



# **Study on the Prevalance, Identification and Antibiogram of Enterococci Isolated from the Heterogenous Clinical Samples in a Tertiary Care Hospital**

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## **Authors' contributions**

*This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.*

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## **ABSTRACT**

The rise in Pantone-Valentine leucocidin (PVL) and gAMMA-haemolysin (Hlg) producing *Enterococcus* spp., among the people is a serious concern on evolution of antibiotic resistance. Recent reports suggested that the synergistic association of PVL toxin and necrotic lesions that lead to infections in skin and mucosa. To study the prevalence of *Enterococcus* isolated from the various clinical samples from both inpatient and out - patient department at a Tertiary care hospital. The resistance to Pantone-Valentine leucocidin (PVL) and gAMMA-haemolysin (Hlg) is further tested by using E-test strips for gentamicin test showed 26.8% of isolated populations had resistance to cell wall active agents. In our study all the intermediately sensitive to vancomycin isolates were found to be vancomycin sensitive by both the Epsilometer test and automated Vitek 2 system. The minimum inhibitory concentration ranging from 0.5 - 4 ug/ml. Hence my study confirmed good activity of the vancomycin, linezolid and Teicoplanin against isolated *Enterococci* spp.

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## 1. INTRODUCTION

The term „enterococcus” has originated from the Greek word enteron meaning „the gut or intestine” and kukkos that means „kernel or a berry”. These are Gram- positive cocci arranged in pairs and short chains, facultative anaerobic organisms that are a part of normal flora of the oral cavity, gastrointestinal tract and genitourinary tract in humans and animals [1,2]. They were previously classified as group D streptococci but were recognized as a separate entity in 1984 by Schleifer and Kilpper –Balz based on genomic analysis [3].

Most common clinical infections caused by Enterococci include urinary tract infections (UTI's), bacterial endocarditis, bacteremia, diverticulitis, wound infections, intra-abdominal infections, catheter related infections, surgical wound infections, pelvic infections and meningitis, respiratory infections, pleural space infections, skin and soft tissue infections have also been reported [4]. Till date around 36 Enterococcal species have been identified and 26 are associated with human infections [5-7]. *Enterococcus faecalis* is the most common human pathogen (70-90%) followed by enterococcus faecium (5-15%) that has been increasingly prevalent in hospital acquired infections. Other Enterococcal species (*E.gallinarum*, *E.avium*, *E.casseliflavs* and *E.raffinosis*) account for less than 5% of the infections [4-8].

Enterococci are emerging as one of the commonest organisms causing nosocomial infections in recent decades [9]. This is attributed by biofilms with multiple antibiotic resistance due to microbial characteristics like such as virulence factors and resistance genes that are magnified by the usage of contamination diagnostic and surgical procedures among the people [8-10]. Enterococcus has both the intrinsic and acquired resistance to many antibiotics that make them an important nosocomial pathogen. Enterococci show intrinsic resistance to cephalosporins, B-lactams, aminoglycosides, lincosamides, Enterococci express low affinity to penicillin binding proteins (PBP"s) that bind weakly to  $\beta$ -lactam antibiotics. For *E. faecium* PBP5 and for *E. faecalis* PBP4. As a result, minimum inhibitory concentrations (MICs) for penicillins are 2 –8 mg/ml for *E. faecalis* and 8–16 mg/ml for *E. faecium* [11].

Thus the accretion and spread of antibiotic resistance determinants among Enterococci, to the point where some clinical isolates that are resistant to all standard therapies, highlight both the vulnerability of our present armament as well as the looming prospect of a "post- antibiotic era". Thus, it results in increase in the cost burden for the healthcare. And it is also responsible to increase the morbidity and mortality. So efforts must be taken to prevent the emerging antibiotic resistance and spread of Enterococcal infections through infection control and stewardship programs implemented in the hospitals as well as in the community [12,13].

So, in context with the above perspective, this study was carried out in Sree Balaji Medical College and Hospital, Chennai.

## 2. METHODS

**Prospective study:** This study was conducted in the Department of Microbiology and central laboratory at the Sree Balaji Medical College And Hospital, Chrompet. Chennai.

