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Research article

Isolation and characterisation of enterococci from a tertiary care centre

Lavanya.T. Kamalasekaran[1], Jhansi Charles[2]
[1]Department of Microbiology, Govt.Kilpauk Medical College, Kilpauk, Chennai
[2]Department of Microbiology, Velammal Medical College Hospital & Research Institute, Velammal Village, Tuticorin Ring Road, Anuppanadi, Madurai

Abstract

Enterococci comprise a significant portion of the normal flora of the gastrointestinal tract (GIT) with some also being found in gastrointestinal, vaginal secretions and in perineal area. Enterococci account for as many as 10% of cases of neonatal bacteremia and septicaemia. Enterococci have become important nosocomial pathogens world-wide and are associated with a high mortality. **Aim:** To isolate and speciate Enterococcus from hetrogenous samples collected from Government Rajaji Hospital (GRH), Madurai. **Methods:** A total of 200 isolates from various clinical samples were included and processed according to standard protocol in different wards at GRH, Madurai. The specimens were plated on Nutrient Agar, MacConkey and Blood Agar plates and incubated at 37 ^{0}C overnight. Presumptive identification of the isolate as Enterococcus was done by Gram's stain, Catalase test, Bile Esculin hydrolysis, Heat resistance and Salt tolerance test. Further speciation was done by carbohydrate fermentanion, pyruvate fermentation, arginine hydrolysis, potassium tellurite reduction. **Results:** Distribution of Enterococcus in this study showed that Enterococcus isolated from blood 20 (55.5%), urine 14 (38.8%), pus 1 (2.7%) and wound swab 1 (2.7%). On speciation, E. faecium 18 (50%), followed by E.faecalis 15 (41.6%) and E.durans 3 (8.3%). Isolates were more from paediatric ward. Of the 33.3% of the Enterococcus isolates from paediatric ward, 27.7% were between the age group 0-1 month. **Conclusion:** In this study distribution of Enterococcus isolates were more from pediatric age group. Mostly isolated from blood specimen 55.5%. E.faecium (50%) was the predominant species isolated

Keywords: Enterococcus, Gastrointestinal tract, Neonatal septicemia, Nosocomial pathogens.

***Corresponding author: Dr. Lavanya T. Kamalasekaran,** Senior Assistant Professor, Department of Microbiology, Government Kilpauk Medical College, Kilpauk, Chennai 600010, India. Email id: manorajanlavanya@gmail.com

1. Introduction

Enterococci have evolved over the past century from being an intestinal commensal organism of little clinical significance to becoming the second most common nosocomial pathogen associated with significant morbidity and mortality [1]. Although about 19 species of enterococci have been identified, *Enterococcus faecalis* and *Enterococcus faecium* are responsible for the majority of human infections [2]. The most common nosocomial infections produced by these organisms are urinary tract infections followed by intraabdominal and pelvic infections. They also cause surgical wound infections, bacteraemia, endocarditis, neonatal sepsis and rarely meningitis [3]. As Enterococcal infections are one of the leading cause of nosocomial infection, and being inherently resistant to many antibiotics, this study was undertaken to isolate and speciate the Enterococci from various clinical samples in our tertiary care hospital.

Aim

To isolate and speciate Enterococcus from various clinical samples collected from Govt. Rajaji Hospital (GRH).

2. Methods

This Prospective study was conducted at GRH, attached to Madurai Medical College after getting ethical clearance from the Human Ethical Committee, GRH, headed by the Dean. The period of study was 6 months. The study population consisted of 200 patients admitted in different wards viz Paediatrics, General Surgery, Urology and Medicine at GRH, Madurai. Various specimens like Blood, Urine, Pus, Wound Swab, and Cerebrospinal fluid (CSF) were collected from the patients depending on the clinical symptoms. All the specimens were collected by standard protocol under aseptic precautions [4].

