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Research Article

ISOLATION AND SPECIATION OF ENTEROCOCCI FROM VARIOUS CLINICAL SAMPLES AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PATTERN WITH SPECIAL REFERENCE TO HIGH LEVEL AMINOGLYCOSIDE RESISTANCE

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ABSTRACT

Background and Objectives: *Enterococci* are important nosocomial agents and strains resistant to penicillin and other antibiotics occur frequently. *Enterococci* are intrinsically resistant to cephalosporins and offer low level resistance to aminoglycosides. In penicillin sensitive strains, synergism occurs with combination treatment with penicillin and aminoglycoside. Serious infections caused by them are treated with penicillin and aminoglycoside combination. But the synergistic effect is lost, when the strain develops high level aminoglycoside resistance. The choice of drug for infections due to such strains is vancomycin. The present study was carried out to isolate and speciate *Enterococci* from various clinical samples, to know the susceptibility pattern of the isolates, to determine the High Level Aminoglycoside Resistance (HLAR) among *Enterococcal* isolates. **Methods:** A total of One hundred *Enterococcal* species isolated from various clinical samples were identified by various biochemical reactions. Antimicrobial susceptibility testing and HLAR were determined by Kirby- Bauer disc diffusion method. **Results:** Out of 100 *Enterococcal* isolates, 59 were *E. faecalis*, 38 were *E. faecium*, 3 were other *Enterococcal* species. Among these 53 isolates showed High Level Aminoglycoside Resistance. **Conclusion:** Present study shows the presence of drug resistance to most of commonly used antibiotics and HLAR is also more in *E. faecium* compared to *E. faecalis*.

Keywords: *Enterococci*, High level aminoglycoside resistance.

INTRODUCTION

The Genus *Enterococcus* consists of Gram positive, aerobic and facultative anaerobic organisms that are oval in shape and may appear on smears in pairs, as singles or short chains. *E. faecalis* is the most common isolate, being associated with 80-90 % of human *Enterococcal* infections¹

Enterococcus species cause urinary tract infections, bacteremia, endocarditis, intraabdominal and pelvic infections, wound and soft tissue infections.² High level aminoglycoside resistance, glycopeptides resistance and beta lactamase production in

Enterococci causing treatment difficulties in hospitals.³

Drug resistant *Enterococci* are due to indiscriminate use of antibiotics, diabetes mellitus, prolonged hospital stay and immunocompromised states.³ *Enterococci* are intrinsically resistant to cephalosporins and also low level aminoglycoside resistance. Infections due to *Enterococci* are treated with penicillin and aminoglycoside. This synergism is lost if the strain develops high level aminoglycoside resistance.⁴ The present study was done to know the antimicrobial susceptibility including HLAR detection in various *Enterococci* species.

MATERIALS AND METHODS

The present study was done in the department of Microbiology, Dr.B.R.Ambedkar Medical College,Bangalore, over a period of one year and four months from September 2012 to December 2013.A total of 100 *Enterococci* isolates from various clinical samples (urine, pus, wound swabs, blood and other body fluids) from both OPD and IPD (Medicine,Surgery,OBG,Paediatrics Departments) were included in the study. Urine samples were inoculated on Cysteine Lactose Electrolyte Deficient (CLED) medium.⁵ Blood samples were processed in blood culture bottles containing glucose broth and the remaining clinical specimens were processed on blood agar and MacConkey's agar. All plates were incubated aerobically at 37°C for 24-48 h and examined for microbial growth. *Enterococci* were identified using standard methods.¹ Based on colony morphology, Gram staining, catalase reaction, bile esculin test, growth in 6.5% NaCl and sugar fermentation reactions. ¹ Isolates were identified by standard biochemical tests.¹

Antimicrobial sensitivity testing was done on Muller-Hinton agar by standard disc diffusion methods as per Clinical Laboratory Standards Institute (CLSI) guidelines.⁶

The antibiotics tested were as follows: Penicillin (10U), Ampicillin (10ug),Ciprofloxacin (5ug), Vancomycin (30ug),Linezolid (30ug)and Tetracycline (30ug).

Quality control :*E. faecalis* ATCC 29212 was used . All the clinical Isolates were detected for HLAR as per CLSI guidelines using high content Gentamicin (120ug) and high content Streptomycin (300ug) discs. A zone of inhibition <6mm indicated as resistant, 7-9 mm inconclusive, >10mm as sensitive.⁶

RESULTS

Of the 100 samples, 61 were males and 39 were females. Various *Enterococcal* species isolated were *E. faecalis* (59), *E.faecium* (38), *E.dispar* (02) and *E.durans* (01).

E.faecium isolates were more resistant to various antibiotics-Penicillin(52%), Ampicillin (58%), Ciprofloxacin(82%), Vancomycin (05%),Linezolid(03%) and Tetracycline(62%).*E.faecalis*were resistant to Penicillin (48%), Ampicillin (40%), Ciprofloxacin (70%), Vancomycin (02%), Linezolid (02%) and Tetracycline (55%).

