

Original Research Article

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Resistance Pattern of *Enterococcus spp.* Isolated from Clinical Specimens

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ABSTRACT

Keywords

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Enterococci are bacteria that are normally present in the human intestines and are often found in the environment. Recently there is an increase in prevalence of Multidrug resistance and other hospital strains like Vancomycin resistant *Enterococcus* and Linezolid resistant *Enterococcus*. Enterococci isolated from clinical samples were identified by standard phenotypic methods and susceptibility testing by Disc diffusion method. *E.fecalis* was common species constitute 80%. Majority of the isolates were multidrug resistant strains. Low resistance was observed against Vancomycin, Teicoplanin and Linezolid. Drug resistance has significantly varies with the different methods in detection of resistance. MIC detection can help in confirming the resistance.

Introduction

Enterococci are bacteria that are normally present in the human intestines and are often found in the environment. In some occasion it can cause infections of the urinary tract, the bloodstream, or of wounds associated with catheters or surgical procedures. Nowadays this bacteria has emerged as a common nosocomial pathogen.

These organisms once considered a harmless commensal has emerged as a medically important multidrug resistant virulent pathogen causing outbreaks of many nosocomial infections. The recent increase of Vancomycin resistant *E.faecium* strains in clinical isolates is especially a cause of serious concern because this glycopeptides

often remains the last treatment available in life threatening infections (Kirshner *et al.*, 2001). Nowadays Linezolid is being used for treating infections caused by VRE. But strains resistant to Linezolid also started emerging throughout the world. First case of Linezolid Resistant *Enterococcus fecium* reported from India in 2014 (Simit Kumar *et al.*, 2014). Our study is aimed to analyse the antibiotic resistance pattern of *Enterococcus* isolated from clinical specimens and also to know the prevalence of Vancomycin Resistant Enterococci (VRE).

Materials and Methods

Enterococci isolated from clinical samples were identified by standard phenotypic

methods and selected for further analysis. Speciation of *Enterococcus* was done by conventional biochemical test using standard methods (Koneman *et al.*, 1997). All isolates were subjected to Routine antibiotic susceptibility testing by Kirby Bauer method according to CLSI guideline (CLSI, 2013).

Results and Discussion

A total of 394 *Enterococcus* were isolated and analysed during a period of one year. Out of this 312 (79.1%) isolates were from urine specimen, remaining 82 (20.8%) from exudates (Pus specimen). About 82 % of them are *E.fecalis* and remaining isolates were *E.fecium*. Susceptibility testing were done by disc diffusion method according to CLSI guidelines using a panel of antibiotic discs. Resistance pattern of these isolates is shown in table 1.

More than 85% of isolates were multidrug resistant strains. Among the antibiotics in the panel lowest resistance was found in Lenizolid, Teicoplanin & Vancomycin in both species. About 93.4% of *E.fecalis* and 97.3% of *E.fecium* were resistant to Penicillin. Resistance to High level gentamicin and Ampicillin was 51.5%. There was not much difference in the pattern of drug resistance between two species.

Clinical significance of *Enterococcus* spp has been increasing for the last few decades. This bacteria is now become one of the common agents causing Urinary tract infection. *Enterococcus* spp. causing nosocomial infections are now become more virulent and showing drug resistance against first line antibiotics. This bacteria is intrinsically resistant to cephalosporins and showing low level resistance to aminoglycosides. Majority of the strains included in our study were multidrug resistant. Among the two species studied,

E.faecium showed more resistance compared to *E.faecalis*, but this is not significant.

Nowadays treatment of choice for any suspected bacterial infections is cephalosporin group. Because of this nosocomial infection by *Enterococcus* becomes common as this bacterium is intrinsically resistant to cephalosporins. Different classes of antibiotics were included in antimicrobial susceptibility testing as per CLSI guidelines. Resistance pattern analysed only by disc diffusion method.

Analysing the resistance to Penicillin group, our study found that 93.4% & 97.3% resistance for Penicillin exhibited by *E.fecalis* and *E.fecium* respectively. But for Ampicillin only 51.5% & 52% resistance shown by those species respectively. This finding correlates with Ira Praharaj *et al.*, study in 2013 where they reported about 46.33% resistance for Ampicillin (Ira Praharaj *et al.*, 2013).

