



RESEARCH ARTICLE

CHARACTERIZATION AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF CLINICAL ISOLATES OF ENTEROCOCCI AT A TERTIARY CARE HOSPITAL IN BHAVNAGAR, GUJARAT.

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Abstract

Enterococcal infection is a life threatening systemic infection prevalent in developing countries like India and is a major public health problem. Enterococci are important nosocomial pathogen of human disease; second most common cause of nosocomial urinary tract and wound infections; third most common cause of nosocomial bacteremias. Because of their resistance to different antimicrobial agents these bacteria are often involved in serious super-infections among patients receiving broad-spectrum anti-microbial therapy. Correct diagnosis is crucial in proper management of Enterococcal infection, so laboratory tests are essential to establish identification of Enterococcal species. The study was conducted to isolate and characterize *Enterococcus* species from different clinical samples and to perform antimicrobial susceptibility testing in all age groups and both genders. Out of 200 Enterococcal species isolated, 164 (82%) were *E. faecalis* and 34 (17%) were *E. faecium*; majority were isolated from urine (60%) and blood (35.5%); they showed more resistance to penicillin (92%), less resistance to vancomycin (1%) and no resistance to linezolid (0%).

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Introduction:-

Enterococci are Gram-positive cocci which are normal residents of the gastrointestinal tract, biliary tract and, in lower numbers, of the vagina and male urethra, in saliva. They are becoming increasingly important nosocomial pathogen of human disease, largely because of their resistance to antimicrobial agents to which other streptococci are generally susceptible.

Enterococci are the second most common cause of nosocomial urinary tract and wound infections and the third most common cause of nosocomial bacteremias. *Enterococcus* species also cause endocarditis, intraabdominal and pelvic infections, soft-tissue infections, neonatal sepsis and rarely meningitis¹.

Because of their resistance to penicillin and cephalosporin group, the acquisition of high-level resistance to aminoglycosides, and now the emergence of vancomycin resistance, these bacteria are often involved in serious super-infections among patients receiving broad-spectrum anti-microbial chemotherapy. Newer agents such as linezolid and daptomycin may also be effective; other agents such as chloramphenicol may be used when multidrug-resistant strains are encountered. For urinary tract isolates, nitrofurantoin, tetracycline, or quinolone may be effective^{1,2}.

In mid-1980s, *Enterococci* were distinguished from *streptococci* and related taxa by their ability to grow at 10°C and 45°C, growth in the presence of 6.5% NaCl, growth at pH 9.6, grow in 0.1% methylene blue milk, ability to hydrolyze esculin in the presence of 40% bile, and production of pyrrolidonyl arylamidase (PYR)^{1,3}.

Enterococcus faecalis is the most common isolate found in human clinical specimens, followed by *Enterococcus faecium*. Other Enterococcal species, including *E. avium*, *E. cassiliflavus*, *E. cecorum*, *E. durans*, *E. gallinarum*, *E. hirae*, *E. raffinosus*, *E. malodoratus*, *E. dispar* and *E. mundtii* are infrequently isolated from human infections.

Risk factors for the development of bacteremias include advanced age, immunosuppression, underlying disease and conditions (e.g.; prematurity, diabetes, malignancy, congestive heart failure, renal insufficiency, deep-seated infections), prior gastrointestinal, genitourinary, or respiratory tract instrumentation, long-term hospitalization, and the use of broad-spectrum antibiotics having little or no antienterococcal activity (e.g., cephalosporin)¹.

Isolation measures to prevent person-to-person transmission according to CDC's Guidelines includes⁴:

- Hand washing with antimicrobial soaps and hand rubs with alcohol-based solutions,
- Wear gloves, Wear a gown,
- Dedicate the use of items such as stethoscopes, blood pressure cuffs, and thermometers to a single patient.

Aims and Objectives:-

- ▶ To isolate Enterococci from different clinical samples and to speciate Enterococci by different tests.
- ▶ To determine antibiotic susceptibility pattern of *Enterococcus* species for proper treatment of patient by specific antimicrobial drugs.

Materials and Methods:-

The study was conducted from May 2014 to August 2015 in Sir Takhtsinhji General Hospital, Bhavnagar, Gujarat, India.

In this study 200 Enterococci species isolated from different clinical samples- Urine, blood culture, pus, catheter tips and body fluids collected in sterile container from all patients attending to Hospital. Samples were collected from patients having risk factors like advanced age, immunosuppression, prematurity, diabetes, malignancy, congestive heart failure, renal insufficiency, deep-seated infections, prior gastrointestinal, genitourinary, or respiratory tract instrumentation, long-term hospitalization, and long- term use of broad-spectrum antibiotics.