Processing of Specimen

The specimens were plated on Nutrient Agar, MacConkey and Blood Agar plates and incubated at 37 ^{0}C overnight. On Nutrient Agar plate, Entercoccus colony was small 0.5 –1 mm transparent, low convex, discrete with glossy surface. On MacConkey Agar, they produced 0.5-1mm sized, magenta coloured lactose fermenting colonies. Blood agar plate showedα, β, or γ hemolysis. The colonies with the above characteristics were presumed to be Enterococcus. Further confirmation of the isolate as Enterococcus was done by Gram's stain, Catalase test, Bile Esculin hydrolysis, Heat resistance and Salt tolerance test. In Grams stain, Enterococci appeared as Gram positive cocci arranged in pairs and short chains with a characteristic leaf shape. Further the organisms were found to be catalase negative. The culture was then inoculated onto Bile esculin agar plates and incubated at 37 ^{0}C for 48 hrs. Enterococci and group D Streptococci produced blackening of the agar plates. The organisms were further subjected to heat test. For this test, 2 or 3 of the isolated colonies were inoculated into glucose broth and incubated at 37 ^{0}C overnight. The growth was judged with the turbidity, sub cultured from the broth onto one half on a nutrient agar plate, then the broth was placed in a water bath set at 60 ^{0}C for 30 minutes. Sub culture was done from the broth on the other half of the nutrient agar medium. After incubation at 37 ^{0}C for 24 hrs, growth was observed on both halves of the plate, which indicates the heat resistant property of Enterococci. Next salt tolerance test was done. This is based on the ability of the Enterococci to grow in the presence of 6.5% NaCl incorporated into broth/agar while other Group D Streptococci are negative for this test [5, 6].

The Enterococcus isolates were further speciated into E. faecalis, E. faecium and E. durans depending upon the fermentation of sugars, motility, arginine decarboxylation, pigmentation, reduction of tellurite and using selective media. The common sugars used were Arabinose, Raffinose, Sucrose, Sorbitol and Mannitol. For sugar fermentation test, Todd-Hewitt broth with the 1% sugar and bromothymol blue as indicator was used. Each tube was inoculated with 2 drops of an 18-24 hrs brain-heart infusion broth culture and incubated at 37 ^{0}C for 24-48 hrs and observed for colour change from blue to yellow. Pyruvate fermentation was tested by inoculating fresh culture into pyruvate broth and incubated at 37 ^{0}C for 24-48 hours. Change in color from blue to yellow showed fermentation of pyruvate.

All the three species did not ferment Raffinose. Arabinose was fermented by E. faecium. Pyruvate was fermented by only E. faecalis. The above three species were found to be non motile by hanging drop and by Mannitol motility medium. All the above three species of Enterococcus deaminated the amino acid Arginine to ammonia resuting in alkalinization of the medium thus changing the colour from yellow to purple. Pigmentation produced was tested by touching the colony grown on Trypticase Soy Agar with a Dacron swab and noting whether yellow pigment was produced or not. All the three species, E. faecalis, E. faecium and E. durans did not produce any pigmentation. The isolates were then tested for reduction of tellutrite. The medium used was human blood agar plate incorporated with 0.04% pottasium tellurite. The isolated colonies were streaked on the plate, incubated at 37 ^{0}C for 24-48 hours. Enterococcus faecalis isolates were identified by the brownish black colored colonies. The other two species did not reduce potassium tellurite into metallic tellurium [7, 8]. Table 1 shows the identification of three species.

Species	Motility	Arabinose	Raffinose	Sucrose	Sorbitol	Mannitol	Pyruvate	Arginnine	Pot.tellurite
E. faecalis	-	-	-	±	±	+	+	+	+
E. faecium	-	+	-	v	-	+	-	+	-
E. durans	-	-	-	-	-	-	-	+	-

3. Result

Among the 200 samples, 50 samples were collected from the Paediatric ward. Out of the 50 samples collected, 24 (48%) were urine, 24 (48%) were blood, 1 each from CSF and pus (2% each). Among the 50 samples collected from the General Surgery, 8(16%) were Urine, 12 (24%) were blood, 17 (34%) were Pus, 13 (26%) were Swab and there was no CSF sample. Among the 50 samples collected from the Medical ward, 19 (38%) were Urine, 26 (52%) were blood, 4 (8%) were CSF and 1 (2%) was from Pus. From Urology ward, a total of 50 samples were collected, out of which 33 (66%) were from Urine, 17 (34%) were Blood and there was no pus/swab/CSF. Table 2 shows distribution of samples ward wise and specimen wise.

Table 2 Ward wise and Specimen wise distribution of sample

SN	Ward	Total no of Specimens	Specimen				
			Urine	Blood	CSF	PUS	Pus/Wound Swab
1	Pediatric	50	24(48%)	24(48%)	1(2%)	1(2%)	-
2	General Surgery	50	8(16%)	12(24%)	-	17(34%)	13(26%)
3	Medicine	50	19(38%)	26(52%)	4(8%)	1(2%)	-
4	Urology	50	33(66%)	17(34%)	-	-	-
Total		200	84(42%)	79(39.5%)	5(2.5%)	19(9.5%)	13(6.5%)

The 36 enterococal isolates were further analysed age wise. It was noted that 10 (27.7%) out of 36 were in the age group 0-1 month, 3 (8.3%) were in the age group of 1 month– 12 months, 2 (5.5%) were between 1 – 12 yrs, 7 (19.4%) were in the age group of 13 – 33 yrs, 8 (22.2%) were in the age group 34 – 54 yrs and 6 (16.6%) were in the age group more than 54. It was noted that maximum numbers of isolates of enterococcus were between the age

group 0-1 month. The age wise distribution of enterococcus is given in Table 3.