Another study from Sweden reported high resistance to ampicillin, about 74% of *E.fecium* were resistant to ampicillin, at the same time zero percentage resistance in case of *E.fecalis* (Anita hallgren *et al.*, 2001). This is statistically significant but compared to our study this report showing high resistance in *E.fecium* and no resistance in *E.fecalis*. The reason could be resistance pattern varies with the region or locality. *Enterococcus* shows low level resistance intrinsically to Penicillin. *E.fecium* has an additional mechanism for Betalactam resistance that they alter cellwall. That also responsible for high resistance in *E.fecium* than *E.fecalis* (Brian Hollenbeck and Louis Rice, 2012) High-level penicillin resistance in *E. faecium* is most commonly due to accumulation of point mutations in the

penicillin binding region of PBP5 (Zapun *et al.*, 2008). A study from turkey where they compared resistance pattern of *E.fecalis* and *E.fecium.*, reported low resistance rate against Penicillin and Ampicillin by *E.fecalis* (20% & 13%) but very high resistance (96%) by *E.fecium* (FiratZafer Mengeloğlu *et al.*, 2011). One of the studies reported 7% resistance by *E. faecium* and 0% resistance by *E. faecalis* towards Penicillin, which is very low probably because the isolates were from green leafy vegetables & other herbs. Though they are pathogenic species not isolated from clinical specimen (Lynette Johnston and Lee-Ann Jaykus, 2004). Study from Brazil showed *E.fecalis* Isolated from endodontic infections were 100% sensitive to Penicillin (Renata Ximenes Lins *et al.*, 2013). These high resistance against Penicillin group is due to their low level intrinsic resistance mechanism.

In our study 76% of *E.fecalis* and 80% of *E.fecium* were showing resistance to Tetracycline. Study by Ira Praharaj *et al.*, showed almost same finding as ours in relation to tetracycline resistance, which was also high (71.38%). Another study also reported high resistance (70%). Tetracyclin or Doxycyclin has a better effect against Gram positive organisms. Tetracycline resistance was frequently noticed in strains from all origins. In one study there was not a single tetracycline-susceptible strain present in the porcine collection (Patrick Butaye *et al.*, 2001). But 100% resistance was not observed in any study with clinical isolates. Study which included environmental samples was reported only 25% sensitivity to tetracycline with no resistance against vancomycin, teicoplanin, linezolid and ampicillin (Malihe Talebi *et al.*, 2015). There are very few studies discussed about Tetracyclin resistance. In general it was noticed that there is an increase resistance to tetracylin.

As for as flouoroquinolone resistance is considered we found that about 75.5% of isolates were resistant to ciprofloxacin. Previous reports also coincide with our study. The degree of resistance to ciprofloxacin was observed as high (74.38%) in a study from Pondicherry, India. They have also noted that all isolates of VRE were resistant to ciprofloxacin and tetracycline (Ira Praharaj *et al.*, 2013). A study from turkey *E faecium* showed 100% resistance and *E.faecalis* showed 55% resistance. So overall resistance reported was 74% in that study which is correlating with our study. In 2001 Anita Hallegran *et al.*, studied *Enterococcus* isolates from ICU patients and observed that 82.5% resistance against ciprofloxacin. They have detected MIC breakpoints for Ciprofloxacin. This resistance rate is higher than that of our study, could be due to MIC detection. MIC detection is always confirmatory to disc diffusion method. Certain environmental isolates were shown less (22%) resistance to ciprofloxacin (Lynette Johnston and Lee-Ann Jaykus *et al.*, 2004).

Enterococcus species are well known for their intrinsic resistance to low level aminoglycosides. High level resistance acquired by *Enterococcus* species either by the production of Aminoglycoside modifying enzyme(AME) or by single point mutation to the ribosome (Brian L. Hollenbeck and Louis Rice *et al.*, 2012). In this study it was found that only 51% resistance to high level gentamicin (HLG). Our study did not include any other aminoglycoside other than HLG. Some studies reported low resistance to HLG.

