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Original article

Prevalence Of Drug Resistance Among *Enterococcus Spp* Isolated From A Tertiary Care Hospital

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ABSTRACT

Introduction: *Enterococci*, initially considered as normal commensal of intestinal tract, has recently emerged as a medically important pathogen causing hospital acquired infections. Incidence is significantly high in debilitated patients. One of the important causes of development of multi drug resistant *enterococci* is antibiotic selective pressure. This study aims to isolate *enterococci* from various clinical specimens of indoor patients and to find out in vitro antimicrobial activity against the isolates.

Materials and methods: Samples were cultured on blood agar, MacConkey's agar and Hi chrome media for *Enterococcus faecium*. Blood samples were collected in blood culture bottles. Isolates were identified up to species level by various biochemical tests as per conventional methods. Antibiotic sensitivity was done on Mueller Hinton agar by Kirby Bauer disk diffusion method. Vancomycin resistant isolates were further tested for minimum inhibitory concentration by E test.

Results: Total number of clinical *enterococcal* isolates was 544, among which 82% was *Enterococcus faecalis* and 18%, *Enterococcus faecium*. Maximum number of isolates was from urine samples. Commonest age group affected was 21 – 30 years. Male: Female ratio was 1: 2.2. Maximum resistance was seen against gentamicin (58%), followed by co trimoxazole (49%), tetracycline (47%) and ampicillin (43%). Nitrofurantoin showed excellent activity against uropathogenic *enterococci*. Newer drugs like linezolid and tigecycline have got important role against multi drug resistant *enterococcal* infection.

Conclusion: In our study, *Enterococcus faecalis* is a predominant species. There is a need for routine surveillance of susceptibility pattern of *enterococcal* infections as they remain a significant clinical problem.

KEYWORDS: *Enterococci*, Hospital acquired infection, Multi drug resistant

INTRODUCTION

Enterococci, initially considered as normal commensal of intestinal tract, has recently emerged as a medically important pathogen, causing hospital acquired infection. Incidence of *enterococcal* infection is significantly high in patients suffering from urinary tract infection, blood stream infection and surgical sites infection. Nosocomial *enterococcal* infection is also common in organ transplantation recipients, cancer patients and debilitated patients receiving broad spectrum antibiotics. [1, 2, 3]

Enterococcal infections usually develop in previously colonized patients and thereafter spread through hands of health care workers and the environment. One of the important causes of development of multi drug resistant *enterococci* is antibiotic selective pressure. This organism is considered as second leading cause of hospital acquired infections. [3, 4]

Enterococcus is a hardy organism and can survive for long period on fomites. Increased use of indwelling medical devices, such as, catheterization and prolonged hospital stay encourages growth of multidrug resistant *enterococci*. [3] Keeping all these things in mind, this study aims to isolate *enterococci* from various clinical specimens from indoor patients and to find out in vitro antimicrobial activity against the isolates.

MATERIALS AND METHODS:

Approval of institutional ethics committee was taken for this study. Study period – one and half year. Study population – patients admitted in a tertiary care hospital, irrespective of age, sex or antibiotic therapy. Specimen, such as blood, urine, pus, wound swab, catheter tip, peritoneal fluid sent in the Microbiology department were processed as per conventional method.

Uncentrifuged urine sample on direct microscopy having ≥4 pus cell/ high power field were further processed. Blood was collected in blood culture bottle. Culture was done on blood agar and MacConkey's agar and incubated aerobically at 37ºC for 24 hours. The isolates were identified by colony morphology, Gram's staining, Catalase production growth on nutrient broth containing 6.5% sodium chloride, aesculine hydrolysis in presence of 40% bile salts, growth at 10ºC, 37ºC and 45ºC and other biochemical reactions. [5], [6]

HiChrom media selective for Enterococcus faecium (E.faecium) was also used for culture.

Following antibiotic disks were used for this study –

Nitrofurantoin (300 µg), ciprofloxacin (5µg), tetracycline (30µg), ampicillin (10 µg), gentamicin (120 µg), chloramphenicol (30µg), teicoplanin (30µg), imipenem (10µg), vancomycin (30µg), linezolid (30µg) and tigecycline (15µg).Nitrofurantoin and ciprofloxacin were only used for urine samples. All antibiotic disks were obtained from Hi Media Pvt Ltd, India.

