MacConkey agar<sup>(8)</sup>. They also grow on media containing high salt concentration of 6.5% NaCl, Hydrolyse esculin in presence of 40% bile and Tolerant heat at 60°C for 30 minutes<sup>(9,10)</sup>. Most often used selective medias are Bile esculinazide agar, Kenner fecal streptococcus broth, Selective media with Vancomycin for VRE, Kanamycin aesculinazide agar, Eosin methylene blue agar, Phenyl ethyl alcohol agar, Cephalixin aztreonam arabinose agar

## INFECTIONS CAUSED BY ENTEROCOCCI

The Enterococci are a dominant bacterial group in the intestinal flora of human and animals and it is recognized that they cause serious infections such as Urinary tract infections, has been reported as the third most common cause of nosocomial UTIs by enterococci<sup>(11)</sup>. 5-20% of Endocarditis, cases of endocarditis and considered as fifth most common cause of prosthetic valve endocarditis<sup>(12,9)</sup>. Bacteremia, according to Nosocomial surveillance data which has conducted in october 1986 to april 1997, Enterococci as the third most common cause of nosocomial bacteremia accounting for 12.8%<sup>(13)</sup>. Intra abdominal and pelvic infections, also produced in significant numbers by these bacteria<sup>(2)</sup>. *E.faecalis* accounts for upto 5% of isolates from skin and soft tissue infections<sup>(12)</sup>. About 0.3% - 4% of all meningitis cases are caused by Enterococcus species. The most commonest species isolated from cases of meningitis is

*E. faecalis*<sup>(14)</sup>. Enterococci causes neonatal sepsis in premature or low birth weight neonates, in infant with nasogastric tubes<sup>(15)</sup>. Enterococci and its species differ in drug susceptibility which emphasizes the need for identification of species in treating Enterococcal infection<sup>(16)</sup>.

## MATERIALS AND METHODS

This prospective study was conducted over a period of 2 years from January 2013 to January 2015. During this period 150 clinical samples were received from Narayana superspeciality hospital to the Department of Microbiology Narayana Medical College.

