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Prevalence of Vancomycin Resistant *Enterococcus* and its Antibiotic Resistance Pattern at a Tertiary Care Hospital

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A B S T R A C T

UTI is one of the most common infectious diseases in people of all age groups from the neonates up to geriatric age groups and geographical locations are affected and thus represent a major source of human discomfort. Since the mid 1990's, hospital-associated *Enterococcal* bacteriuria, has shown a significant increase in frequency over time and is the second most frequently isolated urinary pathogen. *Enterococcus faecalis* is more common in nosocomial infections than *E. faecium*, but *E. faecium* has a great ability to acquire drug resistance. The present study was planned to determine the antimicrobial susceptibility pattern of urinary isolates of *Enterococcus species* and the emergence of vancomycin resistance and high level aminoglycoside resistance. A total number of 187 urinary isolates of enterococci were tested for phenotypic characteristics by conventional methods and growth on hicrome UTI agar. Antimicrobial susceptibility pattern was studied by Kirby-bauer disk diffusion technique, screening agar method and minimum inhibitory concentration (MIC) testing by E test to determine vancomycin resistance. Out of 187 *enterococcal* isolates, 149 (79.67%) were *Enterococcus faecalis* and 38 (20.32%) were *Enterococcus faecium.* Among predisposing factors in the present study, catheterization was found to be the most important predisposing factor where the highest number of patients (43.89%) had enterococcal UTI, followed by diabetes accounted for 31.23 %. 13 enterococcal isolates were resistant to vancomycin by E test and all the isolates were susceptible to teicoplanin, linezolid and tigecycline. Maximum resistance was observed against high level amino glycoside (HLAR), i.e.50.8%. Followed by, most of the isolates were resistant to tetracycline and ampicillin, i.e 44.3% and 42.7% respectively. Multidrug resistant enterococci especially resistant to vancomycin and aminoglycosides have become a threat to patient's safety, making it a formidable nosocomial pathogen.

Introduction

UTI is one of the most common infectious diseases in people of all age groups from the neonates up to geriatric age groups and geographical locations are affected and thus represent a major source of human discomfort (Pankaj *et al.,* 2012). Of the various uropathogens, the most common organisms are *E.coli, Enterococcus spp,*

Staphylococcus aureus and *Klebsiella spp.* (Sivasankari *et al.,* 2013).

Since the mid 1990's, hospital-associated *Enterococcal* bacteriuria, has shown a significant increase in frequency over time and was the second most frequently isolated urinary pathogen (Wazait *et al.,* 2003). In a CDC survey of nosocomial infection, *Enterococci* accounted for 13.9% of urinary tract infections (Desai *et al.,* 2001). This organism is considered as second leading cause of hospital acquired infections (Bose *et al.,* 2012). *Enterococcus faecalis* is more common in nosocomial infections than *E. faecium*, but *E. faecium* has a great ability to acquire drug resistance (Mohammad *et al.,* 2008).

The treatment of these infections poses a great challenge due to the inherent resistance of *Enterococcus* to many antibiotics. A combination of penicillin and gentamicin had been the mainstay of treatment of enterococcal infections till now but with the emergence of high level aminoglycoside resistance (HLAR), vancomycin is the only alternative available. The widespread use of glycopeptides in hospitals has led to the emergence of vancomycin resistant *Enterococcus* (VRE) which is a major concern for health care professionals (Mathur *et al.,* 2003).

In this study, we attempted to determine the antimicrobial susceptibility pattern of urinary isolates of *Enterococcus species* and the emergence of vancomycin resistance and high level aminoglycoside resistance in those isolates recovered from the patients at a Pravara Tertiary Care Hospital, Loni, during March 2011 to August 2012.

Materials and Methods

This study was conducted after approval from institutional ethical committee, in the Department of Microbiology, Pravara Medical College and Hospital and included a total number of 187 enterococci isolated from urine from the patients irrespective of age, sex or antibiotic therapy. Relevant information regarding the patient's history as well as signs and symptoms of patients was collected.

Presence of > 10 pus cells/hpf in centrifuged urine and > 5 pus cells/hpf in uncentrifuged urine is considered to be significant (Taneja *et al.,* 2004). Culture was done on blood agar, MacConkey's agar, CLED agar (Himedia M792**)** and Hicrome UTI agar (Modified M1418) and plates were incubated aerobically at 37ºC for 24 hours (Bose *et al.,* 2012).