The isolates resistant to vancomycin on disk diffusion test were further tested by using vancomycin screen agar. While testing vancomycin against enterococci, plates were incubated for 24 hours and read with transmitted light, as per CLSI guidelines. [7] Minimum inhibitory concentration (MIC) determinations of vancomycin against vancomycin resistant enterococci were done by E test (available from AB Biodisk, Solona, Sweden). MIC value ≤4 µg/ml was taken as susceptible and ≥32µg/ml as resistant. [8, 9]. Enterococcus faecalis (E. faecalis) ATCC 29212 and Staphylococcus aureus ATCC 25923 were used as control strains. [10]

RESULTS

Total 544 *enterococci* were isolated from various clinical samples over a period of one and half year. Maximum number of *enterococci* were isolated from urine samples, i.e.338 (62.36%), followed by 147 (27.02%) from blood, 43 (7.90%) from wound swab, 10 (1.83%) from pus, 4 (0.73%) from Foley's catheter tips and 2 (0.36%) from peritoneal fluids. [Table 1]

Maximum number of age group affected was 21 – 30 years followed by 31 – 40 years. Minimum number of affected age group was 0 – 10 years. [Table 1]Number of females infected with *enterococcal* infection was more, i.e. 375 (68.93%).Number of males affected was 169 (31.07%). Male: Female ratio was 1:2.2. [Table 1]

Among 544 *enterococcal* isolates, 446 (82%) were *Enterococcus faecalis (E. faecalis)* and 98 (18%) were *Enterococcus faecium (E. faecium)*. [Table 2]

Enterococcal isolates from various clinical samples were 100% susceptible to vancomycin, linezolid and tigecycline and maximum resistance was observed against high level amino glycoside, i.e.58%. [Table 3]

Ciprofloxacin and Nitrofurantoin were used for urine samples only and result was quite satisfactory, percentage of resistance being 3.25% and 2.07% respectively. [Table 4]

Two of the clinical isolates of *enterococci* were showing resistance to vancomycin by disk diffusion method however it was found sensitive to the same drug by doing MIC detection, using E- test.

On vancomycin screen agar method, no growth was observed by the two *enterococcal* isolates which were resistant against vancomycin by Kirby – Bauer disk diffusion method. The same two isolates had MIC less than 4 µg/ml, detected by E-test.

Table1: Age, sex and sample wise distribution of various *enterococcal isolates*

Samples	Total no. & %	0 -10 yr		11 -20 yr		21- 30 yr		31- 40 yr		41 –50 yr		51 – 60 yr		>60 yr	
		M	F	M	F	M	F	M	F	M	F	M	F	M	F
Urine	338(62.13%)	0	4	7	18	25	173	21	51	2	8	7	12	4	6
Blood	147(27.02%)	11	2	3	5	6	9	15	8	9	7	11	16	18	27
Wound swab	43(7.90%)	0	0	3	3	6	5	9	6	0	0	2	3	1	5
Foley's catheter tip	4(0.73%)	0	0	0	0	0	2	0	0	0	0	0	0	2	0
Pus	10(1.83%)	0	0	0	0	2	1	1	1	1	2	1	0	1	0
Peritoneal fluid	2 (0.36%)	0	0	0	0	0	0	0	0	0	0	1	0	0	1
Total	544	11	6	13	26	39	190	46	66	12	17	22	31	26	39

Table 2: Species distribution of enterococcal isolates from clinical specimens. (n=544)

Enterococcal species	Number	Percentage(%)
E. faecalis	446	82%
E. faecium	98	18%

Table 3: Antimicrobial resistance pattern of enterococcal Isolates. (n=544)

Antibiotics	Number &Percentage(%) Of Resistant Strains
Tetracycline	255 (47.1%)
Ampicillin	233 (43%)
Gentamicin(high level)	315 (58%)
Chloramphenicol	174 (32.3%)
Teicoplanin	212 (39%)
Imipenem	76 (14.1%)
Vancomycin	0 (0%)
Linezolid	0 (0%)
Tigecycline	0 (0%)

Table 4: Number and percentage (%) resistance of uropathogenic enterococcal isolates against nitrofurantoin and ciprofloxacin.

Antibiotics	Number and % resistance
Nitrofurantoin	7 (2.07%)
Ciprofloxacin	11 (3.25%)

DISCUSSION

We undertook this study to establish the species distribution and antibiotic resistant pattern of *enterococci* from clinical specimens in our setup. During the study period of one and half year, we isolated 544 *enterococcus* species, among which 446 (82%) were *E.faecalis* and 98 (18%) were *E. faecium*. Shouten MA et al also found 83% *E. faecalis* and 13.6% *E. faecium* isolates in their study.[11] Jayanthi S et al reported that 80 – 90% of all *enterococcal* infections were caused by *E. faecalis*.[2]Higher incidence of *E.faecalis* infection might be due to its greater intrinsic virulence. [12] However, Karmarkar et al [4] from Mumbai reported higher isolation of *E. faecium* (80.7%) over *E. faecalis* (19.2%) in their study.