### 2.1 Study Population

The study population included patients in different treating units from both outpatient department and inpatient department attending Sree Balaji Medical College and Hospital during the study period.

### 2.2 Study Period

The study was conducted for a period of one year from December 2015 to December 2016.

### 2.3 Statistics

The statistical analysis was performed using Microsoft Word 2010 and Microsoft Excel 2010 for entering data, creating tables and charts. Reports were expressed in percentage and frequency.

### 2.4 Controls

- *Staphylococcus aureus* ATCC 25923
- *Escherichia coli* ATCC 25922
- *Pseudomonas aeruginosa* ATCC 27853
- *Streptococcus pyogenes* ATCC 19615
- *Klebsiella pneumonia* ATCC 13883

- *Enterococcus faecalis* -In house
- *Enterococcus faecium* -In house

### 3. METHODOLOGY

A total of 17512 heterogeneous clinical samples that is urine, pus, blood, tissue fluids and ear swab taken from both inpatient and outpatient departments, from all the age groups, of both sexes submitted to the Central laboratory, Microbiology laboratory, of Sree Balaji Medical College And Hospital, for a period of one year were analyzed. A total of 190 *Enterococcus* isolates were recovered and further speciation and antibiotic susceptibility pattern was done.

### 4. RESULTS

A total of 17512 samples were analyzed for the prevalence of *Enterococcus* and speciation and antibiotic sensitivity was done after the identification. Further the age-group and sex wise distribution of *Enterococcus* commonly involved are identified.

Out of a total of 17512 heterogeneous samples obtained majority were obtained from inpatients department. 13792 (78.8%) samples were from inpatients and 3720 (21.2%) from the outpatients.

Out of a total of 17512 specimen were obtained that included 7953 of urine specimen, 5441 of pus samples, 3423 of blood samples, 695 of the exudates that included ear discharge, ascitic fluid and pleural fluid. The specimen like sputum, stool and vaginal swab were *Enterococcus* is a normal commensal were excluded from the study. The common *Enterococcal* species responsible for the infection and the other etiological factors related to the *Enterococcal* infections were identified.

Among 190 isolated *Enterococcal* species, predominant isolates that is 110 (57.8%) were recovered from urine, followed by 48(25.2%) in Pus then blood 24(12.6%) and exudates 8 (4.2%) respectively (Table 1).

Total number of enterococci isolated among the various clinical specimens obtained from outpatient department were 33(1.27%) from urine, pus 6 (0.73%) and ear discharge 4(7.4%) (Table 2).

So according to this study the prevalence of *Enterococcus* among in-patients is 1.06% and outpatients is 1.1% (Chart 1). And the total prevalence of *Enterococcus* is 1.08%. In our study among 190 isolated *Enterococcal* samples 67 (35.2%) were collected from the Medicine department, 46 (24.2%) from surgery

**Table 1. IP/OP distribution of the samples**

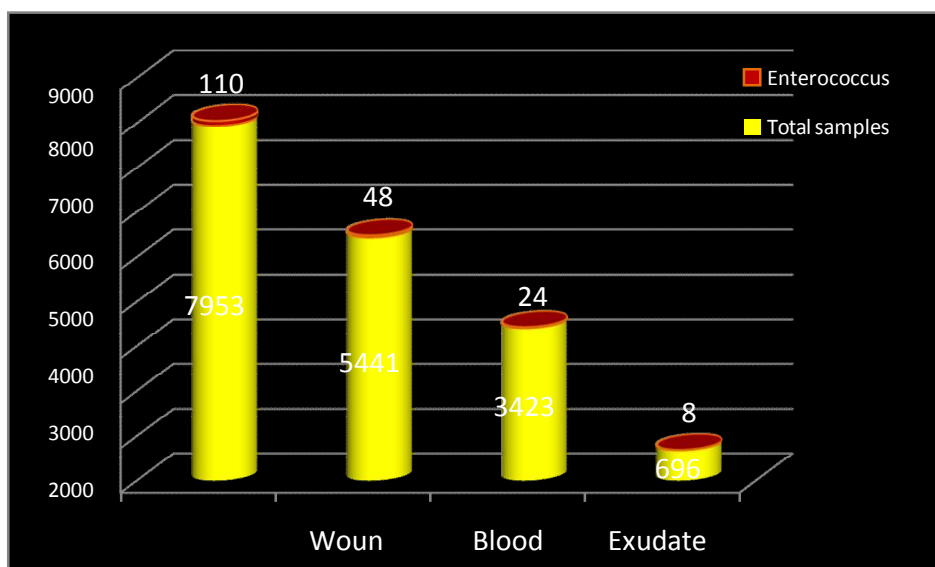
S. No	IP/ OP	Total	Percentage %
1.	Inpatient Department (IPD)	13792	78.8%
2.	Outpatient department (OPD)	3720	21.2%
	Total	17512	