In an Indian study, 37% of all *Enterococcus* isolates were found to show high-level gentamicin resistance by disk diffusion method and 17% for streptomycin (Ira Praharaj *et al.*, 2013). Another researcher

reported 49% resistance (FiratZafer Mengeloğlu *et al.*, 2011). Study from Iran in 2015 reported 44% of clinical isolates and 30% of environmental isolates showed resistance to HLG. Eventhough *Enterococcus* are intrinsically resistance to aminoglycosides there are studies which proved that less than 50% of isolates only showing resistance to aminoglycoside. But other classes of antibiotics like flouroquinolone and tetrayclin were showing high resistance rate. This explains about their acquired resistance mechanisms against those antibiotics, which has more significance.

Resistance to glycopeptides are now started reporting and those are found to be common nosocomial agents also. But very low resistance rate only reported by previous studies.

In our study we have tested sensitivity to vancomycin as well as Teicoplanin. We have noted that 17% resistance to Vancomycin and 12% to Teicoplanin by disc diffusion method. About 9% for vancomycin and 8% for teicoplanin resistance was reported by Ira Praharaj *et al.*, from India. There was not a single VRE reported from Sweden by Hallgren *et al.*, (Anita hallgren *et al.*, 2001) In their study MIC for vancomycin detected which is

always better in relation to disc diffusion method. Another study from Turkey have also not reported any VRE and they have used automation technique for antibiotic susceptibility which could be the reason for very low prevalence (FiratZafer Mengeloğlu *et al.*, 2011). Acquired resistance in Vancomycin is due to synthesis of alternate cell wall (Brian Hollenbeck and Louis Rice, 2012). Studies which included environmental specimen also not reported VRE (Lynette Johnston and Lee-Ann Jaykus, 2004). Study which compared the drug resistance in Biofilm producers and non producers showed that resistance for glycopeptides was more in biofilm nonproducers. In addition to this they have compared clinical isolates with environmental samples for their resistance and it was noticed that resistance seen only in clinical isolates (Malihe Talebi *et al.*, 2015). There was no significant difference in the presence of virulence factors between VRE and Vancomycin sensitive strains (Carolina Baldisserotto Comerlato *et al.*, 2013). Routine disc diffusion method has only minimum role in identifying VRE, without the detection of MIC the prevalence rate analysed may not be satisfactory. Because the method used in studies which showed minimum resistance or no resistance was MIC detection rather than disc diffusion.

Table.1 Resistance pattern of *Enterococcus* spp. expressed in percentage

Drugs	<i>E.fecalis</i> (N=324)	<i>E.fecium</i> (N=70)
Penicillin	93.4	97.3
Ampicillin	51.5	52
Erythromycin	74.9	76.2
Tetracyclin	76	80
High Level gentamicin	50	53
Ciprofloxacin/Norfloxacin	78.5	72
Vancomycin	16.5	18
Teicoplanin	10.7	12.5
Linezolid	11.2	12.2

E.fecalis showed 11.2 % resistance and *E.faecium* showed 12.2% resistance to Linezolid in our study. As resistance to first level antibiotics were increasing the drug of choice for treating *Enterococcus* infections become Linezolid. Here also MIC detection could have been a better option for detecting Linezolid resistance. When comparing with vancomycin resistance Linezolid resistant (LR) strains were less frequently isolated. About 10% resistance in biofilm producing isolates recorded in one study from Iran. None of the isolates were identified as resistant to Linezolid in a study from Pondicherry, India.

Isolates from ICU patients studied also showed no resistance, here also MIC was detected (Anita hallgren *et al.*, 2001). Emergence of Linezolid Resistant *Enterococcus* (LRE) started reporting from many countries. Nine out of 35 found to be LRE in a study which analyse resistant *Enterococcus* isolated from rectal swab (Maria Grazia Bonora *et al.*, 2006).

The present study conclude that *Enterococcus* isolates were responding well to higher antibiotics like Glycopeptides and Oxazolidinone group. Next choice can be High level Gentamicin and Ampicillin.

Other group of antibiotics showed higher rate of resistance that is more than 70%. Another important message to convey through our study is that drug resistance has significantly varies with the different methods in the detection of resistance. MIC detection is required to perform when we planned to identify special isolates like VRE & LRE. Limitation of our study is failure in detecting MIC especially for VRE. In future we would like to collect some more isolates for performing genotypic study for identifying resistance gene.

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