Identification and Characterization:-

Urine samples were inoculated on CLED agar, other mentioned samples were inoculated on Blood agar. After overnight incubation at 37°C colonies were identified. Further identification was done by inoculation on Bile-Esculin agar, PT agar, by doing Gram stain, Catalase test, Growth in 6.5% NaCl and Carbohydrate Fermentation Test using six sugars- Mannitol, Sorbitol, Sucrose, Raffinose, Lactose, Arabinose.

Antimicrobial Susceptibility Testing:-

Antibiotic susceptibility testing of the isolates was performed on Muller Hinton agar by Kirby-Bauer disc diffusion method⁵. The antibiotic concentration of disc used and zone size interpretation was accordance to Clinical Laboratory Standards Institute (CLSI)⁶. These antimicrobial drugs are Ampicillin, Ampicillin-Sulbactam, Penicillin, Piperacillin-Tazobactam, Linezolid, Ciprofloxacin, Tetracycline, Roxithromycin, Vancomycin, High level Gentamycin (120µg), High level Streptomycin (300µg), and Nitrofurantoin. Commercially available six mm disks (Himedia Laboratories, Mumbai) were used.

Quality Controls:-

Standard operative procedures were followed during processing of each sample. *E.faecalis* VRE ATCC 51299 and *E.faecalis* VSE ATCC 29212 were used as a reference strains^{7,8}.

Results:-

Out of 1850 culture positive samples proceeded, 200 Enterococci were isolated from urine, blood, pus, catheter tip (hospital acquired) & body fluid (peritoneal fluid), suggesting 11% prevalence rate of Enterococci.

Enterococci are Gram positive oval cocci, arranged in pairs at an angle to each other; catalase negative; can grow in 6.5% NaCl. After overnight incubation small yellow or pink colonies were formed on CLED medium; small, cream or white or grayish-white, smooth entire, non-hemolytic colonies were formed on Blood agar. As Enterococci hydrolases esculin in presence of 40% bile, blackening on BEA media seen. *E.faecalis* could grow on PT agar producing black colonies, but *E.faecium* could not^{1,3,9}.

Isolated Enterococci species distribution, sample wise, age and sex wise distribution of Enterococci species shown in Table - 1, 2, 3 and 4 respectively.

Table 1:- Species of Enterococci isolated

Enterococci Species	Number	Percentage
<i>E.faecalis</i>	164	82%
<i>E.faecium</i>	34	17%
Other	2	1%

Other Enterococcal species might be *E.avium*, *E.gallinarum*, *E.cassiliflavus*, *E.durans*, *E.raffinosis* or *E.mundtii*.

Table 2:-Samples showing Enterococcal species isolation

Samples	Number	Percentage
Urine	120	60%
Blood	71	35.5%
Pus	3	1.5%
Peritoneal fluid	2	1%
Catheter tip	4	2%

Table 3:- Age-wise distribution of Enterococci Species.

Age Group	Number	Percentage
<1 year	44	22%
1-15 year	32	16%
16-30 year	28	14%
31-45 year	40	20%
46-60 year	34	17%
>60 year	22	11%

Table 4:- Sex-wise distribution of Enterococci Species.

Sex	Number	Percentage
Female	113	56.50%
Male	87	43.50%

The antimicrobial susceptibility pattern of Enterococci species has been shown in Table-5. In spite of predominance of *E.faecalis*, it was observed that *E.faecium* was more resistant than *E.faecalis*.

Table 5:-Antimicrobial drug resistance in Enterococci.

Antimicrobial Drugs	Isolates Resistant to Antimicrobial Drugs (200 species)	Percentage of Resistance
Ampicillin	106	53%
Ampicillin-Sulbactam	83	41.5%
Penicillin	184	92%
Piperacillin-Tazobactam	108	54%
Linezolid	0	0%
Ciprofloxacin	115	57.50%
Tetracycline	136	68%
Roxithromycin	125	62.50%
Vancomycin	2	1%
High level Gentamycin	85	42.50%
High level Streptomycin	118	59%
Nitrofurantoin	76	38%

Results of Sugar Fermentation Test done on Enterococci species has been shown in Table-6. Acid production occurs from sugar fermentation & yellowish color develop.

Table 6:- Sugar Fermentation by Species of Enterococci isolated.

