

Original Research Article

Detection of high-level aminoglycoside resistance by disc diffusion and e-test amongst the enterococcus species isolated from various clinical samples in a tertiary care hospital

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ABSTRACT

Background: The emergence of *Enterococcus* species in causing nosocomial infections poses a therapeutic challenge to clinicians. Enterococci are intrinsically resistance to multiple antibiotics. Acquired resistance to commonly used antibiotics like Ampicillin, Vancomycin and Aminoglycosides have made the situation worse and difficult to treat serious Enterococcal infections. The present study aimed at detection of high-level aminoglycoside resistance by disc diffusion and E-test amongst the *Enterococcus* species isolated from various clinical samples in a tertiary care hospital.

Methods: A total of 102 *Enterococcus* species isolated from various clinical samples and antimicrobial susceptibility was performed by Kirby Bauer disc diffusion method as per CLSI guidelines. E-test was done for all high level aminoglycoside resistance *Enterococcus* species isolated by disc diffusion test.

Results: Among 102 isolates, 81 were *E. faecalis*, 18 were *E. faecium* and 3 were another *Enterococcus*. Their antimicrobial susceptibility pattern shows all isolates were sensitive to vancomycin, linezolid and teicoplanin with HLGR, HLSR detected in 40 and 38 isolates of *E. faecalis*, 17 and 13 isolates of *E. faecium* respectively by disc diffusion whereas by E-test it was detected in 44 and 40 in *E. faecalis* and 17 and 14 in *E. faecium* respectively. *E. faecium* is found to be more resistance to high level aminoglycoside than *E. faecalis*.

Conclusions: Authors hereby conclude that Enterococci being the common cause of hospital acquired infections with their increasing resistance to multiple drugs and acquisition of HLAR; it must be routinely screened for various drugs to prevent drug resistance in hospital settings for serious Enterococcal infections.

Keywords: High level aminoglycoside resistant, High level gentamicin resistant, High level streptomycin resistant

INTRODUCTION

In 1899, France Thiercelin had first used the name “*Enterocoque*” in a published paper.¹ The term *Enterococcus* derived from their presence in the intestinal tract as a normal flora. Enterococci are gram positive bacteria that typically appear as a pair of oval cocci, the cell are arranged at an angle to each other. The term *Enterococcus* was used for organism that grows at 10°

and 45°C, in 6.5% NaCl, and at pH 9.6 and which survived 60°C for 30min.

They are normal resident of gastrointestinal and biliary tracts and in lower numbers in the vagina and male urethra. However, when they colonize where they are not normally found they may become pathogen. They are becoming increasingly important agent of human disease, largely because of their resistance to antimicrobial agents.

Among several species which belong to genus *Enterococcus*, *E. faecalis* the most common isolate, have association with 80-90% of human Enterococcal infection. *E. faecium* isolated from 10-15% of infections.² Other Enterococcal species like *E. malodoratous*, *E. avium*, *E. cecorum*, *E. gallinarum*, *E. raffinosus*, *E. casseliflavus*, *E. dispar*, *E. hirae*, *E. durans*, and *E. mundtii* are infrequently isolated from human infections. Enterococci being 2nd most common cause of nosocomial urinary tract infection and wound infection and 3rd common cause of nosocomial bacteraemia.³ They have emerged an important nosocomial agent due to their colonizing ability and multidrug resistance.^{4,5}

They exhibit resistance to multiple commonly used antibiotics like aminoglycoside and cephalosporin because of their ability to attain and transfer the resistance genes giving rise to resistance to high level aminoglycosides and glycopeptides. Such resistance could be treated with ampicillin or vancomycin with or without aminoglycoside or teicoplanin. High level aminoglycoside resistance HLAR (MIC>2000 microgram / ml) has emerged recently among enterococci, it may be ribosomally mediated or because of production of inactivated enzymes.

The limited choice of efficient therapy in serious Enterococcal infection has been complicated due to resistance to ampicillin, high level aminoglycoside and glycopeptides. This poses therapeutic challenges to physician. Enterococcal infection like bacteraemia and endocarditis needs treatment with combination of antibiotics which includes penicillin group of drugs like ampicillin and penicillin G susceptible to *Enterococcus* species are susceptible and an aminoglycoside like Gentamicin and Streptomycin for which *Enterococcus* isolates do not show high level resistance.

