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Original Article

Microbiology Section

Change in the Prevalence and the Antibiotic Resistance of the Enterococcal Species Isolated from Blood Cultures

ANJANA TELKAR, BARAGUNDI .MAHESH.C., RAGHAVENDRA V. P., VISHWANATH. G., CHANDRAPPA .N .R.

ABSTRACT

Context : Blood stream infections are an important cause of mortality and morbidity. Enterococci have become increasingly resistant to a wide range of antimicrobial agents and enterococcal bacteraemia results in a high mortality of about 54%.

Aim : The present study was done to determine the prevalence of different enterococcal species in blood stream infections and their anti-microbial resistance, with a special reference to vancomycin and high level aminoglycoside resistance.

Material and methods : The study was conducted on 50 enterococcal blood isolates. The isolates were identified by using standard microbiological techniques and antimicrobial susceptibility testing was done by the Kirby Bauer disc diffusion method according to the CLSI guidelines.

Results : The maximum numbers of enterococci were isolated from male patients of the age group of 0-20 years. Most of the enterococcal isolates (78%) were non-haemolytic and multidrug resistant. *E.faecium* (70%) was the predominant species which was isolated. Linezolid showed good antienterococcal activity. Vancomycin resistance was seen in 6 (12%) isolates and high level aminoglycoside resistance was seen in 20 (40%) isolates. The *E.faecium* isolates were more drug resistant than the *E.faecalis* isolates.

Conclusion : Steps should be taken to regularly screen the enterococcal isolates for vancomycin and high level aminoglycoside resistance and to treat the enterococcal infections effectively to limit the spread of multidrug resistant enterococcal infections.

Key Words: Enterococcus, VRE, E.faecium, High level aminoglycoside resistance, Enterococcal bacteremia.

INTRODUCTION

Blood stream infections are an important cause of mortality and morbidity and they are among the most common health care associated infections [1]. The illnesses which are associated with blood stream infections range from self-limiting infections to life threatening sepsis, which require a rapid and aggressive antimicrobial treatment [2]. The enterococci have become major nosocomial pathogens [3]. The enterococci come well equipped with a variety of intrinsic (i.e. naturally occurring) antibiotic resistance. They are also capable of acquiring new resistance genes by gene transfer and/or mutations. The combination of high level resistance to ampicillin, vancomycin and aminoglycosides is now fairly common [4]. *Enterococcal bacteraemia* results in a high mortality of about 54% [5]. Although 12 species in the genus, Enterococcus have been recognized, the most common species which is implicated in human infections is *E.faecalis,* followed by *E.faecium* [6]. Hence, the present study was undertaken to know the prevalence of different enterococcal species in blood stream infections and their antimicrobial resistance, with a special reference to the vancomycin and high level *aminoglycoside* resistance which was seen at our teaching hospital.

MATERIALS AND METHODS

A total of 50 enterococci which were isolated from blood cultures were included in the study. The ethical standards which were laid down by the institutional committee on human experimentation were followed during the study.

The isolates were identified by their colony morphologies, Gram's staining patterns, catalase reactions, growth on bile esculin agar and tolerance to 6.5% NaCl. The species identification was done by using the standard microbiological techniques.

Anti-microbial sensitivity testing was done according to the CLSI guidelines [7] by the disc diffusion method of Kirby-Bauer by using Mueller Hinton (MH) agar. The various antibiotics which were tested were Ampicillin (10µg), Tetracycline (30µg), Erythromycin (15µg), Ciprofloxacin (5µg), Gentamicin (10µg), Vancomycin (30µg) and Linezolid (30µg). For high level aminoglycoside resistance detection, *Gentamicin* (120µg) and *Streptomycin* (300µg) discs were used.

The source of the anti-microbials was Hi-Media Ltd (Mumbai) India. The standard strains, *E.faecalis* ATCC 29212 and *E. faecalis* ATCC 51299 were used as the susceptible and resistant quality control strains.