HLAR was detected in 53% of isolates. HLAR among *E. faecium* isolates (58%) were higher than*E.fecalis* (48%). High level resistance to gentamicin and streptomycin among *E. fecalis* strains were 56% and 40% respectively. High level resistance to gentamicin and streptomycin among *E.faecium* strains were 68% and 48% respectively. Combined resistance to both aminoglycosides was slightly higher in *E. faecium* (58%) isolates as compared with *E. fecalis* (48%).

Table 1: Details of type of specimens from which isolates were obtained

Sr. no.	Specimen(n=100)	<i>E. faecalis</i> (%)	<i>E.faecium</i> (%)	<i>E.dispar</i> (%)	<i>E.durans</i> (%)
1	Urine	38	22	01	01
2	Pus	10	08	01	-
3	Sputum	06	05	-	-
4	Blood	05	03	-	-
5	Total	59	38	02	01

Table 2: Resistance pattern of*E.faecium*

Sr. no.	Specimen(n=38)	Penicillin (%)	Ampicillin (%)	Ciprofloxacin (%)	Vancomycin (%)	Linezolid (%)	Tetracycline (%)
1	Urine	34	40	65	03	01	48
2	Pus	09	08	10	02	01	08
3	Sputum	05	05	04	-	-	03
4	Blood	04	05	03	-	01	03
5	Total	52	58	82	05	03	62

Table 3: Resistance pattern of *E. faecalis*

Sr. no.	Specimen(n=59)	Penicillin (%)	Ampicillin (%)	Ciprofloxacin (%)	Vancomycin (%)	Linezolid (%)	Tetracycline (%)
1	Urine	32	33	54	01	01	44
2	Pus	08	04	11	-	01	05
3	Sputum	04	02	02	01	-	03
4	Blood	04	01	03	-	-	03
5	Total	48	40	70	02	02	55

Table 4: HLAR pattern

Sr. no.	Specimen (n=100)	<i>E. faecium</i> (%)	<i>E. faecalis</i> (%)
1	Urine	45	38
2	Pus	08	06
3	Sputum	02	02
4	Blood	03	02
5	Total	58	48

DISCUSSION

Enterococci are the second most common cause of nosocomial urinary tract and wound infections and third most common cause of nosocomial bacteremias. Because of their resistance to penicillin and cephalosporins of several generations, the acquisition of high level aminoglycoside resistance and now the emergency of vancomycin resistance, these organisms are involved in serious super infections in patients receiving broad spectrum antimicrobial therapy.¹ So it is essential to know the susceptibility pattern of these organisms.

We isolated *E. faecalis* more than that of *E. faecium*. The same results were obtained by Mendiratta DK et al.⁷, Bhat KG et al.⁸ and Gupta et al.⁹ High level aminoglycoside resistance *Enterococci* were first reported in France in 1979 and then have been isolated from all the continents.¹⁰ Our study showed *E. faecium* isolates were more drug resistant compared to *E. faecalis*. This is comparable to the results reported by Anjana Telkaret al.¹¹

In our study majority of the *Enterococcal* isolates were resistant to tetracycline, and ciprofloxacin, which is comparable to the study conducted by Anjana Telkar et al.¹¹

Overall, resistance to penicillin, ampicillin and ciprofloxacin among strains of *E. faecium* is high. Linezolid showed a good sensitivity towards *Enterococci* species, and this can be used as an alternative for the vancomycin resistant *Enterococci*.

In our study *E. faecium* isolates were multi drug resistant as compared to *E. faecalis*, which is comparable to the results reported by Mendiratta et al.⁷ and Bhat KG et al.⁸ Vancomycin resistance detected in 7% of the isolates. Similar results were reported by Bhat KG et al.⁸

In our study HLGR is more in *E. faecium* isolates (68%) compared to *E. faecalis* (56%) strains. Also HLSR is more in *E. faecium* (48%) than in *E. faecalis* (40%). The same results were reported by Mendiratta et al.⁷ and Gupta V et al.⁹ So high percentages of HLAR could nullify efficacy of combination therapy of Beta lactamase, aminoglycosides recommended for the treatment of serious *Enterococcal* infections. Karmarkaret al.¹² also reported greater resistance to vancomycin among *E. faecium*.

The higher antimicrobial resistance rates in the present study may be ascribed to the source of the isolates being from a tertiary care set up and a wider usage of broad spectrum antibiotics.

CONCLUSION

In our study multidrug resistant and HLAR is more in *Enterococcal* isolates. It is essential to screen for the multidrug resistant and HLAR in clinical samples. So proper antibiotic policy and hospital infection control measures can be initiated to prevent the emergence of multidrug resistant strains.

Conflict of interest: Nil

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