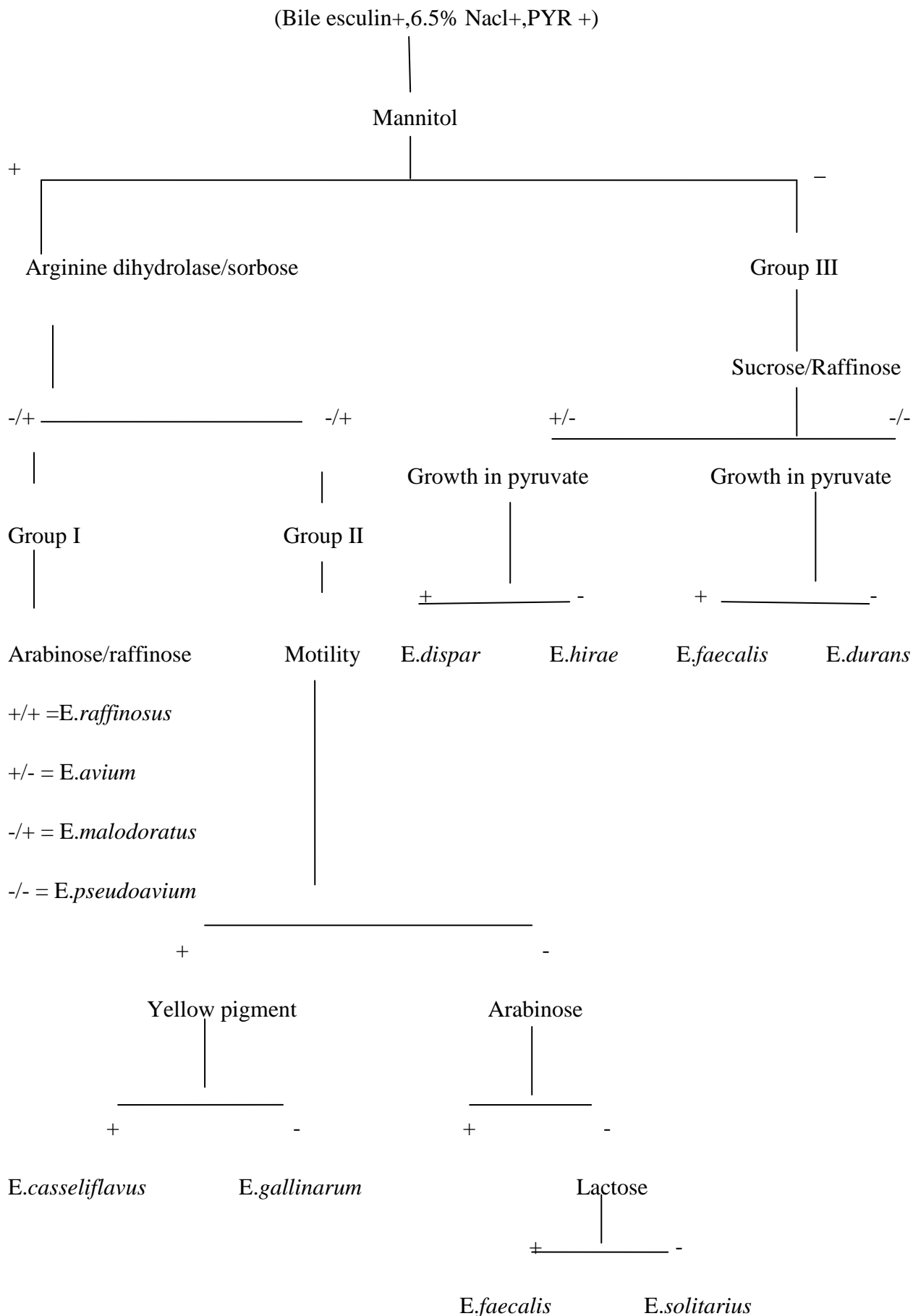
During this period various clinical specimens were collected for species identification such as Pus, Urine, Blood, Burn wound swabs, Ascetic fluids, Peritoneal fluids, Abdominal drain fluids, Synovial fluids obtained in the Microbiology Department were processed. The clinical samples were tested for the presence of Enterococci. Enterococci were identified based on Preliminary tests include Gram staining i.e., Gram positive cocci in pairs and short chains, Catalase test- Negative or Pseudocatalase positive, Bile esculin hydrolysis test- Positive, Growth in Salt Tolerance test (6.5% NaCl), Pyrrolidonyl Beta Naphthylamide hydrolysis test, Heat tolerance test were used for the isolation of Enterococci. Antibiotic sensitivity tests was done by disk diffusion by Kirby Bauer method using following drugs. Pencillin (10IU), Lenozolid(30µg), High level Gentamicin (120µg), Ampicillin (30µg), Ciprofloxacin (5µg), Levofloxacin (5µg), Norfloxacin (10µg), Ofloxacin (5µg), Nitrofurantoin

(30µg), Chloramphenicol (30µg), Erythromycin (15µg), Tetracycline (30µg)

## SPECIES IDENTIFICATION

Enterococcal strains are further identified to species level by using conventional and physiological tests by Facklam and Collins<sup>(3)</sup> which is based on Carbohydrate fermentation using 1% solution of following sugars Glucose, Mannitol, Rabinose, Raffinose, Sorbitol, Sucrose, Lactose, Trehalose, Pyruvate utilization test, Arginine decarboxylation, Motility test, Pigment production detection test on Tryptic Soya agar and Gelatin liquification<sup>(5)</sup>. Haemolysin production was detected in the strains of *E. faecalis* and *E. faecium* by culturing the isolates on Blood agar using 5% Human blood. All the tests were incubated at 37<sup>0</sup>c and read at 24hrs and 7 days.