Enterococcus showed greenish yellow colour on CLED agar and bluish green on Hicrome UTI agar. All of the enterococcal isolates were tested for phenotypic characteristics by conventional methods, on the basis of the following criteria: growth on bile esculin agar and in 6.5% NaCl broth, absence of catalase, and presence of pyrolidonyl arylamidase. Species-level identification was performed by biochemical tests including acid fermentation of mannitol, sorbitol, sucrose, arabinose, and raffinose; motility; and arginine hydrolysis (Mohammad *et al.,* 2008).

For studying the antimicrobial susceptibility pattern in enterococcal isolates, three methods were used Kirby-bauer disk diffusion technique, screening agar method and minimum inhibitory concentration (MIC) testing by E test to determine vancomycin resistance.

Kirby bauer disk diffusion method was used for determining the susceptibility of the isolates to the commonly used antibiotics against *Enterococcus* spp. using the standard guidelines issued by the Clinical

Laboratories Standards Institute (CLSI). The antibiotics tested were nitrofurantoin (300 µg), ampicillin (10 µg), ciprofloxacin (5 µg),gentamicin (high content) (120 µg), tetracycline (30 µg), vancomycin (30 µg), teicoplanin (30 µg), linezolid (30 µg) and tigecycline (15µg). All antibiotic discs were obtained from Hi Media Pvt Ltd, India.

Screening for low level vancomycin resistance for the isolates resistant to vancomycin by disk diffusion method was done by vancomycin screen agar method using 6 µg/ml vancomycin according to NCCLS recommendations. Minimum inhibitory concentration (MIC) of vancomycin was performed by E test (AB Biodisk, Solna, Sweden) (Mendiratta *et al.*, 2008). Susceptibility interpretations followed the guidelines proposed by CLSI ($S \leq 4$; I =8/16; $R \geq 32$ mg/L) (Manpreet *et al.*, 2014). *Enterococcus faecalis (E. faecalis)* ATCC 29212 and *Staphylococcus aureus* ATCC 25923 were used as control strains (Bose *et al.*, 2012).

Results and Discussion

Total 187 *enterococci* were isolated from urine specimens over a period of one and half year. Number of females infected with *enterococcal* infection was more, i.e. 122 (65.24%) and the number of males affected was 65 (34.75%). Male: Female ratio was 1:1.87 as shown in figure 1.

Maximum number of patients affected belongs to 21 –30 years of age group, followed by 31 – 40 years. The age group, 0 – 10 years, has minimum number of patients. (figure 2)

Among 187 *enterococcal* isolates, 149 (79.67%) were *Enterococcus faecalis (E. faecalis)* and 38 (20.32%) were *Enterococcus faecium (E. faecium).* (Table 1)

Among predisposing factors in the present study (Table 2), catheterization was found to be the most important predisposing factor where the highest number of patients (43.89%) had enterococcal UTI, followed by diabetes accounted for 31.23 %. Pregnancy was also an important predisposing factor and 24.88% of pregnant patients showed enterococcal UTI.

Out of 187 enterococcal isolates, 13 were resistant to vancomycin by E test and all the isolates were susceptible to teicoplanin, linezolid and tigecycline. Maximum resistance was observed against high level amino glycoside (HLAR), i.e.50.8%. Followed by, most of the isolates were resistant to tetracycline and ampicillin, i.e 44.3% and 42.7% respectively. (Table 3)

Recent years have witnessed increased interest in enterococci not only because of their ability to cause serious infections but also because of their increasing resistance to many antimicrobial agents (Marothi *et al.*, 2005).

In the present study, of the 187 enterococcal isolates, 79.67% were *E. faecalis* while prevalence of *E. faecium* was 20.32% (Table 1) , similar to the results stated by Marothi *et al* from Madhya Pradesh (Marothi *et al.*, 2005). According to study from Tamilnadu and Jalgaon(Maharashtra) 78.8% and 86% were *E.faecalis* whereas 21.2% and 14% were *E.faecium*, respectively (Sivasankari *et al.*, 2013; Bhardwaj *et al.*, 2013). It is also stated that *Enterococcus faecalis* is more common in nosocomial infections than *E. faecium* (Mohammad *et al.*, 2008).

