Original Article

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Microbiology Section

The Prevalence and the Characterization of the Enterococcus Species from Various Clinical Samples in a Tertiary Care Hospital

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ABSTRACT

Background: Enterococci form a part of the normal flora of the intestinal tract, the oral cavity, and the vagina, but in recent times, they have become emerging nosocomial pathogens. Their increasing importance is largely due to their resistance to antimicrobials. The therapeutic failures in enterococcal infections are mainly due to the intrinsic as well as transferable drug resistance. The main aim of our study was to estimate the prevalence of the *Enterococcus* infection and to determine the antibiogram in a tertiary care hospital .

Method : *Enterococcus* was isolated from a total of 5555 clinical samples like urine , pus, tissue, blood and body fluids during the period from January to December 2008.The isolates were speciated by using conventional biochemical tests (Facklam and Collins). The antibiotic susceptibility testing was done by the Kirby Bauer disc diffusion method. Confirmation of vancomycin susceptibility was done by the Epsilometer test (E test) to determine the Minimum Inhibitory Concentration (MIC).

Result: From various clinical samples, 128 *Enterococcus* species were isolated in a period of one year and the rate of the infection was estimated to be 2.3%. Among the isolates, those of *Enterococcus faecalis (E.faecalis)* were 97(76%) and the remaining 31(24%) were of *Enterococcus faecium* (*E.faecium)*. The maximum number of isolates were from pus 55(43%), followed by the isolates from urine 40(31%). The sensitivity pattern of these isolates showed an increased resistance to penicillin, ampicillin and ciprofloxacin. A High Level of Gentamicin Resistance (HLGR) was present in 60 (47%) isolates of *Enterococcus* and 35(27%) isolates were intermediately sensitive to vancomycin by the Kirby Bauer disc diffusion method. All the intermediately sensitive isolates to vancomycin were further tested by the E test and they were found to be vancomycin sensitive.

Conclusion: Various studies have shown an increase in the rate of infection and the antibiotic resistance in the *Enterococcus* species. There is also a change in the pattern of the *Enterococcus* infection, with an increase in the isolation rate of *E. faecium* and other non faecalis *Enterococcus* species. The Kirby Bauer disc diffusion method is not an accurate method for detecting the Vancomycin Resistant *Enterococci* (VRE).

Key Words: *Enterococci*, Nosocomial pathogen, VRE

INTRODUCTION

Enterococcus, an indigenous flora of the intestinal tract, the oral cavity and the vagina, are known to be relatively avirulent in healthy individuals, but they behave as pathogens in hospitalized patients [1,2]. They have emerged as nosocomial pathogens inspite of the low levels of their virulence [2,3]. Their increasing importance is due to their resistance to many antimicrobials, which include the β lactam antibiotics, the aminoglycosides and most importantly, glycopeptides like vancomycin. The common species of *Enterococcus* which cause human infections are *E.faecalis* (80-90 %) and *E faecium* (5-10%) [4], but now there is an increase in the isolation rate of *E faecium* and other species from various clinical samples [1,2]. The rate of increase in the isolation of *Enterococcus faecium* is a problem, as its intrinsic resistance may lead to a treatment failure [2,3].

The Center for Disease Control and Prevention, in a survey on nosocomial infections, indicated that *Enterococcus* accounted for 13.9% infections, being next to *Escherichia coli* as a causative agent of hospital acquired urinary tract infections [1]. Therefore, the same importance is given to the multidrug resistant *Enterococcus* species, like that of Methicillin Resistant Staphylococcus Aureus (MRSA) and Extended Spectrum Beta Lactamase (ESBL) producers, as nosocomial pathogens. The main aim of our study was to determine the prevalence of *Enterococcus* from various clinical samples and to determine the antibiogram, with special reference to the vancomycin susceptibility.

MATERIALS AND METHODS

The present study was conducted in the Department of Microbiology, M S Ramaiah Medical College, Bangalore, India from January to December 2008. The samples which were included were urine, pus, tissue, blood and body fluids which were collected aseptically and their culture and the antibiotic sensitivity of the organisms which were isolated from them were performed as per the standard recommendations. The isolates of *Enterococcus* from throat swabs, sputum, vaginal swabs and stool were excluded from the study, as they formed a part of the normal flora [4].

The total sample size was 5555, among which 2527 were urine samples, 1230 were pus and tissue samples, 1698 were blood samples and 100 were peritoneal fluid samples. The Cysteine Lactose Electrolyte Deficient Medium (CLED) was used for the semiquantitative urine culture. The latter samples were inoculated on MacConkey's agar and blood agar. After inoculation, the plates were incubated overnight at 37ºC .

The *Enterococcus* species were isolated from 128 samples. They were identified by using standard tests like checking the colony morphology, gram staining, the catalase test, the bile esculin test, the salt tolerance test and the α- pyrrolidonyl β- naphthylamide

test (PYR test) [4,5,6]. Their speciation was on the basis of the sugar fermentation test (Facklam and Collin) [7], their growth in pyruvate broth, their arginine hydrolyzing property and their motility and pigment production [4,7,8]. All the media were purchased from Himedia (Mumbai, India).

The clinical significance of the *Enterococcus* isolates was assessed retrospectively by analyzing the clinical criteria like catheterization in urinary tract infections, the signs of sepsis and other laboratory tests like leucocytosis, the procalcitonin levels, etc. The antimicrobial susceptibility testing was performed by the Kirby Bauer disc diffusion method by using the following commercially available antimicrobial discs from Himedia®. Ampicillin (10 µg), penicillin(10µg), ciprofloxacin (5µg), vancomycin (30 µg), gentamicin (120µg), teicoplanin (30µg), linezolid (30µg) and piperacillin (100µg) [9] were tested on 5% Mueller Hinton blood agar along with a control strain of ATCC *E.faecalis* 29212, as per the Clinical and Laboratory Standards Institute (CLSI) guidelines, 2007. Care was taken to view the vancomycin zone of inhibition in transmitted light after 24 hours of incubation at 37ºC [10].

