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PREVALENCE AND CHARACTERIZATION OF ENTEROCOCCI IN HOSPITAL INFECTION

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ABSTRACT

Introduction: Enterococci are emerging as a significant cause of nosocomial infections accounting for approximately 10% of hospital acquired infections. Hospital infection due to Gram negative bacilli have decreased in incidence while those due to gram positive have increased. **Aims and Objectives**: 1.Find out the prevalence of enterococci in hospital infection. 2.To characterize enterococci isolated from various infection. 3.Find out antibiotic drug resistance pattern in enterococci. **Material and Methods:** Material for this study was collected from patients who had acquired infections while admitted in the hospital and also from the patients acquiring infections outside the hospital. During the study period 300 entercoccal isolates were obtained from same number of patients from various clinical specimens. Each specimen was subjected to various laboratory procedures for isolation, identification and characterization of enterococci. **Results**: Data were collected and statistical analysis was done using Student t test and Chi square test. **Discussion:** 1) Among different types of enterococci, E. faecalis (61%) and E. faecium (20.4%) are the commonest type causing infection. 2) Isolation rate of E. faecalis and E. faecium in the hospital acquired infections varied from place to place, majority from pus. 3) No age is immune to infection with enterococci infections. They can cause infection in all the age group of 21-30 years. 4) They can cause infections in both sexes. Males were 52% and comparatively more affected than females 48%. 5) Only 6.3% isolates of enterococci were sensitive to all 13 drugs tested, whereas 92.3% and 96.7% strains of E. faecalis and E. faecium were resistant to one or more drugs respectively. **Conclusion:** In view of multidrug resistance in septic cases, efforts should be made to eradicate enterococci resistant strains by taking strict aseptic precautions, dressing using chlorohexidine cream and local applications of antibiotics.

KEYWORDS: Enterococci, Nosocomial infections, Hospital infections.

INTRODUCTION

Enterococci are once classified as Group D streptococci (Lancefield classification) and now considered as a separate but related genus of gram positive catalase negative cocci that grow in broth as diplococci and Short chains (Moellering 1992).[1] Enterococci are emerging as a significant cause of nosocomial infections accounting for approximately 10% of hospital acquired infections. Hospital infection due to Gram negative bacilli have decreased in incidence while those due to gram positive have increased. The center for Disease control (C.D.C) have reported enterococci to be the third most commonly reported pathogen from nosocomial infection after Escherichia coil and staphylococcus aureus.[2] Nosocomial infections are defined as infections developing in patient after admission to the hospital which were neither present nor in their incubation at the time of hospitalization . They may, of course, first appear after the patient has stayed for two or three days in the hospital or sometimes even after their discharge depending on their incubation period. For their occurrence there must be a source of infection, transmission of the causative agent and a patient susceptible to that infection. Hospital infection may be endogenous (from the patient own flora, which at the time of infection may include organism brought into the hospital at admission) or exogenous (from another patient or a member of the hospital staff or from the inanimate environment in the hospital) the infecting organism may invade patient tissue spontaneously or be introduced into them by surgical operation, instrumental manipulation or nursing procedure. Enterococci are the second leading cause of urinary tract infection and the third leading cause of wound and blood stream infection in hospital.[3] Enterococci are less susceptible to penicillin than are the related streptococci. This difference is thought to be due to the production of penicillin binding proteins that have lower affinity for

penicillin than do those streptococci. Recently, beta lactamase producing enterococci have also been described as important nosocomial pathogen.

AIMS AND OBJECTIVES

The present study was carried out with the following aims and objectives

1- Find out the prevalence of enterococci in hospital infection.
2- To characterize enterococci isolated from various infection.
3- Find out antibiotic drug resistance pattern in enterococci.

REVIEW OF LITERATURE

The role of enterococci in urinary tract infection was first reported by Andrew and Horder (1906) and was extended in subsequent decades by other investigators (Andrew 1906, Sherman 1937, Evan 1947). Potential virulence factors are haemolysin, aggregative substance and adhesions.[4] Haemoysin production is reported to be more common in the strain isolated from human infections.[5] Linsday et al[6] 1991 obtained 84 strains of E. faecium, these were isolated and collected during 22 year period from 1969 to 1990 (1969 to 1988 n = 48, 1989 n = 15, 19990 n = 21). The original sites of isolation and the number of isolates (1969 to 1988/1989 to 1990) they found as blood 34 (30/4), urine 25 (5/20), wound 9 (7/2) biliary tract 3 (0/3), abdominal fluid 6 (0/6) and unknown (6/1). Earlier ampicillin and penicillin G used to be the most effective drug against all species of enterococci Cherian, Mathai and Sara studied 210 high level aminoglycoside resistant strains of Enterococcus. Of these 152 (72.4%) were from urine, 49 (23.3%) pus and 9 (4.3%) from blood.[7] Among these 210 isolates, 189 (89%) were E. faecalis and 21 (11%) were E. faecium. Kenneth and Zinker[8] and Naknishi et al[9] reported that 10% of E. faecalis obtained from urine specimens were found to be resistant to ciprofloxcacin, norfloxcacin and flouroquinolones. Bhat et al[10] in their study observed that antibiotic resistance was more common in E. faecium than E.faecalis, High level gentamicin reresistance was found in 8.2% and 33.3% isolates of E. faecalis and E. faecium respectively. Of all isolates, only 2.2% strains were found to be resistant to vancomycin.