But this would also be a therapeutic failure, if the isolate is HLAR. In such cases other antibiotics like Vancomycin, Linezolid, Teicoplanin, Quinpristin/Dalfopristin, etc may be useful depending on sensitivity profile.

METHODS

The study was conducted in a tertiary care hospital, on various clinical samples of IPD and OPD patients attending NIMS hospital Jaipur Study Period: January 2015 to June 2016. Study Population: Includes patients of all age group and gender both from outpatients and in patient departments. Inclusion Criteria are including the patient of all age group and all samples (like urine, blood, pus, stool, wound swab, sputum, body fluids, etc) received in our lab and were collected as per standard guidelines only. Exclusion Criteria is Nil. Methodology Followed: Collected samples received in lab and then processed in microbiology lab and isolates were identified and confirmed as *Enterococcus* species by various conventional methods (Gram staining, catalase test, bile esculin test, PYR test, growth at 45°C, salt tolerance test 6.5%, growth at alkaline pH 9.6, arginine dihydrolase test, hippurate hydrolysis test, potassium tellurite reduction test, sugar fermentation test). These isolates were subjected to Antibiotic susceptibility testing using Kirby-Bauer disc diffusion method as per CLSI guidelines 2016. The antibiotics disc used are ampicillin 10µg, nitrofurantoin 300µg, gentamicin (HLG) 120µg, and streptomycin (HLS) 300µg, ciprofloxacin 5µg, vancomycin 30µg, linezolid 30µg, teicoplanin 30µg, quinpristin / dalfopristin 15µg with *E. faecalis* ATCC 29212 as quality control. E-test was done for all high level aminoglycoside resistance *Enterococcus* species isolated by disc diffusion test. Statistical Analysis: Statistical analysis is done by using SPSS software version 17.

RESULTS

Maximum number of patients are in age group 51-60years i.e. 18 (17.7%) followed by 61-70 years i.e. 17 (16.7%), 21-30years i.e. 15 (14.7%), 31-40and <10years i.e. 14 (13.7%) each, 41-50years i.e.13 (12.8%), 11-20 years i.e. 8 (7.8%) and least from age above 70years i.e. 3 (2.9%). (Figure 1).

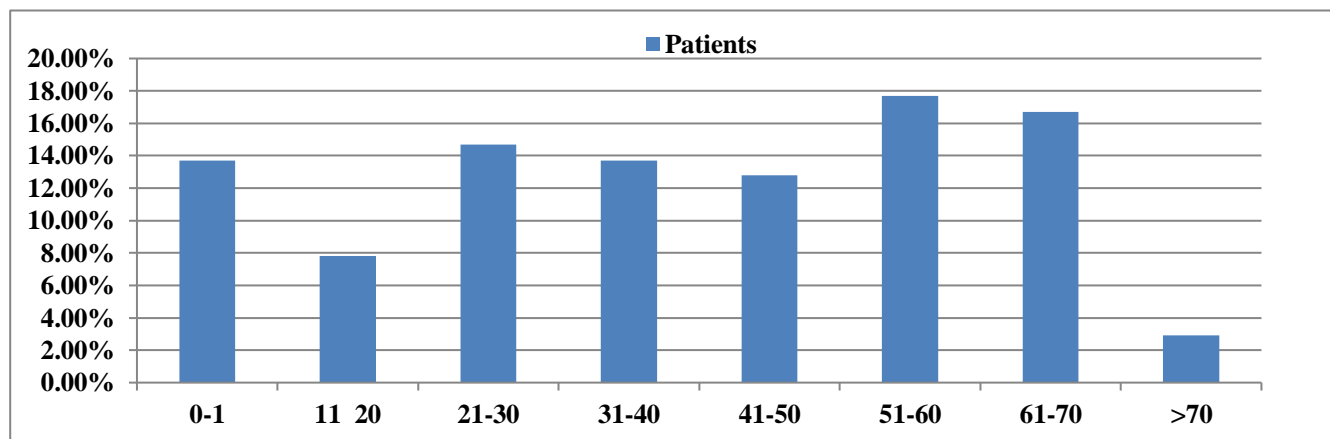


Figure 1: Distribution of patients according to age.