**Table 2. Enterococcus isolated from heterogeneous samples**

S. No	Specimens	Total Number		Isolates		
		OP	IP	Number (N=190)	Percentage %	
1.	Urine	2589	5364	7953	110	57.8%
2.	Pus	812	4629	5441	48	25.2%
3.	Blood	265	3158	3423	24	12.6%
4.	Exudates					
A.	Ear Discharge	54	-	54	4	2.1%
B.	Ascitic Fluid	-	353	353	3	1.5%
C.	Pleural Fluid	-	288	288	1	0.5%
	Total	3720	13792	17512	190	1.08%

**Table 3. Sample wise distribution of enterococcus isolated from outpatient department**

S. No	Specimens	Total no of Samples		Isolates	
		OP	Number (N=43)	Percentage %	
1.	Urine	2589	33	1.27%	
2.	Pus	812	6	0.73%	
3.	Blood	265	-	-	
5.	Exudates				
A.	Ear Discharge	54	4	7.4%	
B.	Ascitic Fluid	-	-	-	
C.	Pleural Fluid	-	-	-	
Total		3720	43	1.1%	



**Fig. 1. Distribution of Enterococcus in various clinical isolates**

**Table 4. ICU And Non-ICU Distribution of Enterococci**

S. No		Total	Percentage %
1	ICU	16	8.4%
2	Non-ICU	174	91.5%

department, 28 (14.7%) from obstetrics /gynecology, 14 (7.3) from urology, 15 (7.8%) from pediatrics department, 16 (8.4%) from ICU and 4 (2.1%) from the department of ENT (Chart 1).

So the overall prevalence of Enterococcus in Intensive care unit at SBMCH is 8.4% of the total enterococcal isolates and 0.9% of the total isolated organisms (Table 3).

Out of a total of 190 Enterococcal isolates majority were from females 102(53.6%) and 88(46.3%) were from males. So, the male female ratio is 1:1.15 (Table 5, Chart 2).

And it is less prevalent in children up to 12 years of age (7.8%) then in adults (92.1 %) of the total isolates (Table 5).

So, the child and adult ratio is 1:11.6 (Chart 3).