Algorithm for Enterococcus species identification

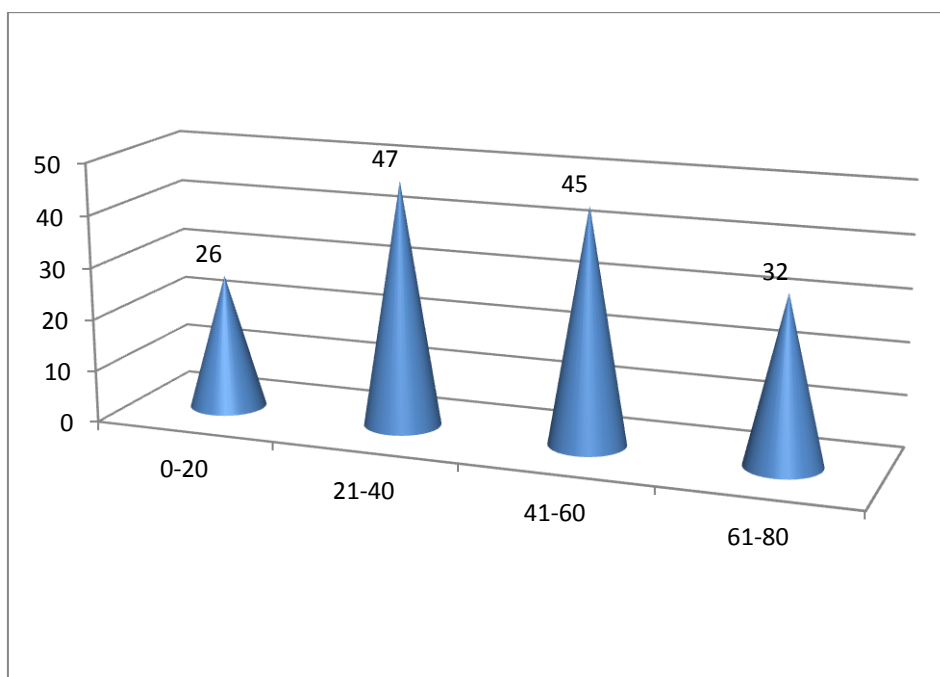


## RESULTS

During the study period extending from over a period of 2 years from January 2013 to January 2015. During this period 150 clinical samples

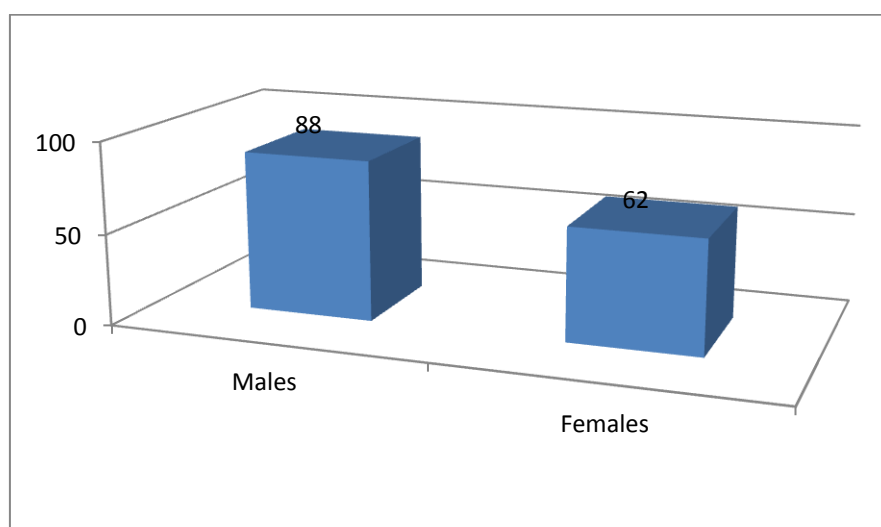
were received from Narayana superspeciality hospital to the Department of Microbiology Narayana Medical College.

