

Original Research Article

Characterization, speciation and antimicrobial resistance pattern of *Enterococcus* species isolated from clinical specimens at a rural tertiary care hospital

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ABSTRACT

Background: *Enterococci* have attracted much attention in recent years due to their increased recognition as a cause of nosocomial infection. They exhibit vancomycin resistance with increasing frequency so that determination of antimicrobial susceptibility pattern is necessary. Aim of present study is to determine the prevalence and susceptibility pattern of *Enterococci* in rural tertiary care hospital.

Methods: Total of 100 *Enterococcus* isolated from various clinical samples were identified and speciated by Vitek®2 compact system (BIOMERIEUX). Antibiotic susceptibility was determined by modified Kirby Bauer disc diffusion method. Results were interpreted as per CLSI guidelines and vancomycin MIC was determined by E-test method.

Results: Out of 100 *Enterococcus* strains 47 were *E. faecalis*, 51 were *E. faecium*, 02 were *E. gallinarum* and 01 was *E. casseliflavus*. Resistance to penicillin, ampicillin, ciprofloxacin, elevated level gentamycin and high-level streptomycin were observed. All isolates were sensitive to linezolid and teicoplanin, 6% strains showed vancomycin resistance by E-test.

Conclusions: High rate of resistance to penicillin, ciprofloxacin and aminoglycosides is observed and emergence of VRE has further worsened this situation. So, there is an urgent need for more rational use of antimicrobials and infection control.

Keywords: *Enterococcus*, E-test, Vitek®2 compact system, VRE

INTRODUCTION

Enterococci are normal commensals of the intestinal tract of humans and animals.¹ Genus *Enterococcus* mostly *E. faecalis* and *E. faecium* cause urinary tract infections (UTI), bacteraemia, intraabdominal infections, and endocarditis. *Enterococci* are now the leading cause of surgical site infection, rank second only to *Staphylococci* as a cause of hospital acquired bacteraemia and rank as the third leading cause of nosocomial UTI. *Enterococci*

are also responsible for 5 to 20% of community acquired endocarditis.²

Enterococci have attracted much attention in recent years due to their increased recognition as a cause of nosocomial infection in patients receiving antimicrobial agents. Serious enterococcal infections are often resistant to antibiotics and mortality is also high.³

By Utley et al, vancomycin resistant enterococcal infections have been a cause of great concern among the

health professionals.⁴ Therefore, VRE along with HLAR is making the treatment of such infections extremely difficult and pose a great challenge to clinicians.

A study is therefore essential to identify *Enterococcal* species causing various infections, and to determine their antimicrobial resistance pattern.

METHODS

The present study was conducted from Jan 2015 to June 2016, in the department of microbiology, UP University of Medical Sciences Saifai, Etawah. Ethical clearance for the study was taken from ethical committee of the institute.

A total of 100 enterococcal strains were isolated from various clinical samples viz. urine, blood, pus, pleural fluid, CSF, high vaginal swab, ascetic fluid, etc. (*Enterococci* isolated from stool samples were excluded) and were identified and speciated by Vitek®2 compact system (BIOMERIEUX). All isolates were stocked in for further testing.

Antibiotic susceptibility testing was done by modified Kirby Bauer disc diffusion method as per CLSI guidelines. Antibiotic discs (Himedia) of penicillin (10U/disc), ampicillin (10µg), elevated level gentamicin (120µg), elevated level streptomycin (300µg), Ciprofloxacin (5µg), vancomycin (30µg), teicoplanin (30µg), and linezolid (30µg) were used.

The minimum inhibitory concentrations of vancomycin were determined by E-test (Himedia) method. *E. faecalis* ATCC 29212 and *E. faecalis* ATCC 51299 were included as a quality control strain.

RESULTS

From various clinical specimens, 100 *Enterococci* were isolated and identified up to species level by phenotypic characters and confirmed by Vitek®2 compact system (BIOMERIEUX). Out of 100 *Enterococci* 75 were from urine, 14 from blood, 03 from pus, 03 from vaginal swab and 05 from other sample pleural fluid, sputum etc. Majority of isolates were *E. faecium* 50% and *E. faecalis* 47%.

Table 1: Species distribution of *Enterococci* in various clinical specimen.

Sample	Total no. of <i>Enterococci</i>	<i>E. faecalis</i>	<i>E. faecium</i>	<i>E. gallinarum</i>	<i>E. casseliflavus</i>
Urine	75	41	34	-	-
Blood	14	2	10	2	-
Pus	3	1	2	-	1
Vaginal swab	3	1	2	-	-
Another sample	5	2	3	-	-
Total	100	47	51	2	1

Out of 75 *Enterococci* isolated from urine 41 were *E. faecalis*, 34 were *E. faecium*. Of the 14 *Enterococci* isolated from blood sample 10 were *E. faecium*, 02 were *E. faecalis* and 02 were *E. gallinarum*. All isolates of

blood were from paediatric ward. 11 isolates were recovered from other samples like pus, vaginal swab and other body fluids. Out of which 04 were *E. faecalis*, 07 were *E. faecium* and 01 was *E. casseliflavus* (Table 1).

