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A Study on the Prevalence of Various Enterococcus Spp. In Various Clinical Infections in Patients Attending Tertiary Care Centre.

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Conflicts of Interest: Nil

Abstract

Background:

Enterococcus spp. normally a commensal, is a potential pathogen of both community acquired and hospital associated infections like pneumonia, endocarditis , bacteremia, meningitis, neonatal sepsis, device associated infections and surgical site infections in both immunocompetant and immunocompromised patients. This study aims to assess the prevalence of various species of *Enterococci* among the various clinical infections in patients attending our tertiary care center.

Materials & Methods:

The study population included both inpatients and outpatient attending medicine, surgery and O&G departments .Our study was approved by the institutional ethical committee. The specimens like blood, urine, pus and wound discharges received in the laboratory were taken for study. The specimens were processed using standard microbiological techniques. The Enterococci isolated from these samples were selected for further processing and were characterized up to species level based on the Faklam and Collins conventional identification scheme for *Enterococci*. The results were analyzed using standard statistical methods.

Results:

A total of about 240 *Enterococci* isolates were recovered from 21,045 clinical samples including urine , blood, body fluids and pus. Majority of the *Enterococcal* isolates were from urine specimens 211 (87%) followed by blood 13(5%), pus 8 (3%), tissue fluids2 (0.8%), feces 6(4.6%). Of the total 240 *Enterococci* isolated,189 (79%), were from adult patients and 51(21%) from pediatric patients. Around 57% (138/240) were isolated from female and 43% (102/240) from male patients. A total of 14 (6%) *Enterococci* isolates were from intensive care units (medicine, surgery). The isolates from other specialties were, 11(4.5%) from nephrology, 92(38%) from urology, 20(8%) from surgery, 50(21%) from medicine , 51(21%) from pediatric departments. *E.faecalis* is the predominant species followed by *E.faecium*. Other *Enterococcal* species such as *E.sulfurous, E.columbae* , have been isolated from urine samples and *E.columbae,E.dispar* from blood,*E.mundtii,E,asini,E.avium and E.durans* from pus samples.

Conclusion:

A total of about 240 *Enterococcal* isolates were recovered from clinical specimens like urine , blood, pus and tissue fluids. Out of the 240 isolates, majority were from adults (79%) and (21%) from children. Higher isolation rate around 57% observed in female patients compared to male patients 43%. Majority of the isolates were from urine 87% followed by blood 5%and pus 3% fluids (0.8%). About (6%) *Enterococcal* isolates were from intensive

care units and the remaining from other specialties - urology(38%), medicine (21%) and pediatrics (21%) surgery(8%) and nephrology (4.5%). *E. faecalis* was the predominant species with an isolation rate of about 52.5% followed by *E.faecium 35%* and *E.raffinosus(3%)* , *E.sulfurous(2%), E.durans(0.8%), E.hirae (0.8%) E.avium (0,4%)and E. mundtii(0.4%)* were the other species isolated.

Keywords: clinical specimens, isolates, *Enterococcus* Species.

Introduction and Background:

The *Enterococcus spp.* a normal commensal and are a part of the indigenous flora of the intestinal tract, oral cavity and genitourinary tract of humans and animals. Now-a-days,it is a potential human pathogen, capable of causing a variety of infections in the community as well as in the hospital. During the past few decades, they have emerged as an important nosocomial pathogen exhibiting multiple drug resistance, contributing significantly to patient morbidity and mortality[1]. They cause serious infections like endocarditis and bacteremia, meningitis, intra-abdominal and pelvic infections, burns and surgical site wound infections in both immunocompetent and immunocompromised individuals. They pose a special risk in causing infection of the catheters and various other implanted medical devices in critically ill patients and also cause late onset sepsis, pneumonia and meningitis in neonates[2]. In a report from Centers for Disease Control and Prevention (CDC) *Enterococci* account for about 12% of health care associated infections (HAI) and ranks third most common multi-drug resistant pathogen causing HAI[3].

