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Original Research Article

Prevalence of Enterococcus Species in Various Clinical Specimens and it's Antimicrobial Susceptibility Pattern in a Tertiary Care Teaching Hospital of Central Gujarat

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ABSTRACT

Background: Enterococci have become common cause of hospital acquired urinary tract infections, wound infections and bacteraemia. The therapeutic challenge of multiple drug resistance (MDR) enterococci have brought their role as important nosocomial pathogens into sharper focus. Hospital acquired wound infections are among the leading cause of morbidity and huge economic burden associated with prolonged hospital.

Material and methods: A descriptive-cross sectional study carried out in the Department of Microbiology in Medical college Baroda & SSG Hospital, Vadodara, Gujarat, India. Various samples like blood, urine, swab, cerebro-spinal fluid (CSF), pleural fluid, body fluid, pus, catheter tip were taken from clinically suspected cases for culture and antimicrobial sensitivity testing according to CLSI guideline. A total of 90 Enterococcus spp. were isolated from these samples, which were included in this study.

Results: Out of 6555 culture positive isolates, 90 were Enterococcus species. The prevalence of Enterococci spp. in our study is 1.37%. Out of total 90 isolates, 71% (64 isolates) were Enterococcus fecalis and 29% (26 isolates) were Enterococcus faecium. The highest number of Enterococcus spp. were isolated from the urine (48%) & in infant (30%). The rate of isolation of Enterococcus was more in males (59%). Enterococcus spp. from various samples show highest sensitivity to Linezolide (99%). Incidence rate of VRE isolates in the present study is 15.5%.

Conclusion: The prevention and control of spread of multi drug resistant Enterococci require co-ordination effort from various departments and can only be achieved by education of hospital staff regarding problem of drug resistance, prudent use of antimicrobials, early detection and reporting and immediate implementation of appropriate infection control measures, improved surveillance for hospital infection control.

Keywords: Enterococcus spp., Multi Drug Resistant (MDR) ,Vancomycin Resistant Enterococci (VRE)

INTRODUCTION

Enterococci contain a C-carbohydrate that reacts with Lancefield group D antisera. Therefore, in the past, they were considered group D Streptococci.[1] In humans, typical concentrations of enterococci in stool are up to 10^8 CFU per gram. Although the oral cavity and vaginal tract can become colonized, enterococci are recovered from these sites in fewer than 20% of cases.[2] Enterococci, leading cause of nosocomial bacteremia, surgical wound infection, and urinary tract infection, are becoming resistant to many and sometimes all standard therapies. The Enterococcus faecalis are the most prevalent species cultured from humans for more than 90% of clinical isolates. Other enterococcal species known to cause human infections include E. faecium, E. avium, E. gallinarum, E. casseliflavus, E. durans, E. raffinosus and E. mundtii.E. faecium represents most vancomycin resistant enterococci (VRE).

Two types of enterococci cause infection : 1) those originating from patient's native flora, which are unlikely to possess resistance beyond that intrinsic to the genus and are unlikely to be spread from bed to bed, and 2) isolates that possess multiple antibiotic resistance traits and are capable of nosocomial transmission. The therapeutic challenge of multiple drug resistant (MDR) enterococci have brought their role as important nosocomial pathogens into sharper focus. [4]

Enterococcus species are hardly, facultative anaerobic organisms that can survive and grow in many environments. They tolerate a wide variety of growth conditions, including temperatures of 10°C to 45°C, and hypotonic, hypertonic, acidic, or alkaline environments. In the laboratory, enterococci are distinguished by their morphologic appearance on Gram stain, culture and their ability to (1) hydrolyze esculin in the presence of bile, (2) grow in 6.5% sodium chloride, (3) demonstrate pyrrolidonyl arylamidase and leucine amino peptidase, and (4) react with group D antiserum.[3]

Enterococci are intrinsically resistant to many antibiotics. Penicillin, ampicillin, piperacillin, imipenem, and vancomycin are among the few antibiotics that show consistent inhibitory, but not bactericidal activity against E. faecalis. E. faecium are less susceptible to Beta-lactam antibiotics than E. faecalis because the penicillin- binding proteins of the former have markedly lower affinities for the antibiotics.[5] Enterococci often acquire antibiotic resistance through exchange of resistance encoding genes carried on conjugative transposons, pheromone responsive plasmids, and other broad host range plasmids. [6]

