



Characterisation of Enterococcal Isolates from a Tertiary Care Centre-A One Year Study

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Abstract

Enterococci form a part of normal flora of intestinal tract, oral cavity and vagina, but in recent times they have become emerging nosocomial pathogens. Enterococcus species have emerged as an important pathogen for urinary tract infections, hepatobiliary sepsis, endocarditis, surgical wound infection, bacteremia and neonatal sepsis. The therapeutic failures in Enterococcal infections are mainly due to the development of antibiotic resistance. The main aim of our study was to estimate the prevalence of the Enterococcus infection and to determine the antibiogram in a tertiary care hospital. 94 strains of Enterococci are collected and characterized from various clinical specimens from Medical College Hospital and Sree Avittom Thirunal (SAT) Hospital, Thiruvananthapuram. The most common human species isolated were Enterococcus faecalis and E. faecium. Enterococcal strains resistant to Ampicillin, Erythromycin and Vancomycin were isolated during the study.

Review of Literature

The Enterococci are Gram-positive, facultatively anaerobic oval cocci that form pairs and chains with ability to survive under high salt concentrations and at a wide range of temperatures (from 10 °C to >45 °C)¹.

Enterococci cause a range of infections including intraabdominal, pelvic, biliary tract, wound, burns and urinary tract infections, bacteremia and infective endocarditis. The increasing number of patients in critical care units within transvascular devices or urinary catheters and on multiple antibiotics favours the development of multidrug-resistant Enterococci¹.

Aim of Study

1. To study the isolation rate of Enterococci from various specimens & to characterize them.
2. To analyze the resistance pattern of various species of Enterococci to antibiotics.

94 strains of Enterococci were collected and characterized from various clinical specimens in Central Microbiology Laboratory, Medical College Hospital and Sree Avittom Thirunal (SAT) Hospital, Thiruvananthapuram during the period March 2012 to February 2013 .

Materials and Methods

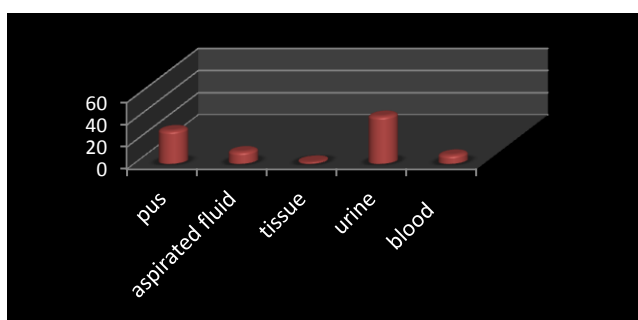
Enterococci isolated from various clinical specimens were inoculated into 5% Sheep blood agar, MacConkey agar, Tellurite blood agar & Glucose broth.

Identification was based on the growth characteristics on culture medium, gram staining, catalase test, PYR Test, sugar fermentation tests. Aesculin hydrolysis, salt tolerance test, heat resistance test & Arginine Hydrolysis test were done for Enterococci isolated.

Results

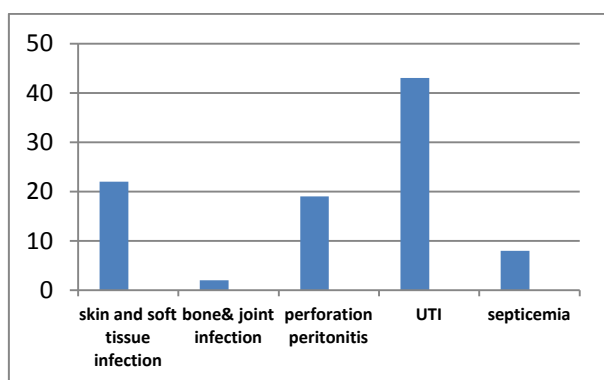
Distribution pattern of Enterococci from various clinical specimens.

SPECIMEN	Number	%
Pus	30	31.91
Aspirated fluid	11	11.71
Tissue	2	2.12
Urine	43	45.74
Blood	8	8.5



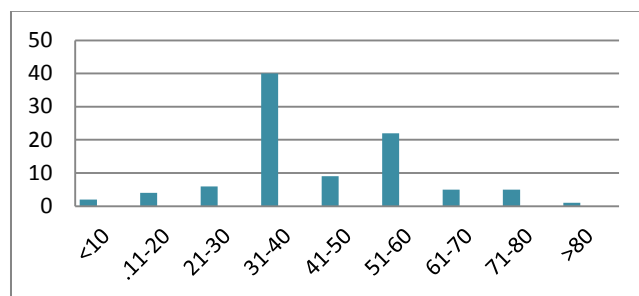
Distribution of Enterococci from various clinical condition

