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ISOLATION, IDENTIFICATION AND SPECIATION OF ENTEROCOCCI AND THEIR ANTIMICROBIAL SUSCEPTIBILITY IN A TERTIARY CARE HOSPITAL

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ABSTRACT: BACKGROUND: Enterococci have emerged as the second most common cause of nosocomial infections over the past 2-3 decades and antibiotic resistance is a major obstacle for treatment. Identification of enterococci to species level is helpful and crucial for proper patient treatment, epidemiologic and infection control purposes. The emergence of vancomycin resistant enterococci is of particular concern as it has limited the therapeutic options available for the clinicians. **AIM:** To speculate and determine the antimicrobial susceptibility of clinical isolates of enterococci with special reference to vancomycin. **SETTINGS AND DESIGN:** A prospective descriptive study was carried out in Rajarajeswari medical college and hospital, Bangalore. **MATERIAL AND METHODS:** The study included a total of 90 clinical isolates of enterococcus from urine, pus, blood and endotracheal aspirates over a period of one year from November 2012 to October 2013. The isolates were confirmed to belong to genus *Enterococcus* and speciation was based on potassium tellurite (0.04 %) reduction, arginine deamination and fermentation of arabinose, mannitol, raffinose, and sorbitol. Antimicrobial susceptibility was determined by Kirby Bauer disk diffusion and MIC of vancomycin was determined by agar dilution method according to CLSI guidelines. **STATISTICAL ANALYSIS:** p value was calculated by Fisher's exact test. **RESULTS:** Amongst the total 90 Enterococcal isolates, 76 isolates (84.5 %) were *Enterococcus faecalis*, 13 isolates (14.4 %) were *Enterococcus faecium* and 1 isolate (1.1 %) was *Enterococcus raffinosus*. Out of the 90 isolates, 65 (72.2 %) were from urine, 15 (16.6 %) from pus, 5 (5.6 %) from blood and 5 (5.6 %) from endotracheal aspirates. High level gentamicin resistance was 22.4 % for *E. faecalis* and 30.8 % for *E. faecium* by Kirby Bauer disc diffusion method. Occurrence of vancomycin resistant enterococci (VRE) in our setting is 2.2 %. Isolates had 100 % sensitivity to linezolid. **CONCLUSION:** *Enterococcus faecalis* was the most common species. Maximum isolates were from urine samples. Higher level of gentamicin resistance could lead to failure of synergistic therapy. Speciation and regular monitoring for antibiotic resistance with special attention to vancomycin and high level aminoglycosides is warranted.

KEYWORDS: Enterococci, speciation, antimicrobial resistance, high level gentamicin, vancomycin, MIC.

INTRODUCTION: Enterococci have evolved over the past century, from being intestinal commensals to becoming pathogens associated with significant morbidity and mortality.¹

Enterococcus species cause urinary tract infections, bacteremia, endocarditis, intraabdominal and pelvic infections, wound and soft tissue infections, neonatal sepsis and rarely meningitis.²

Enterococcus faecalis and *Enterococcus faecium* are the most common species accounting for 90- 95 % of the clinical isolates and the other species *E. gallinarum*.

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E. casseliflavus, *E. durans*, *E. avium*, *E. hirae*, *E. mundtii* and *E. raffinosus* account for 5 % of the clinical isolates.³ Identification of enterococci to species level is helpful and crucial for proper patient treatment, epidemiologic and infection control purposes. Isolates of *E. faecium* tend to be more resistant to penicillin and ampicillin than *E. faecalis* isolates. Majority of vancomycin resistant enterococci are strains of *E. faecium*.²

Enterococci have emerged as second most common cause of nosocomial infections over the past 2-3 decades and antibiotic resistance is a major obstacle for treatment. The organisms display intrinsic resistance to beta lactam agents and low level aminoglycosides. Penicillin and aminoglycoside synergy has been used for treating enterococcal infections. But acquisition of high level resistance to aminoglycosides has made the therapeutic combination of penicillin and aminoglycosides ineffective.

As a result, vancomycin became a first-line drug effective against enterococcal infections. However, in recent years, there has been a rapid increase in the incidence of infection and colonisation of patients by enterococci with vancomycin resistance. The emergence of vancomycin resistant enterococci is of particular concern as it has limited the therapeutic options available for the clinicians.^{1,2,4,5} Moreover, enterococci act as reservoirs of these antibiotic resistance genes and tends to transfer these to other enterococcus species and other bacteria including methicillin-resistant *Staphylococcus aureus*.⁶

Hence in the present study, speciation and determination of the antimicrobial susceptibility of clinical isolates of enterococci with special reference to vancomycin was carried out.

MATERIAL AND METHODS: The study was a prospective, descriptive study carried out in Rajarajeswari medical college and hospital, Bangalore after institutional ethical committee approval. A total of 90 clinical isolates of enterococcus from urine, pus, blood and endotracheal aspirates were included in the study over a period of one year from November 2012 to October 2013.