Table 1 shows distribution of Enterococcal isolates from different clinical samples. Maximum samples from which *Enterococcus* was isolated is urine i.e. 73(71.5%), followed by blood and pus i.e. 12 (11.9%) and 10 (9.9%) respectively.

Table 1: Distribution of enterococcal isolates from different clinical samples.

Samples	No.	(%)
Urine	73	71.5
Blood	12	11.9
Pus	10	9.9
Wound swab	3	2.9
Foley’s tip	3	2.9
Endotracheal tube tip	1	0.9
Total	102	100.0

Figure 2 shows distribution of patients according to OPD and IPD. Maximum patients are from IPD i.e. 74 (72.5%) and OPD i.e. 28 (27.5%).

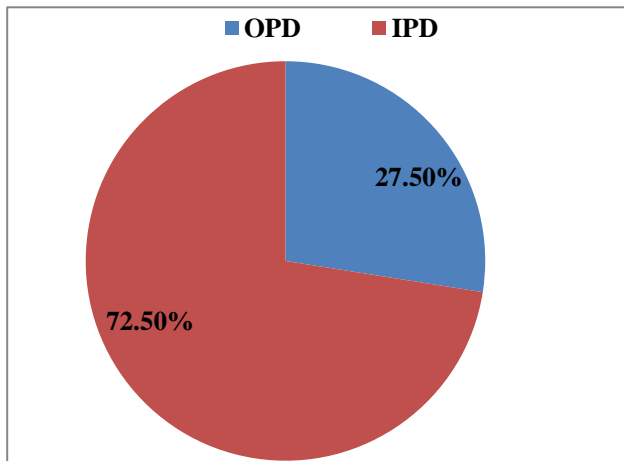


Figure 2: Distribution of patients according to OPD and IPD.

Figure 3 shows distribution of *E. faecalis*, *E. faecium* and other *Enterococci* from various clinical samples. Maximum isolate is *E. faecalis* i.e. 81 (79.4%) followed by *E. faecium* i.e. 18 (17.7%) and other *Enterococcus* i.e. 3(2.9%).

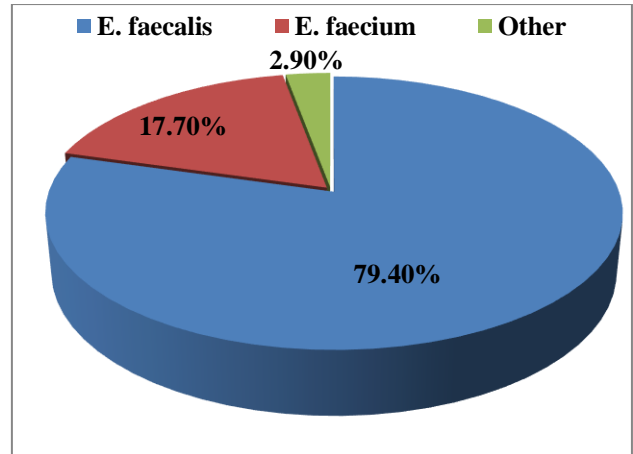


Figure 3: Distribution of *E. faecalis*, *E. faecium* and other *Enterococci* from various clinical samples.

Table 2: Distribution according to susceptibility and resistance pattern of different drugs.

Drugs	Susceptibility		Resistance	
	No.	(%)	No.	(%)
Vancomycin (n=102)	102	100	0	0
Linezolid (n=102)	102	100	0	0
Teicoplanin (n=102)	102	100	0	0
Ciprofloxacin (n=102)	73	71.5	29	28.4
Ampicillin (n=102)	22	21.5	80	78.4
Quinpristin-Dalfopristin (n=102)	11	10.7	91	89.2
Nitrofurantoin(n=73)	60	82.1	12	16.4
High level gentamicin (n=102)	57	55.8	45	44.1
High level streptomycin (n=102)	51	50	51	50

DISCUSSION

During recent year, there is increased interest in Enterococci because of their ability to cause serious infection and their increasing resistance of many antimicrobials. In the present study 102 *Enterococcus* were isolated from 1200 various clinical samples like urine, pus, blood, wound swab, Foley’s tip, Endotracheal tip from patients in OPD, Wards and ICU’s. Bacterial isolates were identified based on colony characters, morphology on gram staining, biochemical reactions, using conventional test scheme by Facklam et al, Antimicrobial susceptibility was done by Kirby Baur disc diffusion method.