### 1. Age wise distribution of patients



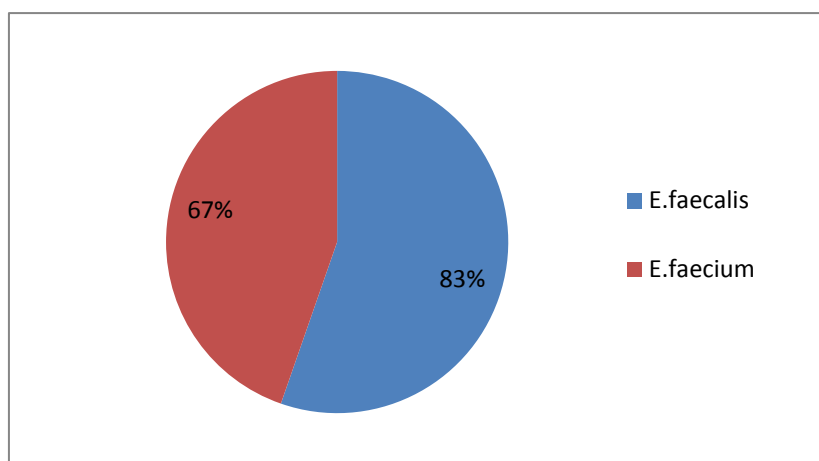
Enterococci infections seen in the age group between 21—60 years followed by 61-80 years comprising 61.3% and 21.3% respectively.

### 2. Distribution of Enterococci infections in relation to sex



Enterococci infection was seen more in Male patients than Females

### 3. Enterococci species distribution



Among 150 isolates of Enterococci were isolated from various clinical samples 83% were *E. faecalis* and 67% were *E. faecium*.

**Table -4** Enterococci species isolation from different clinical samples

Clinical samples(n=100)	n=150	<i>E. faecium</i>	<i>E. faecalis</i>
Pus	76	32 (38.5%)	34 (50.7%)
Urine	39	28 (33.7%)	11(16.4%)
Blood	23	12 (14.4%)	11(16.4%)
Peritoneal fluid	06	100%	-
Synovial fluids	Nil	-	-
Asceticfluid	05	4 (4.8%)	1(1.4%) 20%
Miscellaneous	01	-	100%
<b>Total</b>	<b>150</b>	<b>83</b>	<b>67</b>

The table shows the number of Enterococci isolated from clinical samples. Enterococci isolated from Pus accounting for(76),followed by Urine (39) and blood(23). Among 76 samples 50.7% were *E. faecalis* and 38.5% were *E. faecium*,

Among 39 urine samples 33.7% were *E. faecium* and 16.4% were *E. faecalis*. Similarly among 23 Blood samples 14.4% were *E. faecium* and 38% were *E. faecalis*.

**Table -5** Antibiotic resistance pattern of Enterococci species by Disk diffusion method

Antibiotics	<i>E.faecium</i> (n=83)	<i>E.faecalis</i> (n=67)
Penicillin	76(91.5%)	21(31.3%)
Ampicillin	68(81.9%)	23(34.3%)
High level gentamicin	57(68.6%)	47(70.1%)
Levofloxacin	76(91.5%)	45(67.1%)
Ciprofloxacin	78(93.9%)	55(82%)
Vancomycin	0%	0%
Teicoplanin	0%	0%
Chloramphenicol	48(57.8%)	11(16.4%)
Erythromycin	72(86.7%)	54(80.5%)
Tetracycline	74(89.1%)	56(83.5%)
Linezolid	0%	0%

The table shows that the antibiotic resistance of Enterococci species. *E.faecium* showed more resistance than *E.faecalis*.

**Table -6** Antibiotic resistance pattern of Enterococci isolated from urinary isolates

Urinary isolates	<i>E.faecium</i> (n=28)	<i>E.faecalis</i> (n=11)
Nitrofurantoin	1(3.5%)	1(9.09%)
Norfloxacin	13(46.4%)	5(45.4%)
Oflaxacin	14(50%)	5(45.4%)

The table shows less resistance to Nitrofurantoin in both species and more resistance to Norfloxacin and Oflaxacin.