The isolates were confirmed to belong to genus *Enterococcus* by colony morphology on Mac conkey and blood agar, grams stain, catalase test, bile - esculin hydrolysis. Speciation was based on potassium tellurite (0.04 %) reduction, arginine deamination and fermentation of arabinose, mannitol, raffinose and sorbitol.^{7,8}

Antimicrobial susceptibility to ampicillin, penicillin, vancomycin, teicoplanin, linezolid, ciprofloxacin, high level gentamicin and nitrofurantoin was determined by Kirby Bauer disk diffusion according to CLSI guidelines.⁹ MIC of vancomycin was determined by agar dilution method. *E. faecalis* ATCC 29212 was used as a sensitive ATCC control.¹⁰

RESULTS: Amongst the total 90 enterococcal isolates, 76 isolates (84.5 %) were *Enterococcus faecalis*, 13 isolates (14.4 %) were *Enterococcus faecium* and 1 isolate (1.1 %) was *Enterococcus raffinosus*. *E. faecalis* was the predominant species. (Figure-1)

- Out of the 90 isolates, maximum were from urine samples accounting to 65 isolates (72.2 %) followed by 15 (16.6 %) from pus, 5 (5.6 %) from blood and 5 (5.6 %) from endotracheal aspirates. (Figure-2)
- Amongst the 65 urinary isolates, 57 (87.7 %) were *E. faecalis*, 7 (10.8 %) were *E. faecium*, 1 (1.5 %) was *E. raffinosus*.
- Out of 15 pus isolates 13 (86.7 %) were *E. faecalis* and 2 (13.3 %) were *E. faecium*.

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- Amongst 5 blood isolates 3 (60 %) were *E. faecalis* and 2 (40 %) were *E. faecium*.
- Out of 5 endotracheal aspirates 3 (60 %) were *E. faecalis* and 2 (40 %) were *E. faecium*.

Antimicrobial susceptibility of isolates-

- 38.2 % of the *E. faecalis* isolates were resistant to ampicillin, 44.7 % to penicillin, 22.4 % to high level gentamicin, 43.4 % to ciprofloxacin, 12.3 % to nitrofurantoin, 3.9 % to vancomycin and 2.6 % to teicoplanin by Kirby Bauer disc diffusion method. (Table-1)
- 46.2 % of the *E. faecium* isolates were resistant to ampicillin, 53.8 % to penicillin, 30.8 % to high level gentamicin, 61.5 % to ciprofloxacin, 14.3 % to nitrofurantoin by Kirby Bauer disc diffusion method. (Table-2)
- The isolate of *E. raffinosus* was sensitive to all the antimicrobial agents tested.
- All isolates were sensitive to linezolid.
- Out of a total of 90 enterococcal isolates, two were resistant to vancomycin by both Kirby Bauer disc diffusion and MIC by agar dilution method accounting to 2.2 %.

DISCUSSION: Enterococci are part of the normal intestinal flora. Prior to identification of multiple-antibiotic resistant strains in the late 1970s, enterococci were considered relatively innocuous organisms. Now, Enterococci have been identified as the agents of nosocomial infection with antimicrobial resistance to most currently approved agents. The emergence of vancomycin-resistant enterococci (VRE) has alarmed the global infectious diseases community.⁴

- In our study, *E. faecalis* was the predominant species correlating with other studies.^{3,11,12}
- Maximum number of isolates was from urine which correlates with other studies.^{3,12,13}
- 38.2 % and 44.7 % of *E. faecalis* isolates were resistant to ampicillin and penicillin respectively in our study (Table-1) correlating with the study done by Shrihari et al.³
- Amongst *E. faecium* isolates, 46.2 % were resistant to ampicillin and 53.8 % were resistant to penicillin (Table-2) correlating with study done by Golia et al.¹⁴
- High level gentamicin resistance accounted to 22.4 % and 30.8 % for *E. faecalis* and *E. faecium* respectively by Kirby Bauer disc diffusion method (Table 1&2). Higher resistance to gentamicin leads to the failure of synergistic combination therapy with gentamicin and beta-lactam antibiotics/glycopeptides for serious infections. This correlates with the observation of other studies.^{3,12,15}

Out of the 3 isolates resistant to vancomycin by Kirby Bauer disc diffusion method, one was sensitive by agar dilution method with MIC of 2 µg/ml whereas the other two were resistant with MIC of 64 µg/ml and as high as 128 µg/ml. The 7 isolates which were intermediately sensitive by Kirby Bauer disc diffusion were found to be sensitive by agar dilution method with MIC values less than 4 µg/ml (Table-3) thus suggesting the importance of supplementing Kirby Bauer disc diffusion test with determination of MIC by dilution methods (Fisher's exact test $p < 0.001$) for detection of vancomycin resistance in the microbiology laboratory. Similar observation and suggestion has been made by Rahangadale et al.¹¹ CLSI also recommends MIC determination by dilution methods for detection of vancomycin resistance in enterococci.¹⁶

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Though vancomycin resistance is more common in *E. faecium*², the present study showed that the 2 isolates which were resistant to vancomycin both by disc diffusion and MIC by agar dilution were *E. faecalis*.