As per the correlation of enterococcal UTI with the predisposing factors in our study (Table 2), catheterization was found to be the most important one i.e 43.89%, followed by diabetes (31.23 %) and pregnancy

accounted for 24.88%. Richard Difuku *et al.,* and Robert Orenstein *et al.,* reported the catheter associated UTI constitute 35-40% of all UTIs (Richard Difuku *et al.,* 1984, Robert Orenstein *et al.,* 1999). Closely similar to our result, Patil *et al* in 2012 noted that diabetes found in 36% of all UTIs (Patil *et al.,* 2012). However, studies from Chennai and Iran have reported the incidence of UTI in type 2 diabetic patients was as high as 42-42.8% (Janifer *et al.,* 2009, Richard Daifuku *et al.,* 1984). Changes in the host defence mechanism, the prevalence of diabetic cystopathy and of microvascular disease in the kidneys may play a role in higher incidence of UTI in diabetic patients (Janifer *et al.,* 2009).

The drawback of the control and treatment of enterococcal infections is their intrinsic resistance to various antibiotics, their capabilities to develop new resistance and to live in the external environment for a longer time (Gülçin Baldýr *et al.,* 2013). The emergence and spread of glycopeptide resistance in enterococci has become a substantial clinical and epidemiological concern, (Ojan Assadian *et al.,* 2007).

Vancomycin Resistant Enterococci (VRE) takes place among the important nosocomial pathogens, in that the treatment options are limited, it is likely to transfer vancomycin resistance to other pathogens. VRE is known to spread in the hospital setting through contaminated hands and surfaces (Gülçin Baldýr *et al.,* 2013).

In the present study, from 187 enterococcal isolates, 13 (6.9%) were resistant to vancomycin but susceptible to teicoplanin, similar to the result studied by Hajia *et al.,* (Hajia *et al.,* 2012). The Studies from Puducherry and Chandigarh have reported 8.7% and 5.5% incidence of VRE, respectively (Praharaj *et al.,* 2013). Although the prevalence of VRE infections in India is much lower than in the western world, it has been increasing in the past one decade. (Praharaj *et al.,* 2013). Antibiotic selective pressure exerted by extensive use of third generation cephalosporins and drugs with potent activity against anaerobes have been reported to predispose to VRE colonization and infection (Taneja *et al.,* 2004).

The VRE strains containing vanA and vanB genes carry a high level of resistance to vancomycin, while those carrying VanC gene show a low level of resistance to vancomycin. The strains with vanA gene are resistant to teicoplanin as well as vancomycin (Gülçin Baldýr *et al.,* 2013). And VanB type resistance is characterized by resistance to vancomycin and susceptibility to other glycopeptides like teicoplanin since only the former antibiotic is capable of inducing the vanB resistance type (Guido Werner *et al.,* 2012). So, based on the results of the MIC studies and susceptibility to teicoplanin, in our study, it appears that Van B may be the phenotype for vancomycin resistant *E. faecalis* and *E. faecium* (Taneja *et al.,* 2004; Gülçin Baldýr *et al.,* 2013).

The emergence of VRE had seriously affected the treatment of the conditions caused by this organism. This leaves clinicians a limited choice. For these types of cases, newer antibiotics, such as linezolid and tigecycline are useful. Linezolid was the first oxazolidinone to be available for clinical use in 2000. It has activity against both *E. faecium* and *E. faecalis* (Praharaj *et al.,* 2013). It binds to the domain V region of 23 S rRNA and mutation to that domain causes resistance to the drug (Bose *et al.,* 2012). Another advantage of this drug is that it can be administered both intravenously and orally (Praharaj *et al.,* 2013). Outbreaks due to linezolid resistant enterococci, though rare, have been reported recently. Also the

cases of linezolid- resistant vancomycin-resistant *E. faecium* infection without any prior exposure to linezolid have been reported (Praharaj *et al.,* 2013). Tigecycline is a new glycylcycline derivative of tetracycline. Tissue penetration of tigecycline is excellent and it acts against both Gram positive and Gram negative microorganisms (Bose *et al.,* 2012). All the VRE isolates in our study were found to be sensitive to linezolid and tigecycline.

Gentamicin is one of the most commonly used aminoglycosides against enterococci (Mohammad *et al.,* 2008). The cell wall inhibitors such as penicillin, ampicillin, or vancomycin have been administered in combination with the aminoglycosides such as streptomycin and gentamicin in the treatment of serious infections caused by *enterococci*. A synergistic effect between the cell wall synthesis inhibitors and aminoglycosides disappears in the presence of high-level resistance to aminoglycoside and causes difficulties in the treatment of severe *enterococcal* infections (Gülçin Baldýr *et al.,* 2013).