Clinical condition	Number	percentage
Skin & soft tissue infection	22	23.4%
Bone & joint infection	2	2.12%
Perforation peritonitis	19	20.21%
Urinary tract infection	43	45.7%
Septicemia	8	8.51%



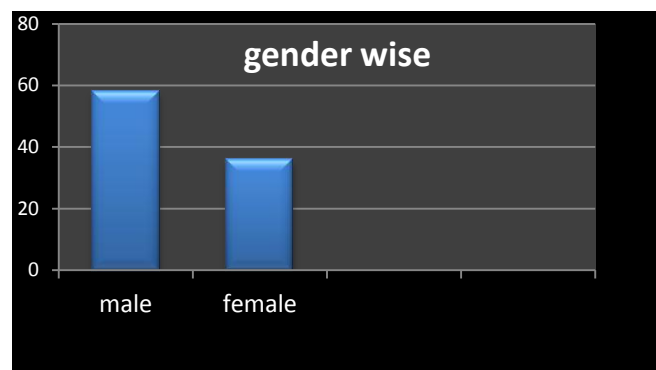
Age wise distribution of cases with enterococcal infection

Age	<10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	>80
No.	2	4	6	40	9	22	5	5	



Gender wise distribution of study group

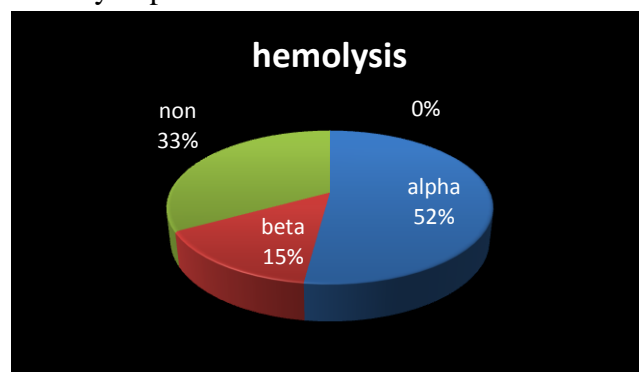
Male	female
58	36



Classification of isolates based on hemolysis of sheep blood agar

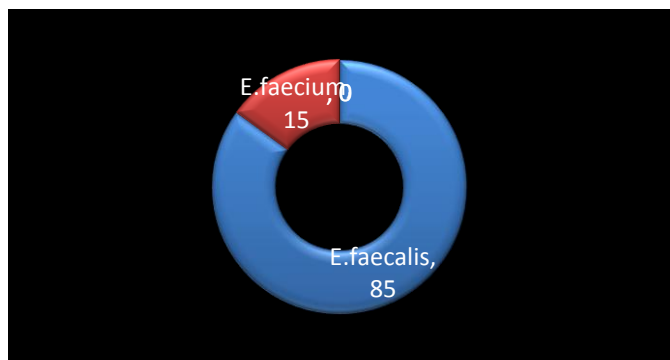
Alpha	Beta	Non hemolytic
49	14	31

Hemolytic pattern



Distribution pattern of two major groups of Enterococci isolated.

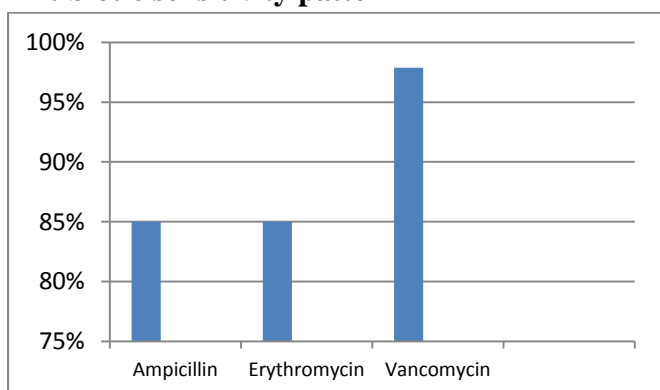
Enterococcus faecalis	85.10%
Enterococcus faecium	14.89%



Biochemical reactions of two different species of Enterococci

Sugars			Total
lactose	80	14	94
sucrose	80	14	94
mannitol	80	14	94
Arabinose	0	14	14
pyruvate	80	0	80
sorbitol	80	14	94
raffinose	0	14	14
inulin	2	0	2
ribose	80	14	94