Among several phenotypes for vancomycin resistance enterococci, Van A (resistance to vancomycin and teicoplanin) and VanB (resistance to vancomycin alone) are most common. Inducible genes encoding these phenotypes alter cell wall synthesis and render strains resistant to glycopeptides. [5]

MATERIAL AND METHODS

A descriptive- cross sectional study carried out in the Department of Microbiology in Medical college Baroda & SSG Hospital, Vadodara, Gujarat, India. The study was conducted over a period of 10 months from December-2021 to September-2022. Various clinical samples like blood, pus and wound swabs, urine, body fluids, sputum, endotracheal tube and secretions were collected from the hospitalized patients under aseptic condition in sterile containers and sent to the Microbiology laboratory for culture and sensitivity testing along with the requisition forms filled with relevant clinical details of patients. During this period 19,069 samples were received and processed at the Diagnostic laboratory. A total of 90 enterococci spp. were isolated from these samples, which were included in this study.

All samples were cultured on Mac conkey agar, Brain heart infusion agar and blood agar . All the

inoculated plates like MacConkey agar, Brain heart infusion agar were incubated aerobically at 37˚C in incubator for overnight (16-18 hours). The inoculated blood agar plates were incubated in candle jar at 37˚C in incubator for 16-18 hours. All the inoculated plates were examined for presence of growth of organism and further identified by Gram staining and bio-chemical reactions like catalase test, 6.5% NaCL Agar, Bile Esculin disc test, Sugar fermentation test, Arginine decarboxylase test.

The antibiotic used for the enterococci isolates were Ampicillin (10µg), Penicillin (10 Units), Vancomycin (30 µg) , Vancomycin MIC (0.016-256 µg/ml) , Linezolid (30 µg) , Erythromycin (15 µg), Doxycyclin (30 µg), Levofloxacin (5 µg), High level Gentamicin (120 µg). Antibiotic sensitivity was done by Kirby Bauer disc diffusion method and their antibiotic sensitivity were done as per CLSI (Clinical and Laboratory Standard Institute).[7] The MIC test were performed only for those isolates resistant to Vancomycin disc in antibiotic susceptibility testing done by disc diffusion method.

RESULTS

In the present study the total of 19069 clinical specimens were processed in the Microbiology Laboratory of Medical College Baroda & S.S.G Hospital during the study period of December 2021 to September 2022. Out of which 6555 isolates were grown.

Out of 6555 grown isolates, 90 were Enterococcus species. The prevalence rate of Enterococcus spp. in present study is 1.37%.

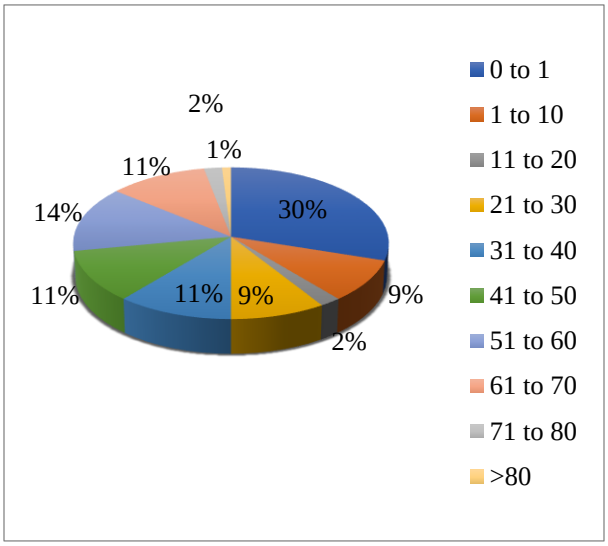

Figure-1: Age distribution of patients from which Enterococci spp. were isolated

In this study, majority of the Enterococcus species was isolated from infants (30%) followed by age group of 51-60 years (14%), 31-30 years, 41-50 years, 61-70 years (11%) and other age groups .

Table-1: Gender wise distribution of patients from which Enterococci spp. were isolated

Gender	Total no. Of Enterococci isolates	Percentage (n=90)
Male	53	59%
Female	37	41%
Total	90	100%

The rate of isolation of Enterococci spp. was more in males (59%) than female (41%).

