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Isolation of *Enterococcus* from Various Clinical Samples and Their Antimicrobial Susceptibility Pattern in a Tertiary Care Hospital

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A B S T R A C T

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 to isolate *Enterococcus* from various clinical samples and their antimicrobial susceptibility pattern in a tertiary care hospital. A total of 102 *Enterococcus* species were isolated from various clinical samples were identified by various conventional biochemical methods. Antimicrobial susceptibility was detected by Kirby Bauer disc diffusion method as per CLSI guidelines. A total 102 *Enterococcus* species isolated from various clinical samples in which 81 were *E. faecalis*, 18 were *E. faecium* and 3 were other *Enterococcus*. Their antibiotic susceptibility pattern is *E. faecium* show more resistance than *E. faecalis*. We hereby conclude that *Enterococcus* isolated from various clinical samples must be routinely screened for various drugs to prevent drug resistance in hospital settings for serious Enterococcal infections.

Introduction

In 1899, France Thiercelin had first used the name "*Enterocoque*" in a published paper (Theircelin *et al.*, 1899). 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 10ºC and 45ºC, in 6.5% NaCl, and at pH 9.6 and which survived 60ºC for 30min. They are normal resident of gastrointestinal and billiary 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 (Washington). 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 bacteraemias are *Enterococcus* (Moellering, 1992). They have emerged an important nosocomial agent due to their colonizing ability and multidrug resistance (Antalek *et al.*, 1995; Buschelman *et al.*, 1993).

They exhibit resistance to multiple commonly used antibiotics like aminoglycoside and cephalosporins 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.

Materials and Methods

The study was conducted in the hospital of National Institute of Medical Science and Research, Jaipur. And was done on various clinical samples of IPD and OPD patients attending NIMS hospital Jaipur during the period of January 2015 to June 2016. The study population includes the patient of all age group and samples collected as per standard guidelines only. Various clinical samples like urine, blood, pus, stool, wound swab, sputum, body fluids, etc were collected by all aseptic technique in sterile container. Then they were inoculated on Blood agar, MacConkey agar and Nutrient agar and incubated at 37˚C for 18-24hrs.

On Blood agar circular, translucent, smooth, convex colonies of 1-2mm in diameter, with regular margins showing either alpha or non-hemolytic colonies. On MacConkey agar they form small, 0.5-1mm magenta coloured colonies. After that colony morphology is observed and processed further. Identification is done on the basis of Gram staining and biochemical reactions as per standard protocol like 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.

Antibiotic sensitivity testing was done using Kirby-Baeur disc diffusion method as per CLSI guidelines. 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. Quality controlled used was *E. faecalis* ATCC 29212.

Results and Discussion

Maximum number of patients are in age group 51-60 years i.e. 18 (17.7%) followed by 61-70 years i.e. 17 (16.7%), 21-30 years 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%) (Fig. 1).

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.

Maximum patients are from IPD i.e. 74 (72.5%) and OPD i.e. 28 (27.5%) (Fig. 2).

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%).

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.

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 (Table 1 and Fig. 3). Bacterial isolates were identified and speciated based on colony characters, morphology on gram staining, biochemical reactions, using conventional test scheme by Facklam and Collins (1989). Antimicrobial susceptibility was done by Kirby Baeur disc diffusion method.

In the present study most of the patients were from age group 51-60years i.e. 17.7% Which is comparable to the study of Palaniswamy *et al.,* (2013) and Sivasankari *et al.,* (2013) whereas in another study by Telkar *et al.,* (2012) showed maximum patients from age group 0-20yrs and Bose *et al.,* (2012) showed most patients from 21-30 years which is slightly lower age group from present study. Majority of patients were 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 51-60 years (10.8%) and female in the age group of 21-30 years and 31-40 years (8.8%) years which is comparable to study of Telkar Anjana *et al.,* (2012) and Golia *et al.,* (2014), whereas Puneet *et al.,* (2014) showed more female to male ratio.