Table 3 Age wise distribution of Enterococcus

Patient's Age in Years	Number	%
0-1 month	10	27.7
1– 12 months	3	8.3
1 – 12 yrs	2	5.5
13 - 33yrs	7	19.4
34 – 54 yrs	8	22.2
> 54 yrs	6	16.6

All the 71 Gram positive cocci were processed for the presence of enterococcus and by confirmation using the various tests and it was found that 36 out of 71 (50.7%) were Enterococci. The other Gram positive cocci isolated were Coagulase Negative Staphylococci (CONS) 24 (33.8%) and Staphylococcus aureus 11 (15.4%). Sexwise analysis of the 36 Enterococcal isolates showed that in Pediatric ward, 7 out of 12 isolates were from males (58.3%) and 5 were from females (41.6%) In general surgery, among the 8 enterococcus isolates, 5 (62.5%) were males and 3 (37.5%) were females. In medical ward, out of 6 isolates 4 (66.6%) were males and 2 (33.3%) were females. In the urology ward, out of 10 enterococcus isolates, 4 were from males (40%) and 6 were from females (60%). Analysis of sexwise distribution of Enterococcus isolates showed that males predominated in all the wards except urology where the females predominated. The isolates were analysed wardwise and it was found that 12 (33.3%) were from pediatric ward, 8 (22.2%), were from Surgery ward, 6 (16.6%) were from Medical ward and 10 (27.7%) were from Urology ward. Thus maximum numbers of isolates of Enterococcus were from Pediatric ward. Wardwise and sexwise distribution of enterococcus isolates is given in Table 4.

Table 4 Wardwise and sexwise distribution of enterococcus

S N	Ward	Total Number	Sex	Number	%
1	Pediatric	12	Males	7	58.3
			Females	5	41.6
2	General Surgery	8	Males	5	62.5
			Females	3	37.5
3	Medicine	6	Males	4	66.6
			Females	2	33.3
4	Urology	10	Males	4	40
			Females	6	60
		36		36	

The enterococcus species were analysed specimen wise and it was found that out of the 36 Enterococcal isolates, 14 (38.8%) were from urine, 20 (55.5%) were from blood, 1 (2.7%) was from pus, 1 (2.7%) was from Swab and no isolate from CSF. Thus maximum numbers of enterococci were isolated from blood samples. Specimen wise distribution of enterococcus isolates is given in Table 5.

Table 5 Distribution of Enterococci Specimen wise

Specimen	Distribution	Percentage
Urine	14 /36	38.8
Blood	20 /36	55.5
Pus	1 /36	2.7
Swab	1 /36	2.7
CSF	-	-
Total	36 /71	50.7

Enterococcus were speciated into E.faecalis, E.faecium and E.durans according to the biochemical reactions and it was found that out of the 36 Enterococcus isolates, 15 (41.6%) were E.faecalis, 18 (50%) were E.faecium, and 3 (8.3%) was E. durans. Thus the most common species of enterococcus isolated was E. faecium. The species wise distribution of Enterococcus is given in Table 6.

Table 6 Species wise distribution of Enterococcus

Species	Isolates in number	Percentage
E.faecalis	15	41.6
E.faecium	18	50
E.durans	3	8.3

Table 7 Ward wise Distribution of Enterococcus Species

SN	Ward	E.faecalis		E.faecium		E.durans	
		Total Number	%	Total Number	%	Total Number	%
1	Pediatric (12)	2	1.8	9	75	1	2.7%
2	General Surgery (8)	4	50	3	37.5	1	12.5%
3	Medicine (6)	3	50	2	33.3	1	16.6%
4	Urology (10)	6	60	4	40	-	-