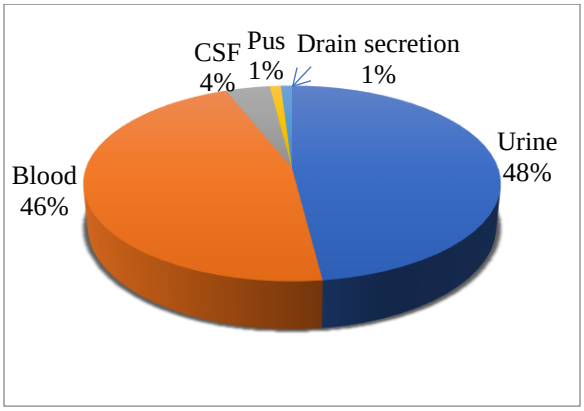

Figure-2: Isolation of Enterococcus species in various clinical specimens

In the present study, highest number of Enterococcus species was isolated from the Urine (48%), followed by Blood (46%), CSF (4%) and other specimens.

Table-2 : Ward wise & Sample wise distribution of Enterococci spp. isolates

Ward	Specimens					Total (n=90)
	Blood	Urine	CSF	Pus	Drain secretion	
NICU	19	0	2	0	0	21(24%)
Pediatric ward	3	4	1	0	0	8(9%)
PICU	3	4	1	0	0	8(9%)
ICCU	1	2	0	0	0	3(3%)
MICU	6	6	0	0	0	12(14%)
Medicine ward	4	16	0	0	0	20(22%)
SICU	1	0	0	0	0	1(1%)
Surgical ward	3	2	0	0	1	6(7%)
TB ward	0	2	0	0	0	2(2%)
Ortho ward	0	3	0	1	0	4(4%)
Gynecology ward	1	0	0	0	0	1(1%)
ENT ward	0	1	0	0	0	1(1%)
OPD	0	3	0	0	0	3(3%)
Total	**31 (46%)**	**43 (48%)**	**4 (4%)**	**1 (1%)**	**1 (1%)**	**90(100%)**

In the present study, highest number of Enterococcus species in Blood were isolated from NICU (46%) whereas highest number of Enterococcus species in Urine were (37%) from Medicine ward & in CSF (50%) from NICU.

Highest number of Enterococcus species were isolated from Medicine wards & ICCUs (39%) followed by Pediatrics Wards & ICUs (32%).

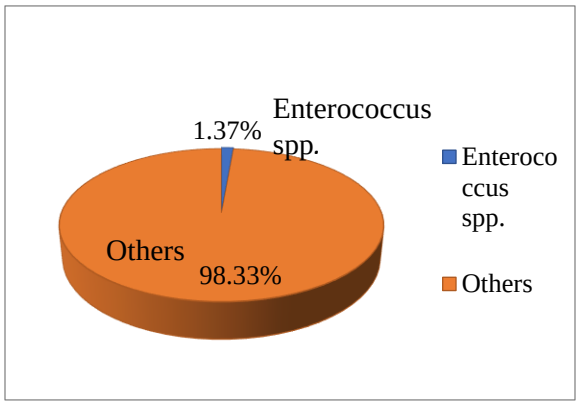

Figure-3: Isolation of Enterococcus spp. among total number of grown isolates

Out of total 90 enterococcal isolates, 71% (64 isolates) were Enterococcus fecalis and 29% (26 isolates) were Enterococcus faecium.

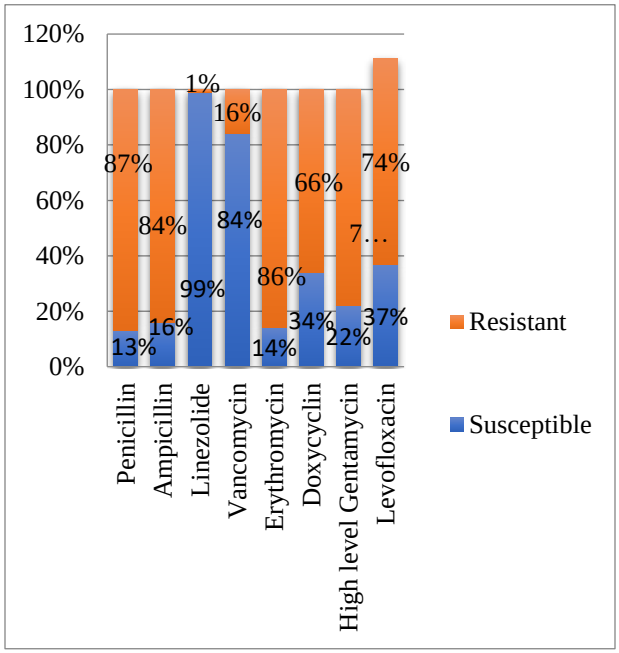

Figure-4: Antimicrobial susceptibility pattern of Enterococcus spp.

