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ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF ENTEROCOCCUS SPECIES ISOLATED FROM CLINICAL SAMPLES IN SOUTH INDIA

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ABSTRACT

Enterococci are common cause of hospital acquired infection and have become progressively more resistant to antibiotic. Among the 13 Enterococcus species ninety percentage of Enterococcal infection are due to Enterococcus *faecalis .E. faecalis* is a causative agent for nosocomial infection. The present study was carried out to asses the changing trends in antibiotic susceptibility of Enterococcus species isolated from clinical samples from Gulbarga region.122 samples were collected from various hospitals and diagnostic centers in Gulbarga region. The clinical isolates were identified by Facklam and Collins conventional method. The susceptibility tests were done against vancomycin, ampicillin, oxacillin, rifamycin, ciprofloxacin, tobramycin, gentamycin, teicoplanin and streptomycin by Kirby bauer method. MIC of resistant *E. faecalis* isolates were determined by NCCLS method. In our study, *E. faecalis* strains found to be 50% multi-drug resistant and the resistance was more in case of vancomycin antibiotic. The *E. faecium* isolates showed high resistance to gentamycin and streptomycin among the few MDR strains. *E. durans* were susceptible to all the antibiotic expect streptomycin and *E. gallinarium* were found susceptible to all antibiotics. The MIC's determined of the 12 strains, showed the prevalence of HLAR and HLGR among the *E .faecalis* isolates. The present study reveals for the first time emergence of vanocmycin resistant enterococci from this part of world and indicates the magnitude of antibiotic resistance in and around the study area.
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KEY WORDS: *Enterococcus spp, Enterococcus faecalis,* multidrug resistance.

INTRODUCTION

Enterococci are Gram positive coccid bacteria that belong to normal microbiota of the gastrointestinal tract of humans, most mammals, birds, and many other species. In the colon of nearly all humans Enterococci can be found in numbers as high as 10^8 colony-forming units per gram of feces [**1**, **2**]. Out of 13 enteroccocal species that are described, *Enterococcus faecalis* and *Enterococcus faecium* are isolated most frequently. In the normal healthy host Enterococci seldom are causing infections, only some urinary tract infections are seen. However, surveillance data indicate that Enterococci are becoming one of the leading causes of nosocomial infections [**3**, **4, 5**] Nosocomial infections with Enterococci are frequently seen in critically ill patients at intensive care units, for example in liver transplant patients, which are often considered especially vulnerable to Enterococcal infections [**6, 7]**. Different studies describe a longer length of stay in hospital and increased mortality due to vancomycin-resistant *E. faecium (*VRE*)* compared to vancomycin-susceptible *E. faecium* [**8**].

Enterococci have an acquired resistance to several classes of antibiotics either by mutation or by receipt of foreign genetic material through the transfer of plasmids and transposon. [**1**].The acquisition of high level aminoglycoside resistance (HLAR) and vancomycin resistance has limited the therapeutic options available for clinicians. The transfer potential of vancomycin resistant genes from Enterococci to *S .aureus* which has been achieved invitro but not yet reported in clinical settings, increases the importance of findings ways to limit the spread of vancomycin resistant Enterococci (VRE). The problem of nosocomial enterococcal infection is compounded by emerging antibiotic resistance. However, resistance alone does not explain the increase of Enterococci in nosocomial infections. Although resistance is relatively uncommon among *E. faecalis* isolates compared to resistance among *E. faecium* isolates [**9**], *E. faecalis* currently accounts for the majority of clinical Enterococcal isolates (up to 90 %), followed by *E. faecium* [**3,10**]. This disparity might be explained by the relative abundance of *E. faecalis* in the gastrointestinal tract [**11, 12**] or enhanced virulence of *E. faecalis*. This report focus on the *E. faecalis* infections as these have

become more prominent among hospital acquired infections. The present study was undertaken to identify the species of the isolates and to evaluate the susceptibility to various antimicrobial.

MATERIALS AND METHODS

Bacterial isolates

All consecutive of Enterococcus were isolated from clinical samples over six months period from September 2008 and January 2009 from district Govt. hospital and diagnostic centers from Gulbarga region were included in the study. The strains were isolated from blood, urine, pus and Cerebrospinal fluid samples.

Identification

The isolates were identified up to the genus and species level by Gram's stain, motility testing and conventional biochemical tests using standard microbiological techniques, these included catalase, growth in the presence of 6.5% NaCl, bile-esculin agar, tellurite reduction, pigment production, arginine dihydroalse reaction and the generation of acid from mannitol, arabinose, sorbitol, lactose and raffinose. The carbohydrate fermentation reactions were performed in brain heart infusion broth containing 1% carbohydrate with bromocresol purple as an indicator [**13, 14**]. *E. feacalis* 5025 (NCIM) and *E. faecium* 2605 (NCIM) were used as control.