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**

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

X^2 =138.1572 P< 0.00001 P< 0.05 significant

Fig.1 Distribution of patients according to age

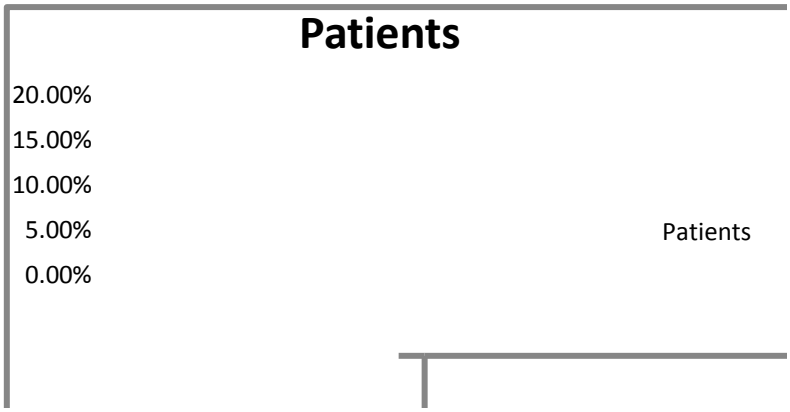


Fig.2 Distribution of patients according to OPD and IPD

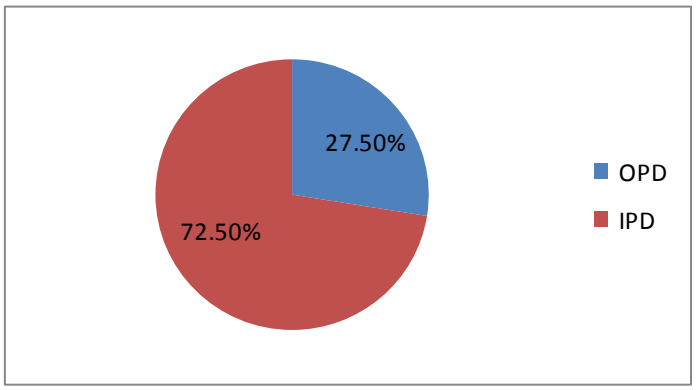
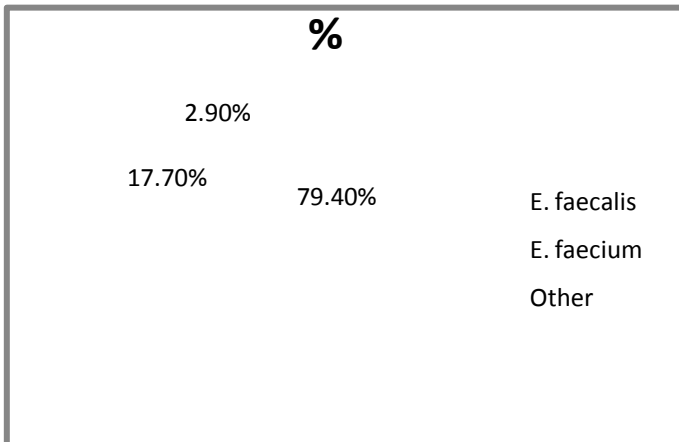


Fig.3 Distribution of *E. faecalis, E. faecium* and other *Enterococci* from various clinical samples



Most of the samples in study from which *Enterococcus* isolated is 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.,* (2016) Lall *et al.,* (2014) Suresh *et al.,* (2013) whereas Golia *et al.,* (2014) reported maximum samples from urine, followed by pus, blood, others, which is slightly different from present study, Sreeja *et al.,* (2012) reported maximum samples blood 58% followed by pus i.e. 43% and urine 31% respectively different from our study. Maximum patients are from wards 72.5% followed by ICUs 28.4% and OPD 27.5%. Similar to the study done by Mittal *et al.,* (2016), Lall *et al.,* (2014), 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. Gangurde *et al.,* (2014), Mulla *et al.,* (2012), Adhikari (2010), whereas Lall *et al.,* (2014), Deshpande *et al.,* (2013), Mendiratta *et al.,* (2008) isolated only two species in their study.

In present study vancomycin, linezolid and teicoplanin shows 100% susceptibility by disc diffusion method. Similar to the study of Suresh *et al.,* (2013), Lall *et al.,* (2014) whereas in the study performed by Mulla *et al.,* shows 100% sensitivity of linezolid and Teicoplanin whereas vancomycin is only 86% sensitive and in study of Puneet *et al.,* (2014) linezolid is 100% sensitive whereas vancomycin and Teicoplanin are 86% sensitive each. Ampicillin, ciprofloxacin, quinpristin-dalfopristin (pristinomycin) and nitrofurantoin shows 78.4% 28.4%, 89.2% and 16.4% resistance respectively similar to study of Lall *et al.,* (2014) whereas Suresh *et al.,* (2013) in his study reported 54% resistance each in ampicillin and ciprofloxacin and nitrofurantoin 100% sensitive and Puneet *et al.,* (2014) showed 95% and 62% resistance in ampicillin and ciprofloxacin respectively which slightly higher than present study with nitrofurantoin 100% sensitive. Out of 102 *Enterococcus* isolated 44.1% were HLGR and 50% were HLSR, 49.3% and 46.9% strains of *E. faecalis* are HLGR and HLSR respectively and 94.4% and 72.2% are HLGR and HLSR of E. faecium respectively. Similar results were shown by Puneet *et al.,* (2014), Adhikari (2010) and Lall *et al.,* (2014). Hence it is concluded that *Enterococci* being the common cause of hospital acquired infections and bacteraemias 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 minimizing the speed of antibiotic resistance in the community and in the hospital.

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