ORIGINAL ARTICLE

PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY OF *ENTEROCOCCUS SPECIES* ISOLATED FROM DIFFERENT CLINICAL SAMPLES IN A TERTIARY CARE HOSPITAL OF NORTH INDIA

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ABSTRACT

Introduction: The present study was carried out to determine the species of *Enterococci* isolated from various clinical samples and to determine its antimicrobial susceptibility pattern. *Enterococci* have emerged as an important cause of nosocomial infections and antibiotic resistance among *Enterococcus* is a major obstacle for treatment.

Material and methods: *Enterococcus spp.* were isolated and identified from different clinical samples between Sept. 2012 to Aug. 2013 by standard biochemical test. Antimicrobial susceptibility testing was performed by modified Kirby bauers disc diffusion method as per CLSI guidelines.

Result: Among the 100 isolates of *Enterococcus* from various clinical samples maximum isolates were from urine sample 70% and *E. faecalis* 92% constituted the predominant isolate. They were found to be susceptible to linezolid and vancomycin with least sensitive to ciprofloxacin and tetracycline.

Conclusion: Routine speciation and in vitro antimicrobial susceptibility testing of *Enterococcus* in various clinical samples is emphasized due to the prevalence of wide variety of *Enterococcus* species and also appearance of high resistant strains.

Key words: *Enterococcus spp.*, Antimicrobial susceptibility testing, Antibiotic resistance

INTRODUCTION

Enterococci have emerged over the last decade as one of the most important nosocomial pathogens worldwide, responsible for increasing number of episodes of bacteremia, endocarditis, meningitis, urinary tract and soft tissue infection.[1,2] Although 19 species within genus are recognized, *Enterococcus faecalis* is the most commonly isolated pathogen, followed by *E. faecium*. Species identification is useful for epidemiological investigation of an outbreak and also for clinical decisions, particularly with regard to therapy, as antimicrobial susceptibility differs by species. [3]Antibiotic resistance among *Enterococci* is a major obstacle for treatment.[4] Thus present study was designed to know the prevalence of *Enterococci* causing variety of infections and its antibiotic sensitivity spectrum.

MATERIALS AND METHODS

The present study was conducted in a tertiary care hospital in north India from June 2012 to May 2013. 100 isolates of *Enterococcus* were collected from different clinical specimens and speciated using extensive phenotypic and physiological tests. All the Gram positive cocci which were catalase negative were confirmed as *Enterococcus* genus with growth on and blackening of bile-esculin agar, growth in the presence of 6.5% sodium chloride (salt tolerance test) and heat tolerance test i.e. growth at 60^0c for 30min. Further *Enterococcus* species were identified by potassium tellurite reduction, arginine dihydrolase test, motility testing and sugar fermentation test including glucose, lactose, mannitol and arabinose.

Antimicrobial susceptibility testing was performed on Mueller Hinton agar as per CLSI guidelines.[5]The following antibiotics were tested- Vancomycin (30μg), Ciprofloxacin (30 μg), Gentamicin (10 μg), Linezolid (30 μg), Netillin (30 μg), Doxycycline (30 μg), Nitrofurantoin (30μg), Cefuroxime (30 μg), Cefoperazone (30 μg), Norfloxacin (10 μg), Piperacillin (100 μg), Amikacin (30 μg), Clindamycin (2 μg), Amoxycillin (30 μg).

RESULTS

A total of 100 *Enterococcus* strains were isolated from various clinical samples during the study period. The major source of *Enterococcus* strains isolated was urine,

followed by stool, sputum, throat swab, C.S.F, high vaginal swab, bile, central line tip, pus, anal discharge and fluid from drain (Table 1).

Table 1: Different *Enterococcus spp.* isolated from various clinical samples.

Sample	Number	*E. faecalis*	*E. faecium*	Percentage
Urine	70	62	8	70%
Stool	6	6	-	6%
Sputum	6	6	-	6%
Throat Swab	4	4	-	4%
C.S.F.	2	2	-	2%
High Vaginal Swab	2	2	-	2%
Bile	2	2	-	2%
Central line Tip	2	2	-	2%
Pus	2	2	-	2%
Anal discharge	2	2	-	2%
Fluid from Drain	2	2	-	2%
Total	100	92	8	100%

Table 2: Distribution of isolates in relation to patient's age

Age group (years)	Number (%)
0-20	38 (38.0)
21-40	40 (40.0)
41-60	20 (20.0)
61-80	2 (2.0)
Total	**100 (100)**

Table 3: Antibiotic sensitivity pattern of *Enterococcus spp.* isolated from urine sample

Antibiotics	Sensitive
Linezolid(LZ)	70 (100%)
Vancomycin(Va)	64 (91.5%)
Nitrofurantion(NIT)	62 (88.5%)
Norfloxacin(NX)	54 (77%)
Gentamicin(G)	42 (60%)
Cefoperazone(CF)	38 (54%)
Netillin(NET)	36 (52%)
Piperacillin(PIT)	32 (46%)
Ciprofloxacin(Cxm)	30 (42%)

Table 4: Antibiotic sensitivity patterns of *Enterococcus spp.* isolated from clinical samples other than urine.