In view of the above perspective, the present study is carried out in our tertiary care hospital in Chennai, South India to assess the prevalence of various *Enterococcus* species in the various clinical infections encountered in patients.

Aims & Objectives:

1. To study the prevalence of *Enterococcus* species from various clinical samples
2. To Isolate and identify the *Enterococci* from various clinical samples by standard techniques.
3. To Characterize the *Enterococococcus* isolates up to the species level by standard microbiological techniques.

Materials and Methods:

Study Design: Cross sectional study

The present study was conducted in Department of Microbiology in Government Stanley Medical College and Hospital, Chennai, India.

Study Population and Period:

Patients attending outpatient department and inpatients of Govt. Stanley Medical College and Hospital, Chennai for a duration of 18 months (2015-2016)

Materials:

A total of about 21,045 clinical specimens such as urine, blood, pus, tissue fluids and feces obtained from all age groups of patients submitted to the microbiology laboratory for bacterial culture, were analyzed for growth. A total of about 240 *Enterococcus isolates* recovered from these clinical samples and were taken for further study.

The study was approved by our Institutional Ethical Committee.

Methodology:

Collection and Processing of samples:

The various specimens such as urine, blood, wound exudates, pus, tissue fluids and feces submitted to the microbiology laboratory from both inpatients and outpatients for bacterial culture were taken for further

study and identified by standard techniques as follows.[4,5,6,7,8]

Identification of the genus:

The suspected *Enterococcus* isolates grown on the primary plating media were selected and subjected to standard techniques of identification such as Gram staining, catalase test and motility test. Isolates which were Catalase negative, Gram Positive Cocci in pairs and short chains were selected and processed further. ATCC *Enterococcus faecalis 29212* was used as the control strain throughout the study.

The suspected isolates were then inoculated onto Sheep Blood agar, MacConkey agar and Bile esculin agar (containing 40% bile) and incubated aerobically at 37ºC overnight. After studying the colony morphology, the isolates which were nonhemolytic on blood agar, showing magenta pink colored tiny colonies on MacConkey agar and black discoloration of the medium in Bile Esculin agar were selected for further processing.

The isolates were tested for heat tolerance[7] by inoculating them into BHI broth and incubating them at 60ºC for 30 minutes in a water bath. Subcultures from the broth were done on blood agar and MacConkey agar before incubation and at intervals of 10 min, 20min and 30 minutes after incubation. ATCC *E.faecalis 29212* was used as a positive control. The isolates showing growth before and after 30min of incubation at 60ºC were taken as heat tolerant *Enterococcal* isolates.

Salt tolerance was tested by inoculating 2 to 3 identical colonies of suspected isolates along with control strains into a tube containing nutrient broth with 6.5% sodium chloride with 1% bromocresol purple as an indicator and incubated at 37ºC for 24-72 hours. The isolates showing turbidity with or without yellow discoloration are taken as salt tolerant and confirmed by subculturing them on Blood agar / MacConkey agar.

Salt tolerant, BEA positive isolates, which were able to grow on MacConkey agar and at temperatures of > 45ºC were identified as *Enterococci* and selected for further speciation.

Species characterization:

Speciation of the *Enterococcus* isolates was done based on the Faklam and Collins conventional identification scheme[10]. *Enterococci* were classified into the physiological groups I-V based primarily on arginine dihydrolysis, fermentation of mannitol and sorbose. Further speciation was based on acid production from specific carbohydrates and motility and pigment production as follows.

1.Arginine dihydrolysis was tested by inoculating the isolate into Moeller's decarboxylase broth containing arginine and incubating it for seven days at 37ºC along with control strains. Development of deep purple color after an initial change to yellow color read as positive reaction. Persistent yellow color indicates negative reaction.

2.The motility and fermentation of mannitol was tested by stab inoculating the isolates along with controls into Mannitol Motility medium and incubated at 37ºC overnight to detect the motility and acid production from mannitol.