Antimicrobial Susceptibility testing

Antimicrobial susceptibility testing was performed on Mueller Hinton agar (Hi-media, India) by the standard disk diffusion method as recommended by the National Committee for Clinical Laboratory Standards [**15**].The antibiotics used for the tests were vancomycin, ampicillin, oxacillin, rifamycin, ciprofloxacin, tobramycin, gentamycin, teicoplanin and streptomycin.

Minimal Inhibitory concentration (MIC): The minimum inhibitory concentration (MIC's) for vancomycin was determined by the broth dilution method. MICs were determined in MH broth containing serial two-fold dilutions of each antibiotic. Bacterial suspensions of 10^4 colony-forming units (CFU)/mL were inoculated into the flasks and results were recorded after overnight incubation at 35°C. The MIC was defined as the lowest antibiotic concentration with no visible growth. [**16**].

RESULTS

Bacterial Isolates

A total of 122 Enterococcus strains were isolated from different clinical samples on bile esculin agar. The species identities of the clinical Enterococccal isolates, includes 76 (62.29%) strains were *E. faecalis* and 27 (22.13%) strains were *E. faecium*, *E. durans* 12 (5.0%) and *E. gallinarum* 7 (5.7%). The *E. faecalis* was the predominant isolates from urine, pus, CSF and blood samples. The *E. faecalis* isolates were Gram positive and were positive for tellurite reduction and arginine hydrolysis and showed negative result for catalase. The carbohydrates like arabinose, raffinose and mannitol were utilized and sorbitol and lactose were not utilized. The *E. faecalis* strains showed non haemolytic on blood agar.

Antimicrobial susceptibility Testing

The results of the susceptibility tests are carried out by disc diffusion method as shown in Table 1. The *E. faecalis* strains showed high antibiotic resistance pattern compared others species. Fifty percent of *E. faecalis* strains were resistant to the different antibiotic like vancomycin (77.63%), gentamycin (64.47%) and oxacillin (55.26%) antibiotics, and were multi drug resistant. The isolates were found sensitive to rifamycin (61.84%), teicoplanin (55.26%) streptomycin 52.63%) and tobramycin (51.13%). *E. faecium* strains showed resistance to more than four antibiotics was 18.51% resistance to gentamycin and streptomycin was 44.4% and 40.8% respectively. Sensitivity was found to rifamycin (88.88%), tobramycin (85.10%), ciprofloxacin (85.18%) and oxacillin (81.48%). *E durans* strains were found sensitive to all the antibiotics except streptomycin (58.8%). *E.gallinarum* strains were sensitive to the all antibiotics tested

Determination of MIC's in *E. faecalis* isolates

MIC's for gentamycin among 12 *E. faecalis* strains were carried out, among them 5 strains showed ≥1024µg/ml and 5 strains had MIC of ≥512µg/ml and 2 strains showed 256µg/ml. The vancomycin MIC for 8 strains showed ≥64µg/ml and 4 strains had ≥ 128µg/ml of the total 12 strains tested as shown in the Table.2.

DISCUSSION

Recently, Enterococci have emerged as important nosocomial pathogens because of their innate resistance to several classes of,

Table1: Antimicrobial susceptibility of *Enterococcus spp*

Antimicrobial agents	*E.faecalis*(76)			*E.faecium*(27)			*E.durans*(12)			*E.gallinarum*(7)		
	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)
Vancomycin	17.10	5.2 6	77.63	62.96	3.70	33.33	100	0.0	0.0	100	0.0	0.0
Ampicillin	36.84	9.21	53.94	77.77	0.0	22.22	100	0.0	0.0	100	0.0	0.0
Oxacillin	38.15	5.26	55.26	81.48	3.70	14.81	100	0.0	0.0	100	0.0	0.0
Rifamycin	61.84	10.52	27.63	88.88	0.0	11.12	100	0.0	0.0	100	0.0	0.0
Ciprofloxacin	43.42	6.57	14.10	85.08	0.0	14.81	100	0.0	0.0	100	0.0	0.0
Tobramycin	51.31	19.73	28.94	85.18	3.94	3.70	100	0.0	0.0	100	0.0	0.0
Gentamycin	23.68	11.84	64.47	17.10	7.40	44.4	100	0.0	0.0	100	0.0	0.0
Teicoplanin	55.26	18.42	26.35	77.77	18.51	3.70	100	0.0	0.0	100	0.0	0.0
Streptomycin	52.36	13.15	32.89	59.25	0.0	40.70	58.33	0.0	41.66	100	0.0	0.0