Antibiotics	Sensitive
Linezolid(LZ)	30 (100%)
Vancomycin(Va)	22 (73%)
Cefoperazone(CF)	22 (73%)
Ofloxacin(OF)	20 (67%)
Doxycycline(Do)	20 (67%)
Clindamycin(CD)	20 (67%)
Ciprofloxacin(CIP)	18 (60%)
Ceftazidime(CAZ)	18 (60%)
Amoxycillin(AMX)	16 (53%)
Tetracyclin(TE)	16 (53%)

Females 72 (72%) were found to be more prone to *Enterococcal* infection as compared to males 28 (28%). High prevalence of *Enterococcal* infection was seen in the age group 21-40 years (40%) followed by 0-20, 41-60 and 61-80 age groups (Table 2). In the present study among the *Enterococcus* isolated *E. faecalis* was the predominant species 92(92%) followed by *E. faecium* 8 (8%).

In the present study all *Enterococcus* strains showed 100% sensitivity against linezolid followed by high sensitivity with vancomycin (Table no. 3, Table no. 4). Among urinary isolates least sensitivity was observed with ciprofloxacin, while isolates from clinical samples other than urine showed least sensitivity with amoxicillin and tetracycline.

DISCUSSION

Enterococci are the commensals of the human intestinal flora. Sites less often colonized by these organisms include the oral cavity, genitourinary tract, and skin, especially in the perineal area. The main sites of colonization in the hospitalized patients are soft tissue wounds, ulcers, and the gastrointestinal tract. *Enterococci* were traditionally regarded as low-grade pathogens, but have emerged as an increasingly important cause of nosocomial infections in recent years.[6]

More females were found to be infected with *Enterococci* as compared to males. This is comparable with the study carried out by Bose et.al (2013) in which number of females infected with *Enterococcal* infection was more (68.93%) than number of males.[7]

In present study the highest prevalence of *Enterococcal* infection was seen in the age group 21-40 (40%) years. Similar findings of higher infection rate in age group 21-30 yrs was reported by Bose et al. (2013) while, in another study Barros et al. (2009) have reported high prevalence of *Enterococcal* infection in the age group of 50-60 years.[7, 8]

In our study maximum number of *Enterococci* were isolated from urine sample followed by sputum, stool, throat swab and one each from high vaginal swab, bile, central line tip, pus, anal discharge, fluid from drain &

CSF. Similar observations were reported by Bose et.al (2013) and Jada et.al (2012) in separate studies.[7, 9]

E. faecalis and *E. Faecium* were the only species isolated in the present study. This is comparable with the findings of Bose et.al (2012) who isolated 82% *E. faecalis* and 18% *E. faecium* in their study.[7] Mickeen et al. (2002) also isolated only three species i.e., *E. faecalis, E faecium* and *E. durans* in their study.[10]

Vancomycin resistant *Enterococci* (VRE) has been increasingly reported from all parts of the world. In present study 9% and 27% of the isolates from urine and other clinical samples respectively were resistant to vancomycin. 10-24% resistance to vancomycin has been reported by Wattal et al. (2010), Butch et al. (2011) and Karmarkar et al. (2004) in separate studies.[11, 12, 13]The emergence of VRE has seriously affected the treatment of the infections caused by this organism. This leaves clinicians with a limited choice. For these types of cases, newer antibiotics, such as linezolid and tigecycline are useful. [7]

In the present study Linezolid show (100%) sensitivity followed by vancomycin(91.5%), nitrofuration(88.5%), norfloxacin(77 %), gentamicin(60%), cefoperazone(54%), nettalin(52%), pepracillin(46%) and ciprofloxacin(42%),

More than 50% resistance with gentamicin was reported by Butch et al. (2011) and Nepal et al. (2013).[12,14] In the present study also more than 40% resistance was observed with aminoglycosides. High sensitivity of 88.5% was observed with nitrofurantoin while Butch et al. (2011) reported more than 60% sensitivity with nitrofurantoin.[12]

Among quinolones least sensitivity was observed with ciprofloxacin. Similar low sensitivity with ciprofloxacin was reported by Butch et al. (2011) and Subbalaxami et al. (2010). [12, 15]

CONCLUSION

Thus, the present study highlights the prevalence of *E. faecalis* in our hospital. The data confirms vancomycin as a drug of choice, limiting the use of linezolid only in case of VRE. We further emphasize the need for constant monitoring of antibiotic susceptibility pattern in defined geographical areas which will be helpful in formulating local antibiotic policies.

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