Enterococci have intrinsic low-level resistance to aminoglycosides and, in addition, have acquired aminoglycoside resistance genes. Along with the rapid rise in incidence of resistance in enterococci to traditional antibiotics, including aminoglycosides, transmission of multiresistant and HLGR strains has been reported (Mohammad *et al.,* 2008).

High level gentamicin resistance (HLGR) was first time reported in *E. faecalis* in the year 1979. Resistance to amino glycoside is often associated with multidrug resistance and is due to various amino glycoside modifying enzymes. Moreover, *E.faecium* has become difficult to be treated by glycopeptides and amino glycosides (Bose *et al.,* 2012).

In our study, 95 (50.8%) *Enterococcal* isolates were resistant to Gentamicin (high level). In another study, from the same place, high level gentamicin resistance was found to be 58% (Bose *et al.,* 2012). A study by MATHUR *et al* from Northen India has stated 26% high level gentamicin resistance. (Mathur *et al.,* 2003) and Praharaj *et al* found 37% of all *Enterococcus* isolates were high-level gentamicin resistant by disk diffusion method (Praharaj *et al.,* 2013).

Recently, Chandrakanth *et al.,* (2012); Shafiyabi *et al.,* (2013), and Jada and Kumar (2013) also reported *Enterococci* to exhibit resistance to Gentamicin. On the other hand, Acharya *et al.,* (2003) and Sreeja *et al.,* (2012) found the isolates to be 62% and 55.2% susceptible to this antibiotic respectively (Manpreet Kour *et al.,*2014). The presence of HLGR is predictive of the loss of synergy between gentamicin and a cell wall-active agent such as ampicillin or vancomycin (Sivasankar *et al.,* 2013).

Ampicillin resistance in enterococci may be caused either by change in penicillin-binding proteins or rarely by production of a beta-lactamase enzyme (Gülçin Baldýr *et al.,* 2013). In the present study, 42.7% enterococcal isolates were resistant to ampicillin. It is related with the one of the studies which shows ampicillin resistance 46.33% (Praharaj *et al.,* 2013). Also a study from Jaipur (Rajsthan) reported 47.16% of Enterococcal isolates were resistance to ampicillin (Manpreet Kour *et al.,* 2014). However some of the studies have noted very low resistance to ampicillin as 12.5%, 18.6% and 28.8%.(Edet Udo *et al.,* 2003; Gülçin Baldýr *et al.,* 2013). And on the other hand, Mathur *et al.,* reported 66% enterococcal strains were resistant to ampicillin (Mathur *et al.,* 2003).

Nitrofurantoin is an excellent drug against *enterococcal* urinary tract infection. It has been used for past many years and still shows very little resistance. It is both bacteriostatic and bactericidal and resistant mutants are very rare. There are no cross resistance between nitrofurantoin and other antibiotics. It is effective against both *E. faecalis* and *E. faecium* including most of the *VRE* (Bose *et al.,* 2012).

In the present study also, 92.6 % of all the enterococcal isolates were sensitive to nitrofurantoin. A study by Bose *et al* have reported only a 2.07% resistance to nitrofurantoin (Bose *et al.,* 2012). Although nitrofurantoin is a good choice of drug for UIT treatment, a study from Puducherry have reported 29% of urinary isolates of enterococci showed *in vitro* resistance to it (Praharaj *et al.,* 2013).

Again in our study 18 enterococcal isolates (9.6%) were resistant to ciprofloxacin and 44.3% showed resistant to tetracycline. It is quite low resistant prevalence as compared to the studies by Praharaj *et al.,* (Praharaj *et al.,* 2013) and Mathur *et al., (*Mathur *et al.,* 2003). Also a study from Puducherry showed resistance to tetracycline as high as 71.38% (Praharaj *et al.,* 2013) which is somewhat related with the study from Kuwait (Edet Udo *et al.,* 2003).