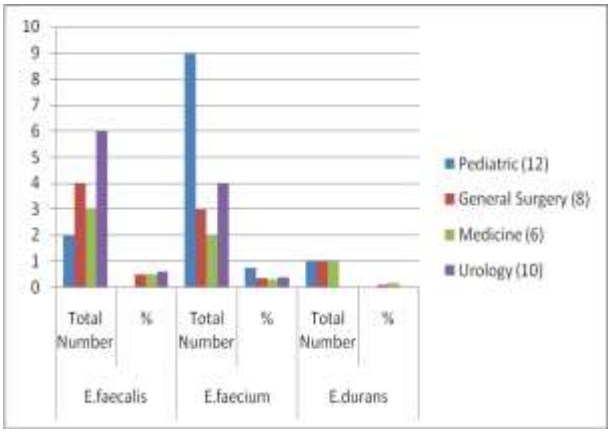

Figure 1 Ward wise Distribution of Enterococcus Species

Table 8 Specimen wise Distribution of Enterococcus Species

Source	E.faecalis	E.faecium	E.durans
Urine	10 / 36 27.7%)	2/ 36(5.5%)	2/ 36 (5.5%)
Blood	4 /36 (11.1%)	14 /36(38.8%)	1/36(2.7%)
Pus	-	2 /36 (5.5%)	-
Swab	1 /36(2.7%)	-	
Total	15/36(41.6%)	18/36 (50%)	3/36(8.3%)

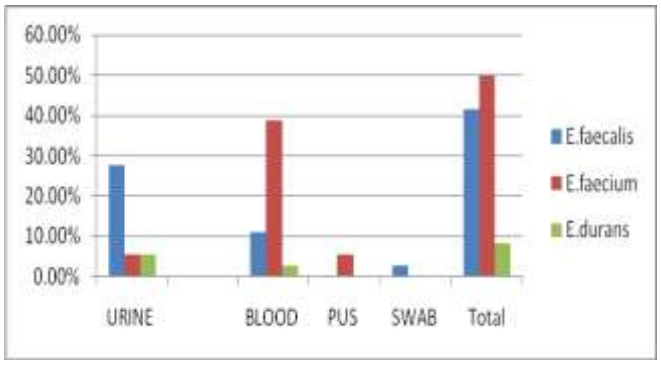

Figure 2 Specimen wise Distribution of Enterococcus Species

Thus, It was noted that E. faecium was the commonest species found in the pediatric ward shown in Figure 1. It was found that E. faecium was isolated more from blood and E.faecalis more in urine shown in Figure 2.

4. Discussion

A total of 200 clinical samples from varied infections of Pediatric, General Surgery, Medicine and Urology wards of (GRH), Madurai were collected and analysed to find the species of enterococcus commonly isolated from Urine, Blood, CSF, Pus and wound Swab. They were further analysed age wise, sexwise and specimen wise to know the common age group, sex and the common infection involved by the species of enterococcus.

In the present study, among the 200 samples collected it was found that 42% of samples collected were from urine and 39.5% samples collected were from blood. This is in accordance with the study by Steven Gordan et al who had shown that 57% of their samples were from urine [9] and Patrick Murray etal who had also shown that 36% of their samples were from blood [10]. As the most common sites for isolation of enterococcus are urinary tract and blood stream, more number of samples collected from these two infections are justified.

In this study it was observed that 50.7% of Gram postive cocci isolated from various infections were enterococcus species. This is in accordance with the study of Louis B. Rice etal who also had shown that Enterococcus infection was responsible for more than 40% in their study on various infections [11]. The higher incidence of Enterococcus among Gram positive cocci may be due to the properties involved in the adherance to host tissue which are considered as important virulent factors for establishing infection by Enterococci. Also enterococci are intrinsically resistant to a wide range of antibiotic which notably include β- lactams and Aminoglycosides frequently used to treat infections with Gram positive organisms. Also they have ability to acquire resistance to antimicrobial agents through plasmids and transposons and chromosomal exchange or mutation.

Age wise distribution of enterococcus in this study showed that 33.3% of them were from the paediatric ward and 27.7% of the enterococci isolated in pediatric ward were between the age group 0-1 month. This is in accordance with the study by Al Otaibi et al who had reported 30% of Enterococcal bacteremia in neonates [12]. The occurrence of enterococcal bacteremia in neonates is obvious because of their poor development of immune system and emergence of virulent antimicrobial resistant enterococci in pediatric wards due to the constant wetting of beds by neonates and irrelevant usage of antibiotics in these wards. In this study, in the paediatric ward, 86.3% males showed enterococcus whereas only 13.7 % females showed enterococci. Similar study by A.S.M. Nawshad Uddin Ahmed in their analysis of cases had reported that 63% of males were with neonatal enterococcal septicemia [13]. This might be due to the better natural immunity shown by female children or by hormonal protection rendered to the female children.