RESULTS

A total of 50 enterococcal isolates were recovered from blood cultures. [Table/Fig-1] shows the age and sex distribution of the isolates. The maximum number of isolates (54%) were seen in the 0-20 years age group, followed by those in the 21-40 years age group. More number of enterococci were isolated from males (64%) than from

females (36%). [Table/Fig-2] shows the haemolytic activity of the enterococcal isolates. Most of isolates were non haemolytic and no isolate was β-haemolytic.

[Table/Fig-3] shows the resistance pattern of the enterococcal isolates. Only three species of enteococci, *E.faecium* (35), *E.faecalis* (14), *E.durans* (1) were isolated from blood cultures. All these three species were multidrug resistant and more than 50% resistance was seen to most of the drugs which were tested, except for Linezolid. Only one *E.faecium* isolate (2.8%) showed resistance to Linezolid.

[Table/Fig-4] shows the vancomycin and the high level aminoglycoside resistance among the enterococcal isolates. Vancomycin resistance was seen in 6(12%) of the enterococcal isolates. 34 (68%) isolates were high level gentamicin resistant and 28 (56%) were high level streptomycin resistant. Both high level gentamicin and streptomycin resistance was seen in 20 (40%) isolates. The *E.faecium* isolates were more resistant to all the drugs as compared to the *E.faecalis* isolates.

Age group (yrs.)	Sex		Total
	Male	Female	
0-20	20 (40%)	7 (14%)	27 (54%)
21-40	7 (14%)	8 (16%)	15 (30%)
41-60	0 (0%)	0 (0%)	0 (0%)
> 61	5 (10%)	3 (6%)	8 (16%)
Total	**32 (64%)**	**18 (36%)**	**50 (100%)**

[Table/Fig-1]: Age And Sex Distribution Of Isolates

Species (no)	Non hemolytic	α-heomolytic	β-hemolytic
E.faecium (35)	28 (80%)	7 (20%)	0(0%)
E.faecalis (14)	10 (71.42%)	4 (28.57%)	0(0%)
E.durans (1)	1 (100%)	0(0%)	0(0%)
Total (50)	**39 (78%)**	**11 (22%)**	**0(0%)**

[Table/Fig-2]: Hemolytic Activity Of Isolates

Antimicrobial agent	*E.faecium* No. (%)	*E.faecalis* No. (%)	*E.durans* No. (%)
Ampicillin	28 (80%)	9 (64.28%)	1 (100%)
Tetracycline	20 (57.14%)	7 (50%)	1 (100%)
Erythromycin	21 (60%)	7 (50%)	0(0%)
Ciprofloxacin	27 (77.14%)	10 (71.42%)	1 (100%)
Gentamycin	27 (77.14%)	10 (71.42%)	0(0%)
Linezolid	**1 (2.8%)**	**0(0%)**	**0(0%)**

[Table/Fig-3]: Anti-microbial Resistance Pattern Of Enterococcal Isolates (N=50)

Enterococcal species	No (%) of isolates resistant to			
	Vancomycin	HLGR	HLSR	HLGR + HLSR
E.faecium (35)	5 (14.28%)	25 (71.42%)	18(51.42%)	14 (40%)
E.faecalis (14)	1 (7.14%)	8 (57.14%)	9 (62.28%)	5 (35.71%)
E.durans (1)	0(0%)	1 (100%)	1 (100%)	1 (100%)
Total (50)	**6 (12%)**	**34 (68%)**	**28 (56%)**	**20 (40%)**

[Table/Fig-4]: Vancomycin And High Level Aminoglycoside Resistance Among Enterococcal Isolates

DISCUSSION

In the present study, we determined the species prevalence and the antimicrobial resistance pattern of the enterococcal isolates from blood cultures.

This study showed the highest number of enterococcal isolates in the 0-20 years age group and males were more effected than females. This could be due to the exposure of these groups to environmental conditions more than others or it may be due to some unexplained cause.

78% of the enterococcal isolates were non-haemolytic and α-haemolysis was seen in 22% of the isolates.