Table 2: Antimicrobial sensitivity pattern of the *Enterococcus* isolates.

Antibiotics	% of sensitive isolates	% of intermediate sensitive isolates	% of resistant isolate
Ampicillin (10µg)	35	2	63
Ciprofloxacin (5µg)	18	1	81
High strength gentamicin (120µg)	42	00	58
High strength streptomycin (300 µg)	64	00	36
Penicillin (10µg)	47	00	53
Vancomycin (30µg)	98	01	01
Linezolid (30µg)	100	00	00
Teicoplanin (30µg)	100	00	00
Nitrofurantoin (300 µg)	74	03	23

High-level gentamycin and high-level Streptomycin resistance were 58% and 36% respectively by disk diffusion method. All isolates were sensitive to linezolid and teicoplanin by disk diffusion. Only 01 isolate speciated as *E. faecium* was resistant to vancomycin by disk diffusion method (Table 2).

Table 3: MIC of vancomycin resistant *Enterococci* isolated in the present study.

Isolate no.	Source	Species	MIC ($\mu\text{g/ml}$) E-test
1	Urine	<i>E. faecium</i>	128
2	Pus	<i>E. casseliflavus</i>	16
3	Blood	<i>E. gallinarum</i>	8
4	Blood	<i>E. gallinarum</i>	8
5	Pus	<i>E. faecalis</i>	8
6	Urine	<i>E. faecium</i>	8

Only 01 isolate speciated as *E. faecium* and 01 isolate speciated as *E. casseliflavus* showed resistance to Vancomycin MIC ≥ 128 and $16\mu\text{g/ml}$ respectively by E-test method isolated from chronically ill patients admitted in ICU. 04 isolates showed MIC $\geq 8\mu\text{g/ml}$ by E-test in which 02 isolate speciated as *E. gallinarum* isolated from IPD patients but not chronically ill. All isolates were sensitive to linezolid and teicoplanin leaving these two drugs as treatment option. (Table 3).

DISCUSSION

Over the last few years, *Enterococcus* has become important nosocomial pathogen due to inherent resistance to antibiotics (cephalosporins), ability to adhere to indwelling medical devices, and ability to survive in adverse environmental conditions. Antimicrobial resistance in *Enterococcus* has been increasing mainly in hospitalized patients.⁵

In present study, most common species isolated was *E. faecium*. In a study by Mendirata DK et al and few studies from north India S. Jain et al Mumbai Karmarkar and South India Telkar et al also found *E. faecium* as predominant species.⁶⁻⁹ In the study isolation rate of *E. faecium* were higher than *E. faecalis* as compare to other studies this may be due to increasing incidence of *E. faecium* infection in hospitalized patients in present circumstances.

In this study isolates were highest from urine 75%, followed by blood and pus. Most of the studies done on *Enterococci* support the same findings as *Enterococci* is identified as one of the most frequent uropathogen.

Beta-lactams along with aminoglycosides are considered as treatment of choice. Therefore, resistance of *Enterococci* against these antibiotics has important clinical implications. Present study showed 53% and 63% resistance to penicillin and ampicillin respectively.

Resistance to penicillin may be due to low affinity penicillin binding proteins or due to production of β -lactamases. In current study, HLAR was seen in 58% of the strains for gentamicin (elevated level) and 36% for streptomycin (elevated level). HLAR was more in *E. faecium* than *E. faecalis*. These finding also reported in some study.^{10,11}

The most recent and important resistance in *Enterococci* is vancomycin resistance and it is being increasingly reported from all parts of the world. In this study 6% the isolates were VRE (1% showed elevated level resistance and 5% showed low level resistance for vancomycin), these strains were isolated from urine, blood and pus specimens in chronically ill and immunocompromised patient admitted in the ICU. This result was comparable with the results of Descheemaeker et al and Salem-Bekhit, et al.¹²⁻¹³

All isolates (100%) were sensitive to linezolid and teicoplanin. All VRE isolates were sensitive to linezolid that is why linezolid can be considered as drug of choice to treat infections with VRE, however resistance to it has been reported in many studies.^{14,15} Hence, its judicious use is recommended.

CONCLUSION

High rate of resistance to penicillin, ciprofloxacin and aminoglycosides is observed in tertiary care hospital and emergence of VRE strains has further worsened this situation. Thus, we suggest more rational use of antibiotics and infection control in our health care settings.

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