Sugars used for fermentation test	<i>E.faecalis</i>	<i>E.faecium</i>
Mannitol	+	+
Sucrose	+	+
Sorbitol	+	-
Lactose	+	+
Raffinose ¹⁰	-	+/-
Arabinose	-	+

Discussion:-

Enterococci colonize the bowels of more than 90% of healthy humans. *E. faecalis* accounts for more than 80% of the Enterococcal isolates in clinical samples, but in recent years *E. faecium* has become more common, probably because of its greater antibiotic resistance to multiple antibiotics. For the last two decades, *Enterococcus* have been the 3rd most common cause of hospital-acquired infections (HAI) after *E.coli* and *Staphylococcus aureus* and ahead of *Pseudomonas aeruginosa*. The most common nosocomial infections caused by Enterococci are urinary tract infections (associated with instrumentation and antimicrobial administration) followed by intra-abdominal and pelvic infections. They also cause endocarditis, neonatal sepsis and rarely meningitis.

In last two decades infections due to Enterococci have increased. Now, it is among the leading causes of bacteremias. Enterococci is the second most common cause of surgical infections and the third most frequently reported cause of HAIs. Gastrointestinal colonization, length of stay in hospital, ICU stay, older age, neutropenia, hematological malignancies and transplantation are risk factors identified for Enterococcal colonization and infection¹¹.

The emergence of high-level resistance to aminoglycosides in Enterococci, particularly *E.faecalis* and *E.faecium*, seriously affected the therapeutic approach; vancomycin become a first-line drug effective against Enterococci¹².

Until the early 1980s, the susceptibility of Enterococci to ampicillin and vancomycin remained fairly predictable. Since that time, *Enterococcus* species resistant to Ampicillin and vancomycin have been reported with increasing frequency. Linezolid seemed to be an appropriate therapeutic option for VRE in our study¹³.

In present study isolated *E.faecalis* and *E.faecium* were 82% and 17%, which is comparable to studies conducted by Bose et al¹⁴ (82% and 18%) and Gupta et al¹⁵ (80% and 20%); In present study only two other species (1%) isolated which might be *E. durans*, *E. raffinosus*, *E. avium*, *E. gallinarum* comparable to study done by Grey et al¹⁶ (1.2%).

In present study, the most common clinical sample from which Enterococci were isolated was urine (60%), comparable to studies conducted by Agrawal et al¹⁷ (62.79%) and Bhatt et al¹⁸ (57%); followed by blood (35.50%), pus (1.50%), catheter tips (2%), peritoneal fluid (1%).

Present study showed that isolation of Enterococci from all age groups, ranging from newborn baby to an old age of 86 years and highest percentage (22%) of Enterococcal isolates was obtained from <1 year age group, similar to observed by Nepal et al¹⁹ (20%).

In present study female predominance (56.5%) over males seen corresponds to the Bhatt et al¹⁸ (54%). Greater vulnerability to acquire urinary tract infection and pelvic infection due to anatomic position of urethra and genital tract might have played a role for predominant female infection by this organism^{11,18}.

In present study, beta-lactam drugs, flouroquinolones, high level aminoglycosides and tetracycline were found to be highly resistant; penicillin was most resistant (92%), comparable to study done by Bhatt et al¹⁸ (97.5%). In present study linezolid has shown 0% resistance, followed by vancomycin shown 1% resistance, comparable to studies conducted by Bose et al¹⁴ (linezolid 0% resistant) and Saraswathy et al²⁰ (vancomycin 1% resistant).

Conclusion:-

- In developing country like INDIA Enterococci are important nosocomial pathogens of human disease; because of their resistance to antimicrobial agents like penicillins, cephalosporins group, the acquisition of high-level resistance to aminoglycosides and now the emergence of vancomycin resistance, these bacteria are often involved in serious super-infections among patients receiving broad-spectrum anti-microbial chemotherapy. Prompt diagnosis is essential for the patient and the community.
- Identification of Enterococci done by urine culture, blood culture, Gram stain and also by other methods like catalase test negative, its ability to grow at 10°C and 45°C, growth in the presence of 6.5% NaCl, ability to hydrolyze esculin in the presence of 40% bile and sugar fermentation test.
- Mainly two species of Enterococci were isolated, *E. faecalis* (82%) & *E. faecium* (17%).
- Out of 200 Enterococcal species 60% were isolated from urine samples, followed by 35.5% were from blood samples.
- As far as antibiotic susceptibility was concerned, resistance to penicillin (92%), suggest that resistance to β -lactam group was more among *Enterococcus* species. Resistance to vancomycin shown in 1% Enterococcal species, linezolid is more effective drug as all isolates are sensitive to it.

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