3.To test the utilization of carbohydrates, the *Enterococcal i*solates were inoculated into carbohydrate fermentation medium containing 1% each of pyruvate, arabinose, sorbitol, sucrose, sorbose and raffinose and incubated at 37ºC overnight to detect acid production indicated by yellow discoloration.

4. Pigment production was tested by growing the isolate in Blood agar and is indicated by yellow/orange tinge on the swab on sweeping a few colonies using a cotton tipped swab[8].

The additional tests performed were production of black colored colonies on 0.04% Tellurite agar, which is a feature of *E.faecalis.*

Enterococcus faecalis isolates were identified by the following characteristics**:** Gram positive cocci in pairs and short chains, catalase negative and nonmotile. Colony morphology on Blood agar (5% sheep blood agar) was nonhemolytic, small, cream colored, smooth colonies with entire edge. β-hemolysis was observed on human blood agar. On MacConkey agar, produced lactose fermenting, magenta colored colonies and blackening of the medium on Bile esculin agar. The isolates were Heat tolerant, Salt tolerant and nonmotile, fermenting mannitol on Mannitol motility medium. The isolates hydrolyzed arginine producing deep purple coloration and produced black colored colonies on 0.04% Tellurite agar. They fermented pyruvate and sorbitol with acid production, arabinose and sorbose were not fermented. No pigment was produced.

Enterococcus faecium isolates were identified by the following characteristics*:* Gram positive cocci in pairs and short chains, catalase negative and nonmotile. Colony morphology on Blood agar(5% sheep blood agar) was non hemolytic, small, cream colored, smooth colonies with entire edge and produced blackening of the medium on BEA. They were heat tolerant, salt tolerant and nonmotile, fermenting mannitol on Mannitol motility medium. They hydrolyzed arginine, fermented arabinose but sorbose not fermented. They didn't produce any pigment and showed resistance to Imipenem. Other species of *Enterococci* were identified based on the following characteristics[6,8,9,10] as per the Faklam and Collins conventional identification scheme for *Enterococci* in the following table.

Enterococcal species	**Arginine dihydrolysis**	**Mannitol Motility medium**	**Fermentation of sugars**	**Motility & Pigment production**
E.raffinosus Group I	Not hydrolysed	Fermented Nonmotile	Raffinose,Arabinose Sorbose fermented.	Nonmotile no pigment
E.sulfurous GroupIV	Not hydrolysed	Not fermented nonmotile	Raffinose,arabinose fermented.Sorbose not fermented	Yellowish pigment produced.
E.columbae Group V	Not hydrolysed	Fermented nonmotile	Raffinose,Arabinose fermented.sorbose not fermented	No pigment
E.durans Group III	Hydrolysed	Not fermented Nonmotile	Raffinose,sucrose pyruvate not fermented.	Nonmotile no pigment
E.hirae GroupIII	Hydrolysed	Not fermented nonmotile	Raffinose&sucrose fermented. Pyruvate not fermented	Nonmotile no pigment
E.dispar Group III	Hydrolysed	Not fermented Nonmotile	Raffinose, sucrose &pyruvate fermented.	Nonmotile no pigment
E.avium Group I	Not hydrolysed	Fermented nonmotile	Arabinose,sorbose fermented. raffinose not fermented	Nonmotile no pigment
E.mundtii Group II	Hydrolysed	Fermented nonmotile	Arabinose fermented	Yellow pigment produced

Results and Discussion:

This study was carried out in the department of Microbiology for aduration of 18 months. A total of about 8774 urine specimens, 6305 pus specimens, 3655 blood specimens, 2183 tissue fluid specimens and 128 feces specimens were analyzed for *Enterococcus spp*. growth. The results were analyzed as follows.