Antibiotic sensitivity pattern



Discussion

During the past few decades, Enterococci have emerged as important healthcare-associated pathogens. The continuing progress of modern medical care toward more intensive and invasive medical therapies for human disease has undoubtedly contributed to the increased prevalence of these remarkable opportunistic pathogens. This trend has also been attributed to the increasing antibiotic resistance among clinical isolates of Enterococci. The rapid spread of resistance Vancomycin Resistant Enterococci (VRE) has been of particular concern. In the present study 85.10% of Enterococci

belonged to Enterococcus faecalis species and 14.89% Enterococcus faecium species. This almost correlates with the study by Bose S et al in which 82% were Enterococcus faecalis and 18%, Enterococcus faecium². The studies done by Parameswarappa J et al, Piekarska K et al, Donald et al and Desai PJ reported the percentage of isolation of E.faecalis (63.3%, 66.2%, 57.2, 49.50%) and E.faecium (36.7%, 29.2%, 42.8%, 35.64%) respectively^{5,6,16,8}. Rudy M et al also reports among the Enterococcal species the most common cause of child urinary tract infection to be E.faecalis¹⁰. Another study conducted by Sawicka et al reports almost equal proportion of isolation of isolation of E.faecalis and E.faecium¹¹.

Out of 94 GDS, 23.44% were from skin and soft tissue infection, from UTI 45.74%, from perforation peritonitis 20.3%, 8.3% from septicemia. The studies done by Bose S et al, Jada S et al, Desai P J et al found out that maximum Enterococcal isolates were from urine (62.36%, 40.30%, 48.2%) followed by pus (8.1%, 31.90%, 29.5%) respectively^{2,7,8}. Another study conducted by Graninger & Ragette 1992 during a 2-year observation period at a 2,200-bed university hospital, bacteremia due to Enterococcus faecalis was observed in 111 patients; out of 55 patients evaluated for nosocomial bacteremia due to E. faecalis the most common entry sites were the urinary tract (25%), the intraabdominal cavity (13%), and burn and decubital wounds (11%).³

The present study showed that Enterococci were isolated mainly from 31-40 age group (42.55%), 23.40% from 51-60 age group, 9.57% from 41-50 age group, 5.31% from 61-70 and 71-80 age group. This almost correlates with study done by Parameswarappa J et al in which 32% of Enterococcal isolates were between 40 and 60 years of age⁴. In another study by Jadan S et al on Enterococcal isolates reported majority of isolates are from middle age group (31-50 age group)⁷. Paulsen LL et al and Barros M et al found out the mean age of Enterococcal UTI as

48 years and 48.9% respectively^{12,13}. Bose S et al found out that the commonest age group affected by Enterococci as 21-40 years².

In the present study out of 94 Enterococci isolated 61.71% were from males and 38.94% from females. This correlates almost the study done by Parameswarappa J et al in which Enterococci were isolated from 68.7% males and 31.3% females⁴. In another study by Jada S et al also reported Enterococci from 55.06% from male patients and 44.94% from female patients⁷. The study by Barros M et al and Bose S et al found out the male: female ratio of Enterococcal infection as 2:1 and 1:2.2 respectively^{13,2}. Poulsen LL et al also reported the cases of Enterococcal UTI were higher in females than males¹²

In present study out of 94 Enterococci, 15% were resistant to Ampicillin and Erythromycin and 2.13% were resistant to Vancomycin. In studies from Lady Hardinge Medical College, New Delhi, Chandigarh and Mumbai reported the percentage of VRE are 8, 5.5 and 23 per cent respectively¹¹ Bose S et al found out that the Enterococcal isolates from various clinical samples were 100% susceptible to vancomycin². Sawicka-Grzelak A et al found out that out of 100 Enterococcus strains, ten isolates demonstrated intermediate susceptibility to vancomycin, 36% and 73% were resistant to Ampicillin and Erythromycin respectively¹¹. Donald et al in SENTRY antimicrobial surveillance program in USA from 1997-1999 observed 17% vancomycin resistant Enterococcal infections reported to CDC-NNIS system increased from 0.3% in 1989 to 7.9% in 1993¹⁶. Chaudery U et al (2007) Post Graduate Institute of Medical Sciences, Haryana reported that the susceptibility of Enterococci to vancomycin was 98%¹⁵. Vidyalekshmi P R et al, Appolo hospital, Chennai reports VRE was isolated in 4% of Enterococci¹⁷. Bose S et al found out that the Enterococcal isolates from various clinical samples were 100% susceptible to vancomycin.² Miskeen PA et al from Bombay Hospital and

Medical Research Centre reported that the magnitude of resistance to ampicillin among Enterococcus species as 23.13% and no resistance as found against vancomycin.¹⁸ Shinde R S et al Mumbai (2012) reported that out of 54 isolates ampicillin resistance was observed in 16.6% and no resistance was found against vancomycin.¹⁹

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