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SPECIATION AND ANTIMICROBIAL SUSCEPTIBILITY OF ENTEROCOCCI FROM PATIENTS IN A TERTIARY CARE HOSPITAL

Microbiology

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ABSTRACT

Introduction: Enterococci have evolved from normal commensals to multidrug resistant pathogen. Enterococci are often reported in association with hospital-acquired infections. Management of Enterococcal infections has become challenging due to the emergence of multidrug resistant strains. **Objectives:** To speciate and detect the antibiotic susceptibility of Enterococci isolated from different clinical specimens.**Materials and Methods:** A total of 100 Enterococci strains isolated from clinical samples were tested using standard microbiological procedures. The minimum inhibitory concentration (MIC) for vancomycin was tested by agar dilution and E-strip methods. **Results:** Enterococci infections were more common in age groups, 21-40 years and 41-60 years with male preponderance. Isolates of Enterococci were more frequently associated with post operative wound infection (26%). *E. faecalis* (55%) was the predominant species, followed by *E. faecium* (45%). *E. faecium* showed 80% resistance to penicillin and ampicillin, while *E. faecalis* showed 38% resistance. All Enterococci isolates were 100% sensitive to vancomycin, linezolid and teicoplanin by the disc diffusion method; however, MIC for vancomycin by agar dilution and E-strip methods showed one strain of *E. faecium* resistant to vancomycin. **Conclusion:** Enterococci identification to the species level should be stressed in routine laboratory practice. Vancomycin resistance detection in Enterococci by the MIC method should be preferred as it helps reduce the morbidity and mortality due to Vancomycin resistant Enterococci.

KEYWORDS

Enterococcus species, Multidrug resistance, VRE

INTRODUCTION

Enterococci are aerobic and facultative anaerobic gram-positive cocci that are considered normal commensals of the gastrointestinal tract of humans and other animals but have recently emerged as multidrug-resistant pathogens causing hospital and community-acquired infections (1).

E. faecalis and E. faecium are the two frequently isolated species accounting for approximately 90-95% of infections caused by Enterococci. Others, such as *E.gallinarum, E.casseliflavus,* and *E. duransare* are isolated less frequently, accounting for 5% of clinical infections (2). Enterococcal species differ in drug susceptibility,which emphasizes the need for the identification of species for treating enterococcal infections (3). Enterococci are intrinsically resistant to many antibiotics. They can also acquire drug resistance by mutation in the chromosome or by acquisition of plasmids or transposons responsible for drug resistance in other bacteria (4). Vancomycin is the drug of choice in many bacterial infections caused by resistant strains of Enterococci. However, there is an increase in the number of vancomycin-resistant enterococci [VRE], and it is a serious problem not only in the treatment of enterococcal infections but also in the risk of transferring this resistance determinant horizontally to other vancomycin-susceptible species (5).

The present study aimed to identify the different species of Enterococci, their antibiotic susceptibility and to determine the prevalence of VRE.

MATERIALS AND METHODS

The study was carried out in a tertiary care hospital over a period of one and half years after obtaining permission from the institutional ethical committee. Clinical specimens received in the microbiology laboratory that yielded Enterococcal isolates were included in the study. Samples from which any other organism was isolated or samples that did not yield any growth were excluded from the study. Identification and speciation of Enterococcal isolates was done by standard bacteriological techniques. Antimicrobial susceptibility testing (AST) was done by modified Kirby Bauers Disc Diffusion method. Clinical Laboratory Standard Institute guidelines (CLSI) 2013 were used for interpretation of AST (6,7,8). Minimum inhibitory concentration (MIC) values were identified by agar dilutions and E-strip methods (9).

Data entry and statistical analysis was done using Microsoft excel 2013.

RESULTS

A total of 100 samples that grew Enterococci were included in the study. Among the 100 Enterococci isolated from clinical samples, *E. faecalis* was the predominant species [55%], followed by *E.faecium* [45%] (Figure 1). A male:female ratio of 1.38:1 was recorded. Fifty-eight percent were male patients, and 42% were females.

The distribution of Enterococci isolation in relation to age groups revealed that it was higher in the age groups of 21-40 years[32%] and 41-60 years [32%]. Age group of 0-20 years had distribution of 16% which included 6 newborns and 3 infants. [Table 1].

Table 1: Age Wise Distribution Of Enterococcal Isolates

Age (in years)	Enterococci isolates N=100
0-20	16
21-40	32
41-60	32
61-80	20

Approximately half of the Enterococci were isolated from pus samples[51%], which includes clinical conditions such as post operative wound infections, wounds, cellulitis and diabetic foot, followed by urine [29%], which includes community acquired and catheter associated urinary tract infections and blood [13%], as shown in the table 2. Table 3 shows the various clinical conditions associated with Enterococci. The maximum number of isolates of Enterococci was associated with post-operative wound infection [26%].