Friedden et al (C.D.C) confirmed the characteristics associated with colonization and infection due to vancomycin resistant enterococci. 1) they are mostly hospital acquired, after a long period of hospitalization 2) they occur mostly in patients who had received intervenous or oral vancomycin or other intervenous antibiotics 3) therefore, is a frequent association with resistance to other antimicrobials 4) there is involment of E. faecium compared with E. faecalis and other enterococal speices.

MATERIAL AND METHODS

The present study was conducted in the Department of Microbiology, Goldfield Institute of Medical Sciences and Research, Ballabgarh, Faridabad, during the period of one year from July 2014 to June 2015.

MATERIAL

Material for this study was collected from patients who had acquired infections while admitted in the hospital and also from the patients acquiring infections outside the hospital.

Specimens

All the clinical specimens including pus, blood, urine, cervical swab, vaginal swab, cerebrospinal fluid (CSF), pleural fluid, continuous ambulatory peritoneal dialysis (CAPD) fluid, ear and conjuctival swab, drain tip, catheter tip, tip, bile, peritoneal fluid and semen were received from the patients admitted in the ward and from patients attending the outpatient department of J.N. Medical college A.M.U Aligarh, subjected to culture examination and sensitivity testing. Enterococcus isolated were included in the study.

Laboratory procedures

Each specimen was subjected to various laboratory procedures for isolation, identification and characterization of enterococci. Smear was prepared from all specimens except blood on a clean sterile glass for *gram staining*. The smear was examined under oil immersion objective for pus cell and gram positive cocci in short chains and pairs. All the above specimens were *cultured* for isolation and identification of enterococci. Culture was done on 5% sheep blood agar and in Robertson cooked meat broth (CMB). The Enterococcal isolates were indentied and characterized according to Sherman's criteria (1937) and criteria given in the Mackie and Mecartney (1996) i.e *colony characters morphology and biochermical tests.*

Statistical analysis

*Student's unpaired test***:** This test was employed to determine whether the difference in the ODI value between the different clinical specimens of enterococcal species and clinical group was statictically significant or not.

Z test for proportion

This test was used to determine whether the difference in proportion of enterococcal isolated between the two categories was statistically significant or not.

OBSERVATIONS AND RESULTS

Observations were made and results were tabulated as follows

(*Figures in parentheses indicate percentages)*

Table 1: isolation of Enterococci in relation to age and sex.

Age group (in years)	No of cases	Male	Females
1-10	72 (24.0)	54 (75.0)	18(25.0)
11-20	35 (11.6)	19(54.3)	16(45.7)
21-30	75 (25.0)	27(36.0)	48(64.0)
31-40	53 (17.6)	20(37.7)	33(62.3)
41-50	34 (11.3)	20(58.8)	14(41.2)
51-60	21 (7.0)	11(52.4)	10(47.6)
61-70	7 (2.3)	3(42.8)	4(57.2)
71-80	3 (1.0)	2(66.7)	1(33.3)
Total	**300**	**156(52.0)**	**144(48.0)**

Table 2: Isolation of Enterococci in relation to source:

Specimen	Total no of isolates
Pus	103(34.3)
Urine	71(23.6)
Cervical swab	53(17.6)
CSF	27(9.9)
Blood	13(4.3)
Semen	9(3.0)
Ascetic fluid	5(2.6)
Catheter tip	4(1.3)
Vaginal swap	3(1.0)
CAPD fluid	2(0.6)
Conjuctival swab	2(0.6)
Pleural fluid	2(0.6)
Bile	1(0.3)
Drain tip	1(0.3)
Peritoneal fluid	1(0.3)
Total	**300**

Table 3: Drug Resistance Pattern in Enterococcal Species.