The MIC of vancomycin was determined by the E test for all the *Enterococci* isolates which showed intermediate sensitivity by the Kirby Bauer disc diffusion method. A lawn culture of Enterococci, 0.5 Macfarland's standard was made on 5% Mueller Hinton blood agar. The E –strip which was obtained from Himedia® was applied with an MIC scale, facing up, by using sterile forceps, with the higher concentration facing the edge of the plate. The plates were examined after 24 hours of incubation at 37ºC. The zone of inhibition was observed in the form of an ellipse. The MIC value is the value at which the zone convenes the comb like projection of the strip [5,6]. The antibiotic susceptibility pattern was interpreted as per the Clinical and Laboratory Standards Institute (CLSI) guidelines, 2007 [10].

RESULTS

The number of *Enterococci* which were isolated from the 5555 clinical samples were 128, accounting for an infection rate of 2.3%. *E. faecalis* amounted to 97(76%) infections and *E.faecium* to 31(24%) infections. The maximum number of *Enterococcus* isolates were obtained from pus-55 (43%), followed by urine-40 (31%). Among the 40 urine isolates, 15(38%) were from catheterized patients. Among the 19 blood isolates, 11 (58%) were from the paediatric age group.

The isolates were predominantly resistant to antibiotics like penicillin, ampicillin and ciprofloxacin [Table/Fig-1]. A high level of gentamicin resistance was seen in 60 (47%) isolates and 35(27%) isolates of *Enterococcus* showed an intermediate sensitivity to vancomycin by the Kirby Bauer disc diffusion method. These isolates were sensitive to vancomycin, with a MIC of less than 4µg which was obtained by the E test [Table/Fig-2]. All the *Enterococci* were sensitive to linezolid.

	Kirby Disc diffusion method	E-test
Sensitive	73%	100%
Intermediate Sensitive	27%	0%
Resistant	0%	0%

[Table/Fig-2]: Vancomycin susceptibility testing

DISCUSSION

The *Enterococcus* species have now emerged as nosocomial pathogens. Hence, it is important to know the changing patterns of the *Enterococcus* infections and the antimicrobial susceptibility patterns.of the isolates [1].

In our study, the maximum number of isolates were obtained from pus (43%), followed by urine (31%). In other studies, the urine isolates were maximum as compared to the isolates from pus, except in P. Vandamme et al's study in 1996, which showed a maximum of 43.4% isolates from pus, which was similar to the picture in our study [8,11]. *E.faecalis* (76%) formed the major isolate, followed by *E. faecium* (24%). The recent studies have shown an increase in the isolation rate of *E.faecium* and other non faecalis species of *Enterococcus* [12].

Our study showed that 47% isolates were resistant to penicillin, 45% to ampicillin, 50% to ciprofloxacin and 47% to high level gentamicin. This was similar to the picture in the study of Biny Thapa et al., 2007 [13]. The recent literature shows a drastic increase in the resistance pattern of the commonly used drugs, an increase in the penicillin resistance to 100%, (12) an increase in the ampicillin resistance to 62% (14) and an increase in the HLGR to more than 50% [12,14]. 27% of the *Enterococcus*, which showed intermediate sensitivity to vancomycin by the Kirby Bauer disc diffusion method, was further tested by the E test. The isolates were found to be sensitive to vancomycin by the E test. Recent studies have shown that the vancomycin resistance can vary between 1.7-20% in the tertiary care hospitals of India [14-17].

The inaccuracy of the disk diffusion method has resulted in an unwarranted utilization of this drug as a part of the treatment regimens. Therefore, a routine MIC monitoring of important antibiotics like vancomycin has to be done, before reporting it as resistant or intermediately sensitive [18]. The emergence of VRE has been attributed to the imprudent use of vancomycin, the colonization pressure and noncompliance with the infection control measures. The usage of vancomycin should be strictly discouraged in the following conditions: as the treatment in response to a single blood culture which is positive for the coagulase negative Staphylococci, as an empirical therapy and as the primary treatment for colitis which is caused by *C. difficile*. Our study did not show *Enterococci* which were resistant to vancomycin, but the inaccuracy of the Kirby Bauer disc diffusion method in detecting the susceptibility to vancomycin was clearly evident. A coordinated effort by various departments should be made in educating the hospital staff

Species	Ampicillin		Penicillin		Ciprofloxacin		Gentamicin 120		Piperacillin		Teicoplanin		Linezolid	
E. faecalis	S	R	S	R	S	R	S	R	S	R	S	R	S	R
%	52.6%	47.3%	51.3%	48.6%	51.3%	48.6%	55.2%	44.3%	77.6%	22.3%	65.7%	34.2%	100%	0%
E. faecium	S	R	S	R	S	R	S	R	S	R	S	R	S	R
%	62.5%	37.5%	58.3%	41.6%	45.8%	54.1%	45.8%	54.16%	70.8%	29.1%	75%	25%	100%	0%
Total%	55%	45%	53%	47%	50%	50%	53%	47%	76%	24%	68%	32%	100%	0%

[Table/Fig-1]: Antibiotic susceptibility pattern of Enterococci

regarding the problem of drug resistance, the vigilant use of antimicrobials by physicians, the prompt reporting and the usage of appropriate procedures by laboratories and an immediate implementation of the appropriate infection control measures. These can further prevent the emergence of VRE and can also reduce the burden of multidrug resistant *Enterococcus* [17].

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