Table.1 Species distribution of Enterococcal isolates from urine specimens (n=187)

***Enterococcal* species**	**Number**	**Percentage (%)**
E. faecalis	149	79.67
E. faecium	38	20.32

Table.2 Distribution of predisposing factors in patients with Enterococcal UTI (n=187)

Sr.No.	**Predisposing factor**	**No. of Enterococcal isolates**	**Percentage (%)**
1	Catheterisation	82	43.89
2	Diabetes	58	31.23
3	Pregnancy	47	24.88

Table.3 Antimicrobial resistance pattern of Enterococcal isolates (n=187)

Sr.No.	**Antibiotics**	**No. of resistant strains**	**% of resistant strains**
1	Tetracycline	83	44.3
2	Ampicillin	80	42.7
3	Gentamicin(high level)	95	50.8
4	Teicoplanin	0	0
5	Vancomycin	13	6.9
6	Linezolid	0	0
7	Tigecycline	0	0
8	Nitrofurantoin	14	7.4
9	Ciprofloxacin	18	9.6

Fig.1 Sex wise distribution of patients with Enterococcal UTI (n=187)

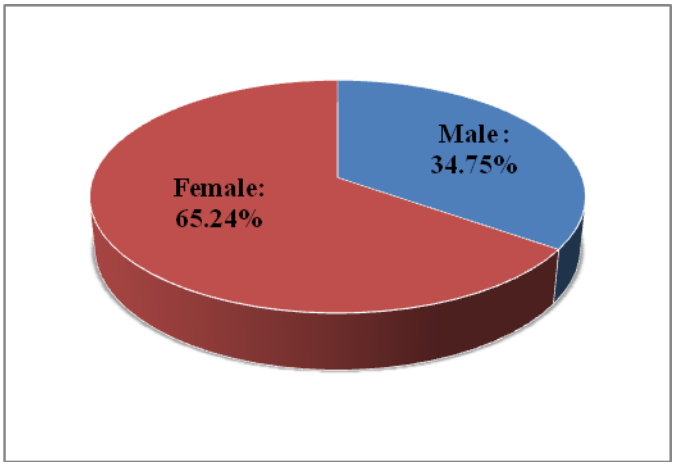
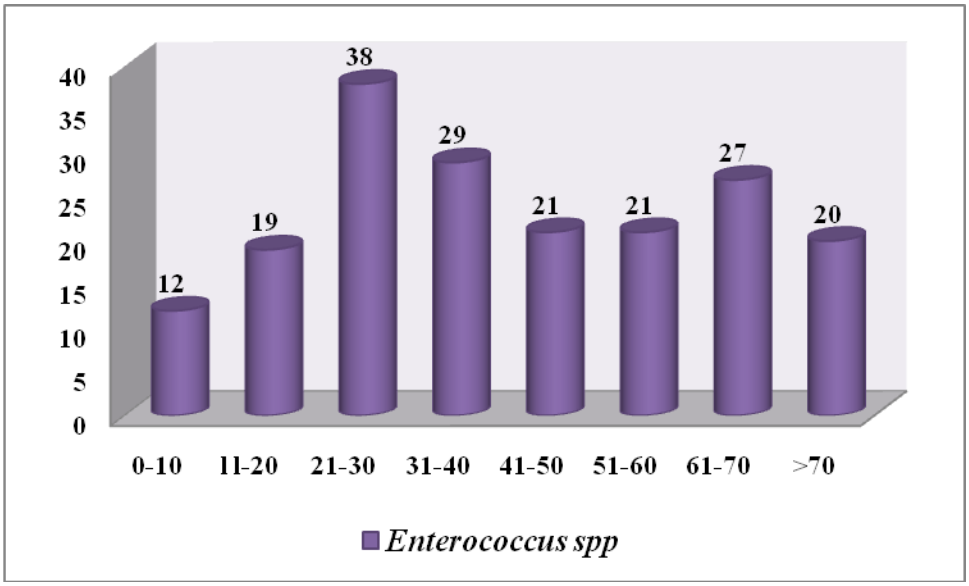


Fig.2 Age group wise distribution of all patients (n=187)



In conclusion, multidrug resistant enterococci especially resistant to vancomycin and aminoglycosides have become a threat to patient's safety, making it a formidable nosocomial pathogen. The rising prevalence of antimicrobial resistance trait among *Enterococcus* spp. has critical outcome on health care system due to increasing in mortality as a result of existence of severe infections such as endocarditis without any effective antimicrobial therapeutic agents. This fact highlights the importance of strict enforcement of antibiotic policies coupled with greater adherence to infection control measures to prevent emergence and spread of antibiotic resistant bacteria.

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