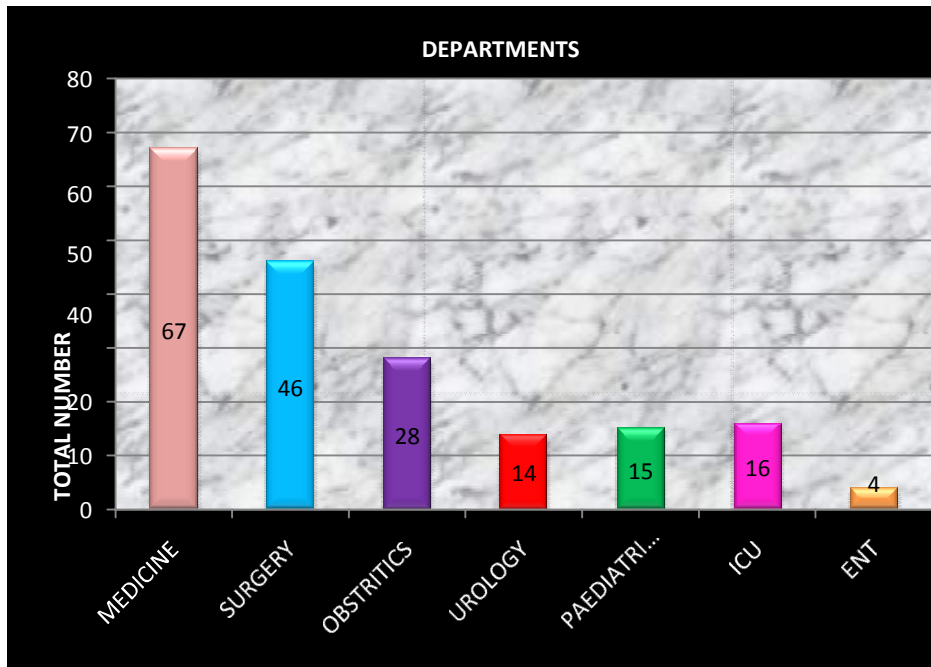


Chart 1. Showing the pattern of Distribution of Enterococci in various Departments

Table 5. Sex-wise distribution of Enterococcal isolates

S. no	Sex	Total number	Percentage (%)
1	Male	88	46.3%
2	Female	102	53.6%
3	Total	190	

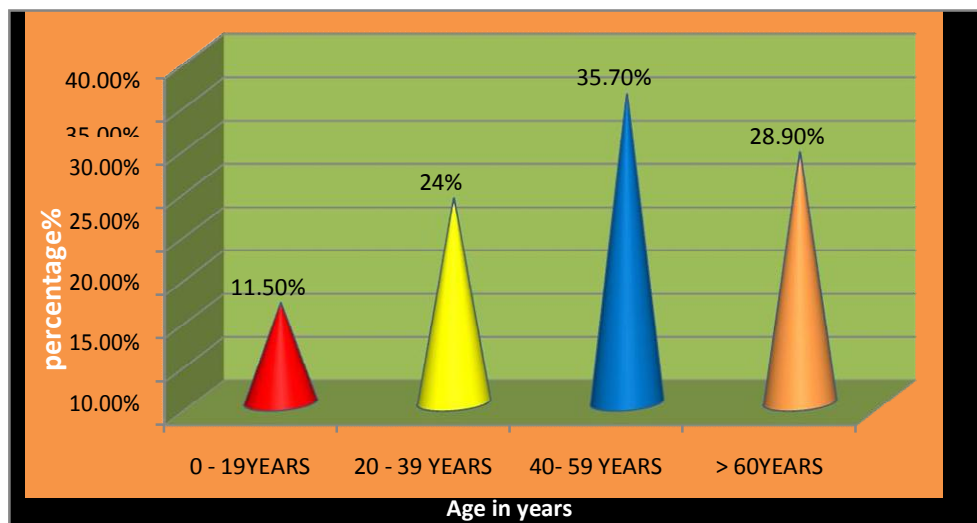
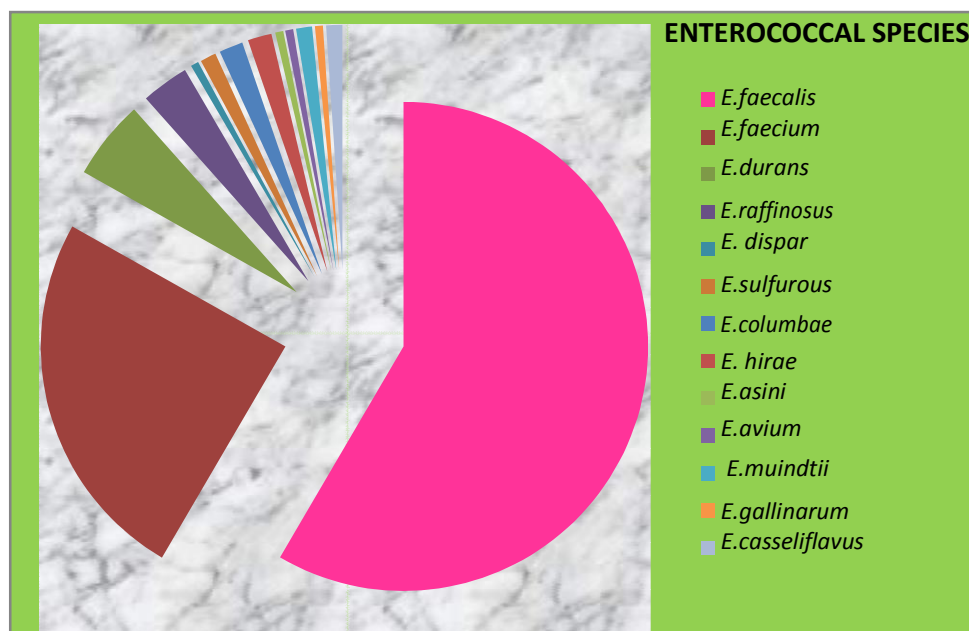