In the present study, 35 (70%) isolates were *E.faecium*, 14 (28%) were *E.faecalis* and 1 (2%) was *E.durans*. Earlier studies from various parts of India [8,9,10,11] had shown E.faecalis as the predominant species which was isolated from humans. A study on hospitalized patients from the United States reported *E.faecalis* to be two times more common than *E.faecium* among the blood culture isolates [12]. But recently, few studies have reported an increase in the number of the *E.faecium* isolates [11,13]. In the present study also, *E.faecium* was the major species which was isolated from the blood cultures. This could be attributed to the geographical variation in the distribution of the enterococcal species in different areas and the increasing drug resistance among the *E.faecium* isolates.

The present study revealed the presence of multidrug resistant enterococcal species. Similar results were obtained in various studies from different parts of world. [6,8,14,15,16], Drug resistance is rapidly acquired by enterococci by plasmids [17], conjugative transposition [18] or by mutations [19], leading to the rapid spread of multidrug resistant enterococcal infections.

The present study also revealed *E.faecium* to be more drug resistant than *E.faeclais*. Similar findings have been reported by other workers. Linezolid showed a good antienterococcal activity,[4,10] and this can be used as a second alternative for the vancomycin resistant enterococci.

Studies [20,21] have shown daptomycin to have a good activity against the enterococcal isolates, but in the present study, we did not test the sensitivity of daptomycin as it was not included in CLSI document.

In the present study, vancomycin resistance was seen in 6(12%) isolates. 7.14% of the vancomycin resistance was seen in *E.faecalis*, whereas 14.28% of the *E.faecium* isolates were found to be vancomycin resistant. Overall, vancomycin resistance was seen in 12% isolates. This was more in comparison with the findings of other Indian studies [8,9,10,18]. which showed between 0-5% vancomyin resistance in the enterococcal isolates. Thus, the present study indicated an increase in the vancomycin resistance of the enterococcal isolates. The vancomycin resistance in enterococci not only leaves fewer options for the disease management, but it is also important due to the potential risk of the vancomycin resistance gene transfer from the enterococci to *Staphylococcus aureus* [6].

A high level of aminoglycoside resistance (HLGR+HLSR) was seen in 20 (40%) isolates and again, this was more in the *E.faecium*

isolates (40%) as compared to that in the *E.faecalis* isolates (35.71%). Our results were comparable to the results of other studies [8,14,15]. The resistance to aminoglycosides is of great concern, since it eliminates the synergy of the aminoglycosides with the β-lactam antibiotics, which is the therapy of choice for the enterococcal infections, thus limiting the therapeutic options.

In conclusion, *E.faecium* was found to be the predominant isolate in enterococcal bacteraemia. Most of the enterococcal isolates were multidrug resistant. Vancomycin and high level aminoglycoside resistance is on the rise in enterococcal isolates. Linezolid has a good antienterococcal activity. Steps should be taken to regularly screen the enterococcal isolates for vancomycin and high level aminoglycoside resistance and to treat the enterococcal infections effectively, to limit the spread of multidrug resistant enterococcal infections.

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AUTHOR(S):

1. Dr. Anjana Telkar
2. Dr. Baragundi .Mahesh.C.
3. Dr. Raghavendra V. P.
4. Dr. Vishwanath.G.
5. Dr. Chandrappa .N .R.

PARTICULARS OF CONTRIBUTORS:

1. Corresponding Author
2. Associate Professor, Dept. of Microbiology, S.N. Medical College, Bagalkot, Karnataka, India.
3. Assistant Professor, Dept. of Anatomy, J.J.M. Medical College, Davanagere, Karnataka, India.
4. Professor, Dept. of Microbiology, J.J.M. Medical College, Davanagere, Karnataka, India.
5. Professor, Dept. of Microbiology, J.J.M. Medical College, Davanagere, Karnataka, India.

NAME, ADDRESS, TELEPHONE, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Anjana Telkar,
Assistant Professor, Dept. of microbiology,
J.J.M. Medical College, Davanagere
Karnataka – Pin 577004, India.
Phone: 9844759777 / 9844759777
E-mail: dranjanarpisale@yahoo.com

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