## DISCUSSION

Enterococci are the normal commensal of the human intestine even though less often colonized in oral cavity, genitor urinary tract, and peri anal region. Enterococci identification upto species level in the clinical microbiology is important because some of the Enterococci species have intrinsic resistance to several antibiotics. So correct speciation can help the clinician to treat the patient with more appropriate antibiotic.

Study was conducted over a period of 2 year from January 2013 to January 2015. We isolated 150 Enterococci from various clinical samples of patients attending Narayana Superspeciality Hospital. Most of the Enterococcal infections are seen in the age group 21-40 years and 41-60 years accounting for 47% and 45% respectively. Most of the patients in our study with Enterococcal infections belongs to 3<sup>rd</sup> and 4<sup>th</sup> decade of life. However study by Modi et al from Ahmadabad showed Enterococcal infection more in 61-75 age group<sup>(17)</sup>. This indicates that all age groups are at risk of acquiring Enterococcal infections.

Of the 150 Enterococci isolates we found predominance among the males patients. Males accounted for 88% and females accounted for 62%. These findings are in concordance with Patel et al from Gujarat and Revathi Shama et al from Navi, Mumbai<sup>(18)</sup>.

Most of the Enterococci isolated from Pus(76) followed by Urine (39), Blood(23) and peritoneal fluid (16). A study from Bangalore by Sreeja et al found higher rate of isolation from pus samples which are similar to our study<sup>(19)</sup>. It was reported previously that *E.faecium* and *E.faecalis* were the

only two species prevalent in India. Various studies conducted from Central and South India found that *E.faecials* was predominant species isolated followed by *E.faecium* but studies carried out in North India have showed Enterococcal infection caused by *E.faecium* was more common than *E.faecalis*<sup>(20)</sup>. In our study *E.faecalis* (83%) was more common than *E.faecium* (67%) and no other species have been isolated.

*E.faecium* showed 91.5% and 85.9% resistance to Penicillin and Ampicillin where as *E.faecalis* showed 31.3% and 34.3% respectively. Studies in Bangalore, Chennai, and Davangere showed 40-45% resistance to Ampicillin. Our studies revealed that both *E.faecium* and *E.faecalis* are resistance to High level Gentamicin 68.6% and 70.1% respectively. Similar findings were reported from Delhi<sup>(1)</sup>.

Resistance to Fluroquinalonesin our study was 91.5% to Levofloxacin and 93.9% to Ciprofloxacin in *E.faecium* and *E.fecalis* showed 67.1% to Levofloxacin and 82% to Ciprofloxacin. But pateletal showed low resistance to Ciprofloxacin (6%) and Levofloxacin(19%). All Enterococci in our study was 100% resistance to Vancomycin, Linezolid and Teicoplanin.

According to the different studies Enterococcal strains with high resistance to multiple drugs not only prevalent in the clinical environment but also in gastrointestinal tract of colonized patients and the healthy individuals. Present study not included the healthy population. This study not detected any VRE, it may be attributed to the method we adopted for detection of vancomycin sensitivity. We might have detected VRE by using MIC or E



strip test which we have not done. Hospitals should keep in mind to implement screening tests for fecal VRE carriage in the hospitalized and in the normal population as these colonized people contaminate themselves as well as environment

## CONCLUSION

Since the enterococci species are common among the health care associated as well as community acquired infections, it is mandatory to know them up to species level, since enterococci species have intrinsic resistance to many beta-lactam antibiotics. According to the studies published, *E.gallinarum* and *E.classeliflavus* show intrinsic resistance to even to vancomycin. So, correct speciation in the clinical microbiology may help us to predict the antibiotic resistance pattern and to guide the clinician for appropriate treatment.

Every clinical microbiology laboratory should adopt appropriate methods like minimum inhibitory concentration (MIC) and E-strip tests for detection of antibiotic resistance apart from routine disc diffusion method. Detection of VRE from the colonized of infected individuals is essential component of any hospital infection control programme, which are necessary for effective therapy and infection control measure.

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