Chart 2. Age wise Distribution of Enterococci

**Table 6. Distribution of enterococci adult: Children ratio**

S. no	Age	Total	Percentage
1	Children ≤12 years	15	7.8%
2	Adults ≥13	175	92.1%
	Total isolates	190	



**Chart 3. Distribution of various Enterococcal species at the Tertiary Care Hospital**

**Table 7. Vancomycin susceptibility testing By different methods**

Tests	Sensitive	Intermediate Sensitive	Resistant
Kirby Disc diffusion method	88.9%	11.1%	0%
Epsilometer - test	100%	0%	0%
Vitek -2 system	100%	0%	0%

So, the predominant species isolated from urine is *E. faecalis* 61 followed by *E. faecium* 27. And followed by other species like *E. durans*, *E. sulfurous*, *E. columbae*, *E. dispar*, *E. durans*, *E. casseliflavus*, *E. gallinarum* and *E. hirae* (Table 7, Chart 3).

Since all the isolates showed sensitivity to vancomycin with MIC ranging from 0.5 - 4 ug/ml by both the methods, Henceforth they are interpreted as vancomycin sensitive as per CLSI guidelines (Table 7).

## 5. DISCUSSION

Enterococci is distributed widely in nature and normally it constitutes a part of mixed flora in the

gastrointestinal tract so it is difficult to differentiate the pathogen from the normal colonization [14]. Enterococci are one of the leading cause of nosocomial infections and opportunistic infections in the immune compromised patients worldwide that is attributed to the intensive use of broad spectrum antimicrobial agents [8,15-17]. The various life threatening infections caused by Enterococci include bacteremia, endocarditis, peritonitis, urinary tract infections, wound infections and device related infections [10]. It possesses specific traits that enable the organism to survive in the adverse environmental conditions including the hospital conditions. Any isolate that is suspected to be Enterococcus is a gram –

positive cocci in pairs, facultative anaerobe, catalase negative, grows in 6.5% NaCl, 40% bile salts, pH of 9.6, grows at 10°C and 45°C and resists 30 min at 60°C [18]. Serious Enterococcal infections are difficult to treat as it has tremendous ability to acquire resistance to penicillin, high level aminoglycosides and glycopeptide resistance. So, it possess a great challenge for the clinicians and health care institutions as the multidrug resistance complicates the treatment and therapeutic spectrum becomes limited [19]. In this context our study aimed to detect the prevalence, conventional methods of isolation, identification and speciation, done by using the conventional tests proposed by R.R.Facklam and Collins, enterococci in different clinical isolates along with the in vitro susceptibility testing [5]. In our study a total of 190 enterococci were isolated from a total of 17512 heterogeneous clinical samples both from outpatient and inpatient departments. The total of 21.2% Enterococcal isolates were obtained from outpatient department with a prevalence of 1.1% while 78.8% of the total isolates were obtained from inpatient department and a prevalence of 1.06%. As majority of specimen were obtained from inpatients. The results are similar to the findings of Acharya et al. who has reported 28% from outpatients and 72% among inpatients [20].

According to our study, age wise distribution showed maximum number of isolates were obtained from the age group 40-59 years of age (35.7 %). Which differs from the studies by Preeti et al that showed highest prevalence of 40% between 21-40 years of age followed by 0 -20 and according to the study by Saroj et al and Parameswarappa et al that showed its predominant ≥61 years of age. Majority of the cases were isolated from adults patients 92.1% and only 7.8% from pediatric patients that does not resemble the study by Acharaya et al who has isolated 30.5% of enterococci from pediatric patients [21-23].