The sex wise distribution of Enterococcal isolates showed that 55.5% were males and 45% were females. In all wards except Urology, more than 50% isolates were from males and in Urology ward more than 50% isolates were from females. Vittal Prakash et al in their study also demonstrated that 56.5 % were males and 43.5% were females [14]. The increased incidence of enterococcal isolation among females in Urology ward may be attributed to the more number of Urine samples collected in ward and enterococcus is a known urinary pathogen. It is more common in females mainly due to the anatomical built of the female urethra. The less incidence in males may be due to the drier environment surrounding the meatus and antibacterial effect of prostatic fluid.

It was also shown in this study that 33.3% of Enterococcus were from Pediatric ward whereas Wisplinghoff et al has demonstrated only 9% Enterococci from Pediatric ward[101]. Louis B. Rice et al in their study proved that relative proportion of enterococcus infection had increased in some instances to even more than 40% [15]. This sudden increase might be due to the sudden emergence of antibiotic resistant Enterococci in hospitals especially in pediatric ward.

In this study, it was observed that Enterococcus was commonly isolated in blood (55.5%) and the same was supported by A. Bedini et al who had reported in their study an incidence of 42.9% enterococci in blood stream infections [16]. Patrick Murray had explained that most of the enterococcal bacteremias were of nosocomial origin because the enterococcus has the character of showing

multiple drug resistance especially due to heavy use of antimicrobial agents [10].

It was also observed in this study that 77.7% of Enterococcus isolated were from inpatients. This in accordance with the study by Martinez Odriozola et al who had reported that 68% of enterococcal isolates were hospital acquired [17] and Patterson et al who had reported that 61% of enterococcal infections were nosocomial [18]. The period of study was post monsoon period in which many diarrhoeal cases were reported in the pediatric ward. Obviously, enterococci, a commensal of Gatrointestinal tract would have occured as a nosocomial agent in the pediatric ward where neonates with poor immunity and heavy doses of irrelevant antibiotics got admitted.

Species wise distribution of enterococcus showed that 50% of enterococcal isolates were E.faecium. It was noted in this study that 75% of E.faecium were from the paediatric ward and 90% of E. faecium in the pediatric ward were in the age group 0-1 month and 71.4 % pediatric cases were in males. This is in accordance with the study by Lata Kapoor et al who had also isolated 66% of E.faecium from paediatric cases from males [19]. Prematurity, low birth weight, increased number of days of hospitalisation, treatment via central venous line, parentral nutrition and antibiotic abuse might have attributed to the increased colonisation of neonates by enterococcus.

Specimen wise distribution showed that Blood was the most common specimen from which it was isolated (38.8%). Similar studies by M.G Karmarkar et al have reported 80.7% of cases due to E.faecium [20]. Uma Chaudhary et al have stated that E.faecalis was the most common species in all clinical specimen except in blood where E.faecium was the most common isolate (50%) [21]. Mohanty et al in their study recovered 42.9% of E. faecium [22], which was the predominant isolate from blood wheras E.faecalis was mostly isolated from urine and pus. Thus the above studies correlated well with the present study. The increasing predominance of E. faecium as a cause of bloodstream infections may partly reflect the high rate of antimicrobial resistance in this species. E.faecium more commonly acquires resistance to ampicillin and glycopeptides relative to other enterococcal isolates. Since ampicillin was one of the common antibiotics used in our hospital during the study period, the increased incidence of enterococcus faecium during that period was justified.

Conclusion

The study on the speciation of Enterococci in varied infections of GRH revealed that out of 200 samples from varied infections at GRH, 42% were urine samples and 39.5% were blood samples. Among them, 52% were gram positive organisms from the pediatric ward in blood samples. Of the gram positive organisms, 50.7% were enterococcus isolates and 33.3% among them were from pediatric wards. Males predominated in 55% of the isolates and females only in 45%. More than 50% enterococci were found in males in all wards except urology where only females showed more than 50%. Enterococci were isolated in 55% of the blood samples, 33.3% of them were from pediatric ward, 27.7% in the age group 0-1 month, 58.3% showed male predominance and 77.7 % were inpatients.

The Enterococci on speciation showed that 50% were E. faecium. Analysis of E.species showed that 38.8% were from blood, 75% were from pediatric ward, 90% were in the age group 0-1 month and 71.4 % were males.

- Enterococcal infections contributed to a significant proportion of infection in the population under study.
- The most common species was E.faecium.
- The patients infected with E.faecium were mostly children between 0-1 months and septicemia was the most common infection.

Therefore this study has revealed that Enterococci could emerge as a significant agent of nosocomial infections especially in neonatalogy wards contributing to significant morbidity and mortality by virtue of Multi Drug Resistance.

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