Drugs	E. faecalis (N=169)	E .Faecium (N=59)	E.liquefaeciens (N=20)	E.durans (N=18)	E.zymogenes (N=15)
Cotrimoxazole	52(30.76)	32(54.23)	4(20.0)	7(38.8)	5(33.3)
Amikacin	44(26.03)	32(54.23)	1(5.0)	7(38.8)	6(40.0)
Gentamicin	76(44.9)	35(59.3)	6(30.0)	11(61.1)	5(33.3)
Ciprofloxcacin	52(30.76)	27(45.76)	6(30.0)	7(38.8)	4(26.6)
Erythromycin	54(31.9)	32(54.23)	4(40.0)	9(50.0)	3(20.0)
Tetracycline	59(34.9)	31(52.54)	7(35.0)	7(38.8)	6(40.0)
Vancomycin	3(1.77)	3(5.08)	0(0.0)	1.5.6)	0(0.0)
Cephalexin	79(46.7)	35(59.9)	5(25.0)	10(55.6)	6(40.0)
Tobramicin	57(33.7)	28(47.4)	2(10.0)	8(44.4)	5(33.3)
Chloremphenicol	57(33.7)	26(44.06)	5(25.0)	5(27.7)	6(40.0)
Cefozoline	87(51.4)	44(74.57)	11(55.0)	7(38.8)	9(60.0)
Norfloxcacin	69(40.8)	39(66.1)	15(75.0)	7(38.8)	8(53.3)
Ampicillin	62(36.68)	39(66.1)	12(60.0)	6(33.3)	6(40.0)

Table 4: isolation of vancomycin resistant enterococci in relation to palce and source.

Source	Vancomycin resistant Strains (VRE) (N=7)	Indoor spccimens (N=4)	Outdoor specimens (N-3)
Urine (N=71)	2.(2.8)	1	1
Cervical	2(3.7)	1	1
Pus	0(0.1)	1	1
CSF	1(3.7)	1	0
Semen	1(11.1)	0	1

DISCUSSION

During the study period 300 entercoccal isolates were obtained from same number of patients from various clinical specimens. Patients studied belong to the different *age groups* i.e. 0-10 to 71-80 years. The youngest patient was one day old and the oldest was of 80 years. The mean age was 26.6 years. Isolation rate of enterococci varied in each age group. Maximum number of entercoccal isolates 75 (25%) were found in the age group of 21-30 years. Minimum number of isolates 3 (1.0%) were in the age group of 71-80 years. Among both the sexes, the isolation rate of enterococci was higher in the males 156 (52%) than in females 144 (48.0%) but this was statistically not significant. In relation to *source of infection* majority of the enterococci were isolated from pus (34.33%), urine (23.6%) and cervical swab (17.66%), followed by cerebrospinal fluid (9.0%), blood(4.3%), semen (3.0%), ascetic fluid (2.66%), catheter tip (1.33%) vaginal swab and ear swab (1.0% each), pleural fluid, conjunctival swab and CAPD (continuous ambulatory peritoneal dialysis) fluid (0.66% each) and only one isolate (0.33%) each from drain tip, bile and peritoneal fluid in descending order.

In our study 300 entercoccal isolates were obtained out of which 183 (61.0%) were identified as *E. faecalis*, 61 (20.4%) as E. faecium, 22 (7.3%) as E. liquefaeciens, 18 (6.0%) and 16 (5.3%) as E. durans and E. zymogenes

respectively. In the present study, out of 183 isolates of E. faecalis, majority 61 (33.33%) were found from pus followed by urine 45 (24.5%), cervical swab 30 (16.3%), blood 10 (5.4%), semen 7 (3.8%), catheter tip and ascetic fluid 4 (2.18%) each, CAPD fluid 2 (1.09%), vaginal and peritoneal fluid 1 (0.54%) each in descending order. None of the isolates were fluid in conjuctival swab, pleural fluid and bile.

Out of the total 61 isolates of *E. faecium*, majority of the isolates were found pus and urine 17(27.8%) each, followed by cervical swab 12(19.67%), cerebrospinal fluid 6(9.8%),blood 3(4.9%),ear swab and conjunctival swab 2(3.2% each),semen and bile 1(1.6%).

Among 22 isolates of *E.liquefaeciens*, majority of the isolates were found from pus 12(54.54%), followed by urine 4(18.18%), cervical swab 2(9.0%)and ear swab, cerebrospinal fluid, semen and pleural fluid 1(4.5%) each.

Out of 18 isolates of *E.durans*, majority 6(33.33%) of the isolates were from cervical swab followed by pus 4(22.22%), cerebrospinal fluid 3(16.66%), urine 2(11.11%), ascetic fluid, vaginal swab, pleural fluid 1(5.5%) each.