A total of about 240 *Enterococci* isolates were recovered from the above samples, of which majority were from urine specimens-211(87%), 13 from blood specimens(5%), 8(3%) from pus specimens , 2 (0.8%) from tissue fluids (bile, Bronchial secretion) and 6(4.6%) isolates from feces specimens as depicted in table 1, which is higher than the findings of V.Gupta. et al[11] who have reported *Enterococcus* isolation rate of 49% from urine,5% from blood.

Table .1.Distribution of samples showing growth of *Enterococci*

Specimen type	OP		IP		Total	
	samples analyzed	Positive *Enterococcal* growth	samples analyzed	Positive *Enterococcal* growth	samples analyzed	Positive for *Enterococcal* growth
Urine	4169	113 (2.7%)	4605	98 (2.1%)	8774	211 (2.4%)
Pus	586	-	5719	8(0.001%)	6305	8 (0.001%)
Blood	319	-	3336	13 (0.003%)	3655	13 (0.003%)
Tissue fluids	3	-	2180	2 (0.009%)	2183	2 (0.009%)
Feces	60	-	68	6 (8.8%)	128	6 (4.6%)
Total	5137(24%)	113 (2.1%)	15908(76%)	127 (0.007%)	21045	240(1.1%)

OP- outpatients, IP – inpatients

The majority of the specimens were from inpatients (76%) than from outpatients (24%) which is in correlation with the findings of Acharya[14] et al,who have reported 72% specimens from hospitalized patients and 28% specimens from outpatients.

Table 2: Distribution of *Enterococcus* isolates in various clinical specialties.

Specimens	ICU/ IMCU	Non ICU					Total
		Nephrology	Urology	Surgery	Medicine	Pediatrics	
Urine	9	11	92	12	38	47	211
Blood	4	-	-	-	7	2	13
Pus	-	-	-	8	-	-	8
Tissue fluids	1	-	-	-	1	-	2
Feces	-	-	-	-	4	2	6
Total	14(6%)	11(4.5%)	92(38%)	20(8%)	50(21%)	51(21%)	240
	ICU14(6%)	Non ICU –226(94%)					

ICU- Intensive care units, IMCU-Intensive medical care unit.

In our study, a total of 14 (6%) *Enterococci* isolates were from intensive care units (medicine, surgery). The isolates from various specialties were 11(4.5%) from nephrology, 92(38%) from urology, 20(8%) from surgery, 50(21%) from medicine and 51(21%) from pediatrics

Suzanne.L.F et al[12]. Has reported 13.9% of *Enterococcus* isolates from ICU and 12% from non ICU patients. In our study, the isolation rate from ICU patients is lower about 6%. The risk factors we observed in ICU patients were presence of intravenous catheters, one patient on endotracheal intubation, two patients were transplant recipients under immunosuppression, one patient on urinary catheterization and presence of comorbid conditions such as Diabetes and heart disease, prolonged hospitalization and broad spectrum antibiotic usage such as third generation Cephalosporins were also noted. From nephrology 3 patients were on chronic ambulatory peritoneal dialysis. In the surgical wards, presence of comorbid conditions like diabetes, prolonged hospitalization, broad spectrum antibiotic usage (third generation Cephalosporins, Metronidazole) were the associated risk factors. In medicine patients comorbid conditions like Diabetes, broad spectrum antibiotic usage and recurrent infections were the associated risk factors. In pediatric patients, malnutrition and prolonged hospitalization were the associated risk factors observed. The feces samples were processed as surveillance cultures. We also observed that the isolation rate in patients of medicine was 21%, whereas it was 8% from surgery patients and 21% from pediatrics .. The present study showed varying isolation rates with other studies such as MM. Salem-Bekhit et al[13], who have reported the *Enterococcal i*solation rate of about 85% from ICU, about 27.9% from surgical patients and11.3% from internal medicine ward.

Table 3.Age and sex distribution of *Enterococci* isolates (n-240).