And from ICU a total of 8.4% of enterococci were isolated. The study differs from Salem Bekhit MM. et al that has reported 85% of isolates from ICU. And corresponds to the study by Paule et al who have reported only 13.9% of the enterococcal isolates from ICU [24]. As per our study on the antibiotic susceptibility pattern we found that most of the Enterococcal isolates are highly resistant to penicillin 86.8%. The resistance among *E. faecalis* is 90% and that of

*E. faecium* is 95.7%. This suggests that *E. faecium* is highly resistant to penicillin. The finding is consistent with the reports from Gordon et al. [6]. Resistance to ampicillin is 35.7%. *E. faecium* shows 36.1% resistance to ampicillin. So 60.3% of *E. faecalis* and 63.8% of *E. faecium* were sensitive to ampicillin.

Erythromycin resistance is 55.2% which is less in comparison to the other studies by Mathur et al and Agarwal et al. [25]. According to our study the resistance of the Enterococcal isolates to ciprofloxacin is 72.1%, levofloxacin is 27.3%, tetracycline is 50.5%, Norfloxacin is 81.5% this is comparable to the study conducted by Mendiratta et al. Our study showed high sensitivity to nitrofurantoin 76.3%. Among which *E. faecalis* shows a sensitivity of 83.6% and *E. faecium* of 59.2% respectively. This is similar to the study of Preeti et al that showed 88.5% sensitivity to nitrofurantoin [26].

According to my study the isolates that showed intermediate sensitivity that is a zone size of 15 - 16mm by Kirby Bauer disk diffusion test were further tested by E- Test to determine the MIC value. And the E-Test results showed MIC values 0.5 – 4µg/ml. Thus all the isolates showed 100% sensitivity to vancomycin. This is comparable to the study by Shreeja et al that showed 100% sensitivity to vancomycin after all the intermediately sensitive isolates to vancomycin were further tested by the Epsilometer test [24].

Since the identification and susceptibility are detected within 3 -6 hours by Vitek 2 System it appears as the most reliable and fast method and similar results as that of E- test were obtained after the isolates were subjected to Vitek 2 System that is 100% sensitivity to vancomycin and teicoplanin. This is comparable to the study by Nicole Van den Braak et al the overall agreement of vancomycin susceptibility testing with the Vitek 2 compact system (biomeriux) compared with the reference agar dilution method detecting MIC was 94% (184 of 195) and the overall agreement of the teicoplanin testing results between the two methods was 97% [26]. However vancomycin resistant strains have been increasingly reported worldwide but in our study no such resistant strains were isolated. So, the judicious use of these antimicrobials is recommended for the multidrug resistant isolates of Enterococci in order to restrict the emergence of resistance to these drugs.

## 6. CONCLUSION

Enterococcal infections are emerging as the significant pathogen responsible for both nosocomial infections as well as community acquired infections. There is an immense increasing multidrug resistance among Enterococci that contributes significantly to the mortality and mortality among the patients. There has been a change in species isolated from various clinical specimens as more of the *E. faecium* is isolated that suggests the change in the pattern of the infections caused by Enterococci. According to the study *E. faecium* is highly multidrug resistant and this suggests the species level identification of the Enterococcal isolates is important to treat the Enterococcal infections as well as to limit the emerging resistance as it can predict the patterns of antimicrobial susceptibility. There is a stringent need for prevention and control of spread of multidrug resistant Enterococci that could be achieved by the vigilant use of antimicrobial agents, close monitoring of higher drugs like vancomycin with its minimum use and looking for patients compliance, early detection and reporting by the laboratories, antibiotic prescribing policy and the audit, educating and screening of the healthcare workers and hospital staff, maintaining the corrective reflexive practices such as proper hand washing technique and immediate implementation of the appropriate infection control measures that will reduce the mortality and morbidity that can further prevent the organism to become a formidable clinical problem in the years to come.

## CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

## ETHICAL APPROVAL

The study is approved by the institutional ethical committee, Sree Balaji Medical College and Hospital.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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