In the present study most of the patients were from age group 51-60 years i.e. 17.7% Which is comparable to the study of Palaniswamy et al, and Sivasankari S et al, whereas study by Telkar et al, showed maximum patients from age group 0-20yrs and Bose et al, showed most patients from 21-30yrs which was slightly lower age group from present study.⁶⁻⁹ Majority of patients were

Table 2 shows distribution according to Susceptibility and Resistance pattern of different drugs. The susceptibility and resistance pattern of drugs used in the study was depicted, in which Vancomycin, Linezolid and Teicoplanin shows 100% susceptibility. Ciprofloxacin, Ampicillin, Quinpristin-Dalfopristin, Nitrofurantoin, high level Gentamicin and Streptomycin shows 71.5%, 21.5%, 10.7%, 82.1%, 55.8% and 50% susceptibility and 28.4%, 78.4% and 89.2%. 16.4%, 44.1% and 50% resistance respectively. The findings were found to be statistically significant.

males 53.5% in the study compared to females 45.7% with a male female ratio of 1.17:1. Most of the male patients belong to age group of 51-60years (10.8%) and female in the age group of 21-30 years and 31-40 years (8.8%) years which was comparable to study of Telkar et al, and Golia et al, unlike study of Bhatt et al, with higher female to male ratio.^{8,10,11} Most of the samples in study from which *Enterococcus* isolated was urine 71.5% followed by blood 11.9%, pus 9.9%, others like wound swab 2.9%, Foley's tip 2.9% and Endotracheal tip 0.9%. Similar results were shown by other authors Mittal et al, Lall et al, Suresh et al, whereas Golia et al, reported maximum samples from urine, followed by pus, blood, others, which was slightly different from present study, Sreeja et al, reported maximum samples blood 58% followed by pus i.e. 43% and urine 31% respectively different from our study.^{10,12-15} Maximum patients were from Wards 72.5% followed by ICUs 28.4% and OPD 27.5%. Similar to the study done by Mittal et al, Lall et al, Agarwal et al, 79.4% *E. faecalis*, 17.7% *E. faecium* and 2.9% other *Enterococcus* was isolated in this study, nearly similar results were obtained by different authors.^{12,13,16} Gangurde et al, Mulla et al, Adhikari et al, whereas Lall et al, Deshpande et al, Mendiratta et al, isolated only two species in their study.^{13,17-21}

Antimicrobial susceptibility showed vancomycin, linezolid and teicoplanin to be 100% susceptibility by disc diffusion method similar to the study of Suresh et al, Lall et al, whereas in the study performed by Mulla et al, showed 100% sensitivity of linezolid and Teicoplanin whereas vancomycin was only 86% sensitive and in study of Bhatt et al, linezolid is 100% sensitive whereas vancomycin and Teicoplanin are 86% sensitive each.^{11,13,14,18}

Ampicillin, ciprofloxacin, quinpristin-dalfopristin (pristinomycin) and nitrofurantoin showed 78.4% 28.4%, 89.2% and 16.4% resistance respectively similar to study of Lall et al, whereas Suresh et al, in his study reported 54% resistance each in ampicillin and ciprofloxacin and nitrofurantoin 100% sensitive and Bhatt et al, showed 95% and 62% resistance in ampicillin and ciprofloxacin respectively which slightly higher than present study with nitrofurantoin 100% sensitive.^{11,13,14} Out of 102 *Enterococcus* isolated 44.1% were HLGR and 50% were HLSR, 49.3% and 46.9% strains of *E. faecalis* were HLGR and HLSR and 94.4% and 72.2% were HLGR and HLSR of *E. faecium* respectively. Similar results were shown by Bhatt et al, Adhikari et al, Lall et al.^{11,13,19}

Hence it is concluded that *Enterococci* being the common cause of hospital acquired infections and bacteraemia with their increasing resistance to multiple drugs, the treatment has become a challenge for the physician. So it is important to know the susceptibility pattern of the organism and routine screening should be done in patients suffering from Enterococcal infections as it will support appropriate treatment strategies in cases of Enterococcal infection particularly life threatening

infection and will help the clinician in treating such patients and in minimising the speed of antibiotic resistance in the community and in the hospital.

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