Of 16 isolates of *E. zymogenes* majority of the isolates were found from pus 9(56.25%) followed by urine & cervical swab 3(18.76% each) and vaginal swab 1(6.25%).

The isolation rate of enterococci from *hospitalized* patient was 178(59.3%) isolates, whereas 122(40.7%) from *outdoor* patients. Of these 178 isolates from indoor patients, 108(60.6%) were of biotype E. faecalis, 32(17.9%) were E. faecium, 15(8.4%) were E. durans, 13(7.3%) were E. liquefaeciens, and 10(5.6%) were E. zymogenes. Among 122 isolates from outdoor patients, 75(61.5%) were E.faecalis, 29(23.8%) E.faecium, 9(7.3%) E.liquefaeciens, 6(4.8%) E. zymogenes and 3(2.4%) were E. durans. A significant correlation was found in isolation of E.durans.

Enterococcal isolates were resistant to one or more antibiotics, highest resistance was found with cefozoline 159(56.5%) followed by norfloxcacin (49%), cephalexin (48%), gentamicin (47.3%), ampicillin (44.5%) tetracycline (39.1%), erythromycin (36.3%), Cotrimoxazole (35.5%), chloremphenicol (35.2%), less than 35% isolates were resistant to ciprofloxcacin, tobramicin, amikacin and only (2.49%) isolates were found to be resistant to vancomycin.

Among 183 isolates of E. faecalis 7.6% strains were found to be sensitive to all thirteen antibiotic tested. Out of 169 drug resistant isolates, maximum number of isolates were resistant to cefozoline 87(51.47%), followed by cephalexin 79(46.74%), gentamic in 76(44.97%), norfloxcacin 69(40.82%), ampicillin 62(36.68%) and resistance to drugs like amikacin, cotrimoxazole, ciprofloxcacin, erythromycin, tetracycline varied from 26% to 35%. Only 3(1.77%) strains were found to be vancomycin resistant.

Among E.faecium isolates 3.2% each were sensitive and resistant to all antibiotics used in the study. Majority of strains were resistant to cefozoline (77.96%), followed by ampicillin and norfloxcacin (66.10%) gentamicin and cephalexin (59.32%), Cotrimoxazole, amikacin and erythromycin (54.23%), tetracycline (52.54%) and less than 50% drug resistant were found in tobramicin, ciprofloxacin, chloremphenicol and only 3(5.08%) strains were resistant to vancomycin.

Out of 22 isolates of E. liquefaciens 2(9.09%) were sensitive to all drug resistant strains maximum number were resistant to norfloxcacin (75%) followed by ampicillin (60%),cefozoline (55%), less than 35% were found with tetracycline, gentamicin ciprofloxacin each cephalexcin and chloremphenicol, erythromycin, Cotrimoxazole, tobramicin and amikacin. None of the isolate was resistant to vancomycin.

Among E. durans in the present study, none of the strains were sensitive to all antibiotics. Among resistant strains maximum number of strains were sensitive to gentamycin(61.1%) followed by cephalexin (55.5%), erythromycin (50%) and less than 50% drug resistance were present in tobramicin, norfloxcacin, ciprofloxacin. Cotrimoxazole, amikacin, cefozoline and tetracycline, ampicillin and only 1 (5.5%) strain of E. durans was resistante to vancomycin.

Out of 16 isolates of zymogene none of the strains were resistant to all 13 antibiotics used where as only one (6.3%) was sensitive. Among drug resistant isolates maximum number of strains were resistant to cefozoline (60%) followed by norfloxcacin (53.3%) and less than 41% were resistant to amikacin, tetracycline, cephalexin, chloremphenicol, ampicillin, Cotrimoxazole, gentamicin, tobramicin, ciprofloxacin, and erythromycin. None of the strains were resistant to vancomycin.

Methicillin and oxacillin sensitivity was found in 1.1%, 6.5%, 6.25%, and 6.3%, strains of E. faecalis, E. faecium, E. liquefaciens and E. zymogenes respectively. But none of the isolates of E. durans were found sensitive.

Among enterococal isolates 59.33% and 40.66% were present in outdoor and indoor patients respectively. Majority of isolates from indoor patients were from pus (37.1%), followed by cervical swab 18.5%, urine 14.6%, and cerebrospinal fluid 14.0% whereas among isolates from outdoor specimens majority of isolates were from urine 36.9%, pus 30.3% and cervical swab 14.4%.

Among 63 multi drug resistant E. faecalis isolates, majority (71.4%) of isolates were from indoor patients

and 28.5% from outdoor patients. Maximum number of isolates was from pus 28.5%, followed by urine 20.6%. From indoor specimens, maximum isolates were from pus 33.0%. From outdoor specimens, maximum isolates were from urine 44.4%.