Table 2: Enterococcus Species From Different Clinical Samples

Samples	Total n=100	*E. faecium* n=45	*E. faecalis* n=55
Pus	51%	29%	71%
Urine	29%	59%	41%
Blood	13%	62%	38%
Peritoneal fluids	06%	100%	-
Miscellaneous(semen)	01%	-	100%

Table 3: Enterococcus Species From Different Clinical Condition

Clinical conditions	% of Enterococci isolated
Post op wound	26
Urinary tract infection –UTI	18
Wound infection	13
Bacteremia/Sepsis	13
Catheter associated with UTI	11
Diabetic foot	6
Cellulitis/Furnculitis	4
Device associated (JJ feeding,VP shunt)	2
Others	7

Figure 1 depicts the antibiotic susceptibility pattern of Enterococci species. E. faecium is found to be more resistant than E. faecalis. Urinary isolates of Enterococci were least resistant to nitrofurantoin: 6% in E. faecium and 2% in E. faecalis. All Enterococci isolates in our study were 100% sensitive to vancomycin, linezolid and teicoplanin by the disk diffusion method. However, one strain of E. faecium isolated from blood sample showed an MIC of 32 µg/ml for vancomycin by both agar dilution and E-strip methods.

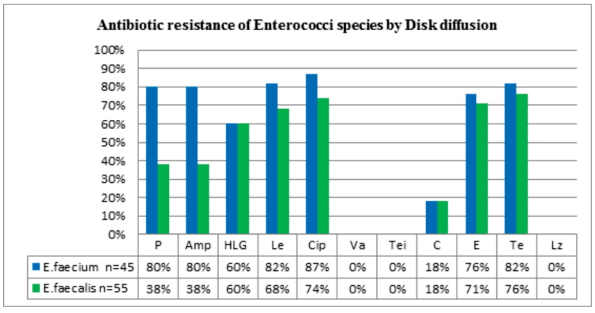

Figure1: Antimicrobial Resistance Pattern of *E. faecalis* and *E. faecium*

DISCUSSION

Enterococci are commensals of the human gut and are also less often seen as colonizers in the oral cavity, genitourinary tract and perianal area (10). Enterococci were previously considered low-grade pathogens, but recently,they gained importance because of changing trends in infections caused by different Enterococci species and the emergence of multidrug resistant strains (11). Most laboratories identify the organism only up to the genus level. Enterococci identification to the species level in clinical microbiology is important because some of the Enterococci species have intrinsic resistance to antibiotics, such as beta-lactams (cephalosporins) and low-level aminoglycosides. Species such as *E. gallinarum* and *E.casseliflavus* show intrinsic resistance to vancomycin (11). Hence, correct speciation can help to predict antibiotic resistance patterns and early treatment.

In the present study, Enterococcal infections were more common in the age groups of 21-60 years, followed by 61-80 years. Studies from Pondicherry (12), Jaipur(10) and Gujarat (13) showed higher infection rates in the 21-40 years age group. However, astudy by Modi et al from Ahmadabad showed that Enterococcal infections were more common in the 61 to 75year age group (14). These findings indicate that all age groups are at risk of acquiring enterococcal infections.

Our study showed a slight preponderance in male patients over female patients, but there was no statistically significant difference in the gender-wise distribution of Enterococci infections.

Isolation of Enterococci from various clinical samples showed that pus was the common source, followed by urine and blood samples, which was similar to the study conducted from Bangalore by Sreeja et al (15). However, this is in contrast to other studies, where the maximum numbers of isolates were from urine (10,16).

E. faecalis [55%] was the predominant species, followed by E. faecium[45%]; however, E. faecium was the common isolate from most of the clinical samples, such as urine, blood and peritoneal fluid, whereas E. faecalis was predominant only in pus samples. Other species of Enterococci were not isolated in this study, similar to the studies conducted from Bangalore (15), Chennai (17) and Davangere (18).

E. faecium showed 80% resistance to penicillin and ampicillin, whereas E. faecalis showed 38% resistance to penicillin and ampicillin. Both E. faecalis and E. faecium showed resistance to high-level gentamicin [60%], which makes the combination therapy of ampicillin and high-level gentamicin ineffective in treating serious enterococcal infections such as endocarditis.

In recent years, multidrug-resistant Enterococci have been reported to have an increasing trend worldwide. Most of the Enterococci isolated in our study also showed multidrug resistance, and E. faecium was more resistant to antimicrobial agents than E. faecalis. This could be due to changes in the patient population in the hospital and antimicrobial use, coupled with the greater antibiotic-resistant nature of *E. faecium, which* might have conferred a greater selective survival advantage compared to *E. faecalis* (19). Urinary isolates were less resistant to nitrofurantoin, whereas a study from Jaipur by Preeti et al showed 88% resistance to nitrofurantoin (10). All Enterococci isolates in our study were 100% sensitive to vancomycin, linezolid and teicoplanin by the disk diffusion method. However, one strain of E. faecium isolated from a blood sample of a term baby with severe sepsis showed vancomycin resistance by both agar dilution and E-strip methods with an MIC value of 32 µg/ml. Hence, there are chances of obtaining false sensitive results with the disk diffusion method, which can lead to inappropriate selection of vancomycin as a therapeutic option leading to treatment failure.

The VRE prevalence in our region is approximately 1%, which is much lower than that in other studies,whereVRE prevalence ranged from 10-24% (10,21). Linezolid is the drug of choice for VRE infections; however, recently, there have been reports of Enterococci developing resistance to linezolid, thus limiting therapeutic options (22).

CONCLUSION

Enterococci identification to the species level in clinical microbiology should be stressed in routine laboratory practice. Vancomycin resistance detection in Enterococci by the MIC method should be preferred. It helps reduce the morbidity and mortality due to VRE.

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