Table 2: MICs determination of Gentamycin and vancomycin resistance *Enterococcus faecalis* isolated from different clinical samples, using micro broth dilution method

Strains	Specimen	MIC µg/ml	
		Gentamycin	Vancomycin
121	Urine	1024	128
122	Urine	1024	128
123	Urine	1024	128
124	Urine	1024	64
125	Urine	1024	64
136	Blood	512	64
137	Blood	512	64
138	Blood	512	64
139	Blood	512	64
140	Blood	512	64
141	CSF	256	128
142	Pus	256	64

antibiotics, such as cephalosporins, and their ability to acquire additional resistance, such as glycopeptide resistance. **[17]** The present study emphasizes on the antimicrobial susceptibility

pecies level distribution of clinical Enterococcal isolates from Gulbarga region The majority of the clinical isolates (98%) were *E. faecalis* or *E. faecium*, while other Enterococcus *spp*. accounted for only 2% of isolates, comparable to the distribution of species in previous studies [**12,18, 19,**].Our study reveals that most of the strains were *E. faecalis* from the urine sample and *E. faecium* were found in the blood cultures .The relatively high proportion of *E. faecalis* among the hospitals was from the urine cultures. Changes in the hospital patient population and antimicrobial use patterns couples with a greater antibiotic resistant nature of the *E. faecalis* [**20**]. In our study the cultures were found to be non hemolytic on the blood agar and showed high degree resistant to the antibiotics used. Similar results were obtained as the strains were resistant to more than four antibiotic inturn the strains were more resistant to the vancomycin, gentamycin and oxacillin antibiotic **[21].**

The results of this study confirms that *E. faecalis* were more resistant to the vancomycin (77.63%), gentamycin (64.47%) and oxacillin (55.26%) and were sensitive to rifamycin (61.84%), teicoplanin (55.26%) and

streptomycin (52.63%) The multidrug-resistant Enterococci are being increasingly reported from all over world. Many studies have demonstrated that *E. faecium* is comparatively mores resistant than *E. faecalis*. [**20, 22**] However, in our study *E. faecium* strains showed (18.21%) multidrug resistant which was very less compared to the fifty percent resistance of *E. faecalis*.*E. durans* strains were susceptible to all antibiotics except to streptomycin (59.25%) and *E.* gallinarium were susceptible to all antibiotics tested.

High level aminoglycoside resistant Enterococci were first reported in France in 1979 and since then resistant have been isolated from all the continents [**23**].In India (Nagpur) a study was reported, prevalence of high level gentamycin resistant enterococci (7.8%) and streptomycin (24.7%) with regard to earlier antimicrobial resistance pattern **[24].** Our studies showed higher rates of gentamycin resistance (64.47%) and streptomycin is (32.89%) for *E. faecalis*. In case of the *E. faecium* the rate resistance for gentamycin (44.4%) and streptomycin (40.70%). For the *E durans* the streptomycin rate is higher compared to *E. gallinarum* and *E faecium* of (58.33 %).In our study about 12 strains showed raised MIC of ≥512 to ≥1024 µg/ml of gentamycin antibiotic. The prevalence of colonization or infection by VRE has dramatically increased in many countries. The first report of VRE infection was reported in 1988 **[25].**Indian studies have reported vancomycin resistance in 0-5% of enterococci, the 15% of colonizing enterocococcal strains in a pediatric hospital to be vancomycin-resistant **[26]**. In present study about 77.64 % of *E. faecalis* strains showed high resistant to vanocmycin by disk diffusion test and MIC of 12 strains as showed ≥64 to ≥128 µg/ml for vancomycin respectively. The last therapeutic resort for Enterococci was vancomycin. Unfortunately, recently VRE have taken firm hold and have become epidemic in some hospitals and regions.

In India the incidence of Enterococcal infections is not thoroughly investigated. *E. faecalis* most revalent species cultured from humans accounting for 80-90 percent of clinical isolates in other studies [**27, 6**] same results as been obtained in our studies. Our study signals the emergence of Vancomycin resistant enterococci in Gulbarga region. Thus, a more detailed study is necessary using phenotypic and genotypic methods to have a better picture of enterococcal infections. In conclusion, *E. faecalis* was observed as the predominant isolate from enterococcal bactermia in clinical samples of Gulbarga, India. Entercococci revealed an alarming rate of resistance to the standard antimicrobial agents used for therapy and MIC values to vancomycin. The importance of rational use of antimicrobials in patient management and infection control is to be emphasized.

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