Occurrence of VRE has varied between 1 % to 21 % in various studies.^{11,12,17} In our study also, the occurrence of VRE accounted to 2.2 %. Regular monitoring for vancomycin resistant enterococci is warranted, especially because they act as reservoirs of resistance genes and tend to transfer these to other Enterococcus species and other bacteria, including methicillin-resistant *Staphylococcus aureus*.⁶

All isolates were sensitive to linezolid.

CONCLUSION:

- *Enterococcus faecalis* was the most common species.
- Maximum isolates were from urine samples.
- Higher level of gentamicin resistance could lead to the failure of efficacy of synergistic combination therapy with gentamicin and beta-lactam antibiotics or glycopeptides.
- Speciation of the enterococcal isolates and regular monitoring for antibiotic resistance with special attention to vancomycin and high level aminoglycosides is warranted.

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Antibiotic	Number of isolates-Sensitive (%)	Number of isolates-Intermediately sensitive (%)	Number of isolates-Resistant (%)
Ampicillin (10 µg)	45 (59.2 %)	2 (2.6 %)	29 (38.2 %)
Penicillin (10 U)	39 (51.3 %)	3 (4 %)	34 (44.7 %)
Gentamicin (120 µg)	55 (72.4 %)	4 (5.2 %)	17 (22.4 %)
Ciprofloxacin (5 µg)	38 (50 %)	5 (6.6 %)	33 (43.4 %)
Nitrofurantoin (300 µg)*	47 (82.4 %)	3 (5.3 %)	7 (12.3 %)
Vancomycin (30 µg)	68 (89.5 %)	5 (6.6 %)	3 (3.9 %)
Teicoplanin (30 µg)	74 (97.4 %)	0 (0 %)	2 (2.6 %)
Linezolid (30 µg)	76 (100 %)	0 (0 %)	0 (0 %)

Table 1: Antimicrobial susceptibility pattern of *E. faecalis* (76 isolates)

*Nitrofurantoin was used for urine isolates (57 isolates).

Note: Resistance to high level gentamicin is 22.4 %.

Antibiotic	Number of isolates – Sensitive (%)	Number of isolates-Intermediately sensitive (%)	Number of isolates-Resistant (%)
Ampicillin (10 µg)	6 (46.2 %)	1 (7.6 %)	6 (46.2 %)s
Penicillin (10 U)	6 (46.2 %)	0 (0 %)	7 (53.8 %)

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Gentamicin (120 µg)	9 (69.2 %)	0 (0 %)	4 (30.8 %)
Ciprofloxacin (5 µg)	5 (38.5 %)	0 (0 %)	8 (61.5 %)
Nitrofurantoin (300 µg)*	6 (85.7 %)	0 (0 %)	1 (14.3 %)
Vancomycin (30 µg)	11 (84.6 %)	2 (15.4 %)	0 (0 %)
Teicoplanin (30 µg)	13 (100 %)	0 (0 %)	0 (0 %)
Linezolid (30 µg)	13 (100 %)	0 (0 %)	0 (0 %)

Table 2: Antimicrobial susceptibility pattern of *E. faecium* (13 isolates)

*Nitrofurantoin was used for urine isolates (7 isolates)

Note: Resistance to high level gentamicin is 30.8 %

Isolate	Kirby Bauer disc diffusion method		Agar dilution method	
	Zone of inhibition (mm)	Interpretation	MIC (µg/ml)	Interpretation
1	15	I	2	S
2	15	I	1	S
3	16	I	1	S
4	15	I	2	S
5	16	I	1	S
6	15	I	1	S
7	16	I	2	S
8	13	R	2	S
9	12	R	64	R
10	7	R	As high as 128	R

Table 3: Comparison of enterococcal isolates showing intermediate sensitivity or resistance to vancomycin by Kirby Bauer disc diffusion method with MIC by agar dilution method.

Note: Kirby Bauer disc diffusion method: Sensitive ≥ 17 mm, Intermediately sensitive 15-16 mm, Resistant ≤ 14 mm. Agar dilution method: MIC ≤ 4 µg/ml Sensitive, 8 to 16 µg/ml Intermediate, ≥ 32 µg/ml Resistant. S = Sensitive, I = Intermediate, R = Resistant

Note: Confirmation with MIC by agar dilution test showed that all 7 isolates that were intermediately sensitive by Kirby Bauer disc diffusion were sensitive with MIC ≤ 2 µg/ml and out of 3 isolates that were resistant, 1 isolate was sensitive with MIC 2 µg/ml (Fisher's exact test, p value < 0.001) suggesting that determination of MIC is important for detecting VRE.

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Note: *Enterococcus faecalis* is the predominant species.

Note: Maximum isolates are from urine.

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