Age	sex		Total
	Male	Female	
Adults(≥13yrs)	75 (40%)	114 (60%)	189 (79%)
Children(≤12yrs)	27 (53%)	24 (47%)	51 (21%)
Total	102 (43%)	138 (57%)	240

Out of the total 240 *Enterococci* isolated, majority were isolated from adult patients 189 (79%), however around 51(21%)of isolates from pediatric patients. Acharya,A. et al[14],has isolated about 30.5% *Enterococci* from pediatric patients a which is in close resemblance to our study.

A higher isolation rate of about 57% (138/240) was observed among the female patients than 43% (102/240) from male patients. This is in contrary to the findings of MM Salem – Behkit et al[13], who have reported a male preponderance of about 91% in their study.

Table. 4. Distribution and prevalence rate of various *Enterococcus* species.

Enterococcal Species	*Urine*	*Blood*	*Pus*	*Tissue fluids*	*Feces*	*Total no*	*Prevalence value per 100subjects*
E.faecalis	115	6	2	-	3	126 (52.5%)	52.5%
E.faecium	72	5	2	2	3	84 (35%)	35%
E.raffinosus	8	-	-	-	-	8(3%)	3%
E.sulfurous	5	-	-	-	-	5(2%)	2%
E.columbae	3	1	-	-	-	4(1.6%)	1.6%
E.CDC PNSE2	3	-	-	-	-	3(1.2%)	1.2%
E.durans	1	-	1	-	-	2(0.8%)	0.8%
E.hirae	2	-	-	-	-	2 (0.8%)	0.8%
E.dispar	1	1	-	-	-	2(0.8%)	0.8%
E. asini	1	-	1	-	-	2(0.8%)	0.8%
E.avium	-	-	1	-	-	1(0.4%)	0.4%
E.mundtii	-	-	1	-	-	1(0.4%)	0.4%
Total	211	13	8	2	6	240	

E.faecalis is the predominant species followed by *E.faecium*. Other *Enterococcal* species such as *E.sulfurous, E.columbae* , have been isolated from urine samples and *E.columbae,E.dispar* from blood,*E.mundtii,E,asini,E.avium and E.durans* from pus samples .

In our study we observed that *E.faecalis* was the predominant species 126 (52.5%) followed by *E.faecium* 84 (35%) *E.raffinosus*(3%), *E.durans(0.*8%) E.hirae 0.8% , *E.avium(0.4%) and E,mundtii (0.4%)* .In other studies, Perlada.D,et al[15]. have reported,69%E.faecalis,29% *E.faecium* and 1% each of *E.avium and E.durans* .Vittal P Prakash et al[16] have reported 2.5% *E.raffinosus* and 2.5% *E. hirae* , 1.7% *E.mundtii* and MM Salem-Behkit et al.[13]reported 2.1% *E.avium* and 0.8% of *E.hirae* which correlates with the findings of our study.

Conclusions:

- A total of about 21,045 clinical specimens like urine, blood, pus and tissue fluids were analyzed for *Enterococcal* isolates.Majority of the specimens were from inpatients (76%) than from outpatients.
- A total of 240 *Enterococcal* isolates were recovered from these specimens. Majority of the isolates were from urine 87% followed by blood 5%and pus 3% fluids 0.8%.
- Around 6% of *Enterococcal* isolates were from intensive care units and the isolation rate from other specialities were Urology (38%), Medicine (21%) and Pediatrics (21%) Surgery (8%) and Nephrology (4.5%).
- Majority of the isolates were from adults (79%) and 51 (21%) from children. Higher isolation rate of

about 57% was observed in female patients, compared to male patients43%.

- *E. faecalis* was the predominant *Enterococcal* species with an isolation rate of about 52.5% in our study, followed by *E.faecium 35%.*

Other than these two species, *E.raffinosus(3%)* , *E.sulfurous(2%), E.durans(0.8%), E.hirae (0.8%) E.avium (0,4%)and E. mundtii(0.4%)* were also recovered from the clinical specimens.

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