Most common isolation of *enterococci* were from urine samples (62.13%), followed by blood, wound swab, pus, catheter tip and peritoneal fluid. McNamara EB et al also described urinary tract as the commonest site of isolation of *enterococci* in their study. [13]

In our study, *enterococcus* isolates showed high number of resistance against high level amino glycoside, i.e. 58%. Resistance to other drugs was also relatively high, such as, tetracycline 47.1%, ampicillin 43%, chloramphenicol 32.3%,

teicoplanin 39% and imipenem 14.1%. As per guide lines of clinical and laboratory standard institute, *enterococci* species against cephalosporin, amino glycoside (except for high level resistance screening), clindamycin and trimethoprim – sulfamethoxazole may appear susceptible in vitro, but may not be effective in vivo. Therefore, these drugs should not be reported as susceptible against *enterococci*. [7] *Enterococci* demonstrate both intrinsic as well as extrinsic types of resistance to antibiotics causing them an important etiological agent of hospital acquired infection. Because of low affinity of penicillin binding proteins, they tolerate β – lactams. *Enterococci* also use pre formed folic acid, thereby, bypassing inhibition of folate synthesis causing resistance to trimethoprim and sulfamethoxazole. Acquired resistance to penicillin, chloramphenicol, tetracycline, fluoroquinolones, amino glycoside (high level) and vancomycin were also reported in *enterococcal* infection. High level gentamicin resistance (HLGR) was first time reported in *E. faecalis* in the year 1979. Resistance to amino glycoside is often associated with multidrug resistance and is due to various amino glycoside modifying enzymes. Moreover, *E.faecium* has become difficult to be treated by glycopeptides and amino glycosides. [3, 10, 14, 15]

We used ciprofloxacin and nitrofurantoin only for urine samples. Nitrofurantoin is an excellent drug against *enterococcal* urinary tract infection. It has been used for past many years and still shows very little resistance. It is both bacteriostatic and bactericidal and resistant mutants are very rare. There are no cross resistance between Nitrofurantoin and other antibiotics. It is effective against both *E. faecalis* and *E. faecium* including most of the *VRE*. [16] Nitrofurantoin can be given in early pregnancy also. [17]

In our study, all the isolates were susceptible to vancomycin, linezolid and tigecycline. Two of our *enterococcal* isolates showed intermediate resistance against vancomycin, detected by disk diffusion method but showed susceptibility to the same drug by vancomycin screening agar test and E test (for MIC detection).There are ample chances of getting error associated with disk diffusion susceptibility testing against vancomycin. Therefore, to depend only on report of disk diffusion test against vancomycin may result unnecessary elimination of the antibiotic as a part of treatment schedule. [13] Although, at present, *VRE* is not a problem in our set up, its routine monitoring is essential, since it appears to be an emerging pathogen in India. [10]

The emergence of *VRE* had seriously affected the treatment of the conditions caused by this organism. This leaves clinicians a limited choice. [3] For these types of cases, newer antibiotics, such as linezolid and tigecycline are useful. Tigecycline (GAR – 936) is a new glycylcycline derivative of tetracycline. Tissue penetration of tigecycline is excellent and it acts against both Gram positive and Gram negative microorganisms. [9] Linezolid is the first oxazolidinone introduced in 2000. It acts effectively against various Gram positive organisms, including *VRE*. It binds to the domain V region of 23 S rRNA and mutation to that domain causes resistance to the drug. Resistance to linezolid is extremely low. [18, 19] However, few reports regarding microorganisms resistant to linezolid and tigecycline have been reported by various researchers. [20, 21, 22] Antibiotics stewardship programme should be made to prevent emergence of multidrug resistant microorganism. [23]

VRE bacteremia prolongs the duration of hospital stay by an average of two weeks and mortality rate up to 30 – 50% was reported from this infection. [3]

On the other hand, blood cultures that grow the *enterococci* without any evidence of ongoing infection may be positive because of skin contamination. Those types of cases should be carefully re evaluated.

CONCLUSION:

Infection due to multidrug resistant *enterococci* is not uncommon in our set up. Multi resistance and cross resistance shown by the microorganisms result in limited options of drugs for treatment. This emphasizes the need for speciation and in vitro antibiotic susceptibility testing with alternative chemotherapeutic regimens for treatment of serious *enterococcus* infections. For *enterococcal* urinary tract infection, Nitrofurantoin is an excellent choice. Against multidrug resistant *enterococcal* infection, linezolid, tigecycline and vancomycin are very effective. To prevent the emergence of multidrug resistant bacteria, judicious use of antibiotics to treat the patients today and preservation of newer drugs for future generation should be adopted, whenever possible.

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