In this study, Enterococcus spp. from various samples show highest sensitivity to linezolide (99%), followed by vancomycin (84%), levofloxacin (37%), doxycyclin (34%), high level gentamycin (22%), ampicillin (16%), erythromycin (14%) and penicillin (13%).

Incidence of VRE in the present study is 15.5%.

Incidence of high level Gentamycin resistant (HLGR) in the present study is 77.8%.

DISCUSSION

Enterococci are emerging as one of the most common agents of nosocomial infections in the hospital and also cause opportunistic infections in immunocompromised individuals. It is a well-known fact that they can cause a variety of serious life-threatening infections such as endocarditis and bloodstream infections and also cause wound infections and urinary tract infections. [7]

The prevalence of Enterococcus spp. in present study is 1.37%. With this context, the present study was attempted to assess the incidence of multidrug resistance among the clinical isolates of enterococci recovered from the patients of a tertiary care hospital located in Central Gujarat.

Out of the total 90 cases, 71% of isolates were of Enterococcus fecalis and only 29% isolates were Enterococcus faecium which was in agreement with the findings of the studies by Chakraborty A et al [9], Jaiswal S et al study [10], Sangwan J et al study [11], which shows highest number of isolates were Enterococcus fecalis. While in Ohri S et al study[8], Enterococcus faecium were 59% & Enterococcus fecalis were 41%.

In present study, majority of the Enterococcus species were isolated from infants (30%) followed by age group of 51-60 years (14%), 31-30, 41-50, 61-70 years (11%) and other age groups which was differ from Yilema A et al study [12] & Sangwan J et al study [11] . Enterococcus species were isolated from <5 years (32%) & 60 years (40.4%) respectively.

In presnt study, the rate of isolation of Enterococcus was more in males (59%) than female (41%). In Yilema A et al study [12] isolation

rate was higher in male (54.3%). In Sangwan J et al study [11] isolation rate was higher in female which was 56%.

In the present study, highest number of Enterococcus species were isolated from the Urine (48%) which was similar to the Ohri S et al study [9] (54%) & Chakraborty A et al [9] (66%) study & Yilema A et al study [12] (41%) & Jaiswal S et al study [10] (75%).

The present study shows that the strains were sensitive to Linezolide (99%) which was around similar with the study done by Chakra-borty A et al [10], Sangwan J et al study [11] (100%).

Sensitivity pattern for Vancomycin was 84% in present study which was higher than study done by Yilema A et al study [12] **(**59%) which is higher in Vijyalakshmi P et al [13] (100%), Chakra-borty A et al [9](100%), Agrawavl J et al [14](95.5%), V A Rahangdale et al [15](91.9%),

Sensitivity pattern for High level Gentamycin was 22% in present study which was lower than study done by Chakraborty A et al [10] (55%), Sangwan J et al study [12] (34.8 %), Agrawavl J et al [15] (90.69%), V A Rahangdale et al [16] (50.5%).

The major limitations of this study are:

Failure to perform molecular techniques by gene level detection for better results.

Biofilm production in enterococci spp. can be studied further in ICU pt's samples.

CONCLUSIONS

The main concern in Enterococci is the high amount of drug resistance that has been reported in present study and according to our study Vancomycin remains the drug of choice.

The present study also revealed that despite recent trends of increasing resistance to Aminoglycosides, a combination therapy of β-lactam and Aminoglycoside as first-line drugs would be currently the best choice. Vancomycin or linezolid therapy should be restricted for use in patients infected with MDR strains only. Judicious use of vancomycin and linezolid in serious infections and appropriate infection control measures would probably recede the possible emergence of VRE outbreaks in our geographical area.

Patients attending health facilities for the cases of UTIs, wound infections and sepsis have to be critically examined for enterococci infection. Attention has to pay for inpatient, patients having history of any antibiotics or urinary catheterization for the suspension of enterococci infections.

Thus prevention and control of spread of multi drug resistant Enterococci require co-ordination effort from various departments and can only be achieved by

1. Education of hospital staff regarding problem of drug resistance

2. Prudent use of antimicrobials

3. Early detection and reporting and immediate implementation of appropriate infection control measures

4. Improved surveillance for hospital infection control.

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