Out of 39 multi drug resistant E. faecium isolates in present study 24 (64.86%) and 15 (38.4%) were from indoor and outdoor patients respectively. Majority of these isolates were from urine 30.7%. From indoor specimens, maximum number of isolates was from pus 29.1%. whereas among outdoor specimens maximum isolates were from urine 40.0%.

Of the six isolates of multi drug resistant E. liquefaeciens, of these 4 isolates (66.66%) were from indoor and 2 (33.33%) from outdoor specimens. Both the isolates from outdoor specimens were from pus whereas among indoor isolates 2 were from pus and one each from urine and CSF specimen.

In our study 7 multi drug resistant isolates of E. zymogenes were obtained from indoor and outdoor patients. Of these 4 isolates (57.1%) were from indoor and 3 (42.9%) from outdoor specimens.

Of the 12 multi drug resistant E. durans in our study 10 and 2 were obtained from indoor and outdoor patients respectively.

In our study only 7 isolates (2.3%) were found to be vancomycin resistant. Of these 1 (1.6 these 3%) was E. faecalis, 3 (4.9%) were E. faecium and 1 (5.5%) was E. durans. The VRE strains of E. faecalis, E. faecium and E. durans were found to be resistant to gentamicin, Amikacin and tobramicin also.

CONCLUSIONS

On the basis of our findings and available knowledge following conclusions were drawn.

1. Among gram positive bacteria, enterococci are one of the leading causes of hospital and community acquired infections. Among type of enterococci E. faecalis (61%) and E. faecium (20.4%) are the commonest type causing infection, where as other types were less commonly isolated i.e. E. liquefaeciens (7.3%), E. durans (6%) and E. zymogenes (5.3%) in the study.
2. Isolation rate of E. faecalis and E. faecium in the hospital acquired infections varied from place to place, majority from pus. In the present study it was 61.0% and 27.8% respectively.
3. No age is immune to infection with enterococci infections. They can cause infection in all the age group of 21-30 years, whereas minimum number 1% of patients were in the age group of 71-80 years.
4. They can cause infections in both sexes. Males were 52% and comparatively more affected than females 48%.
5. Indiscriminate and wide spread use of antibiotics lead to emergence of multiple drug resistance in various infections. All types of enterococci were equally resistant (both newer and older) except vancomycin, majority of enterococci were multiple drug resistant. Among vancomycin resistant strains of enterococci (VRE) 57.15% vancomycin resistant strains were also resistant to all other antibiotics tested where as 42.85 % were multidrug resistant. E. faecium was found to be more antibiotic resistant than E. faecalis.
6. Only 6.3% isolates of enterococci were sensitive to all 13 drugs tested, whereas 92.3% and 96.7% strains of E. faecalis and E. faecium were resistant to one or more drugs respectively, of both the types (37.3% and 66.1%) multi drug resistant. These multi drug resistant strains were isolated from almost all clinical specimens in our study. This indicates that a greater caution is required in selection of antibiotic therapy to avoid selection of resistant strains and treatment failure.
7. E.faecalis and E.faecium have emerged as one of the worst and dangerous nasocomial pathogens and increased incidence of multiple drug resistant E. faecalis and E. faecium were reported from many parts of the world. The vancomycin resistant strains colonize in the hospital and transfer multidrug resistance to other related species and to staphylococcus aureus commonly in the hospital. Often these VRE are responsible for outbreaks of nosocomial infections. Hence antibiotics should be used more rationally in order to prevent a possible outbreak of nosocomial infection.
8. Mere isolation of multidrug resistant strains of E. faecalis and E. faecium and other species from a patient does not necessarily warrant therapy as colonization with these organism are frequent. A colonized patient may serve as a source of infection to other patients also a reservoir for antibiotic resistant genes in hospital. Therefore infection control measure should be strictly adhered to in dealing with colonized patients.
9. In view of multidrug resistance in septic cases, efforts should be made to eradicate enterococci resistant strains by taking strict aseptic precautions, dressing using chlorohexidine cream and local applications of antibiotic. Nursing staff should take special care about strict hand washing, topical alcoholic preparation of chlorohexidine, use of glove and other measure to prevent spread of such strains.
10. Once introduced, the eradication of multidrug resistant strains becomes difficult. Therefore, constant periodic surveillance in form of isolation, prompt identification and later search for environmental and personal sources and proper monitoring for emerging resistance and carriage rate in patients, hospital staff and environment is important for every hospital and microbiology laboratory to control spread of the strains in the community.

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