A COMPARATIVE STUDY ON ANTIMICROBIAL RESISTANCE PATTERN INCLUDING HIGH LEVEL AMINOGLYCOSIDE AND IMIPENEM RESISTANCE OF ENTEROCOCCAL URINARY AND NON URINARY ISOLATES

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Abstract:

Enterococci are the second leading cause of nosocomial infections. During the past decade, Enterococci resistant to multiple antibiotics (Vancomycin, lactams and Aminoglycosides) have been recognized making it a formidable nosocomial pathogen. It is crucial for laboratories to provide accurate antimicrobial resistance patterns for Enterococci so that effective therapy can be instituted. UTI is the commonest form of presentation of Enterococcal infections. Aim The present study was undertaken with the objectives to isolate, identify and speciate the Enterococci isolated from different specimens and to compare antimicrobial resistance pattern of different Enterococcal species isolated from UTI with those isolated from other sources with special reference to High level Aminoglycoside (HLAR) and Imipenem resistance.

Materials and methods

A total of 100 non repetitive Enterococcal isolates, 50 consecutively from urine and 50 consecutively from various other sources (pus, wound swab, sterile fluids, blood etc.) were included in this study. Antimicrobial susceptibility testing was performed as recommended by CLSI for various antibiotics. Gentamicin (120 mg) and Streptomycin (300 g) was used to detect High level Aminoglycoside resistance. For Imipenem sensitivity, Ampicillin (10 g) and Imipenem disks (10g) were used. Vancomycin MIC was determined. Results E. faecalis was the commonest species isolated (63) in this study. E. faecium was the commonest isolate in bloodstream infections and was also more resistant to antimicrobials. Identification to species level is important for deciding treatment options. Imipenem Ampicillin resistance was noted in 46 of the total isolates. 70 of the Enterococci showed HLAR. Combined HLGR and HLSR was higher in E. faecium (48) than E. faecalis (42). Resistance to lactam antibiotics was higher in Enterococci isolated from urine than those isolated from other sources where as resistance to High level Aminoglycosides was found more in Enterococci isolated from other sources than those isolated from urine.
HLAR Enterococci were found to be resistant to multiple antibiotics. The overall resistance to Chloramphenicol and Rifampin was found to be 24 and 20 in our study and stills remains as a treatment option of enterococcal infections. VRE was noted in 2 of cases in this study.  

**Keyword**: Enterococcus spp, UTI, Antimicrobial susceptibility testing, VRE.

**INTRODUCTION:**
Enterococci are the commensal microorganisms that act as agents of infection, particularly in hospitalized patients and people with serious underlying conditions. Enterococci are intrinsically more resistant to antimicrobial agents that are commonly used in hospitals. In addition, these organisms are capable of acquiring and exchanging genes that encode resistance to antimicrobial agents. Enterococci are also the first clinically relevant group of Gram positive Cocci to acquire and disseminate resistance to Vancomycin, the single cell wall active agent available for use against Gram positive cocci resistant to Lactams. Multiple factors contribute to colonization and infection with Vancomycin resistant Enterococci ultimately leading to environmental contamination and crossinfection. Spread of this troublesome resistance marker from Enterococci to other clinically relevant organisms like *Staphylococcus aureus* is a serious public health concern.

Enterococci are the second leading cause of nosocomial infections. Urinary tract infections (UTI) are the most common of Enterococcal infections and are implicated in 10% of all UTIs and 16% of all nosocomial UTIs. They also account for 20% of native valve and 6-7% of prosthetic valve endocarditis. They are also implicated in Bacteremia, intra abdominal and pelvic abcess, biliarytract infections, Surgical site infections and rarely in otitis, sinusitis, septic arthritis and endophthalmitis.

*E. faecalis* is the most frequent Enterococcal spp. isolated which constitutes 80-90% of isolates, followed by *E. faecium*, which is constitutes 5-10% of enterococcal isolates. Prevelance of high level Aminoglycoside resistance (HLAR) among Enterococci (17-66% in various studies) is of concern knowing the limited options at hand to tackle the same. Various levels of resistance is already known for Gentamicin (HLGR), Kanamycin (HLKR) and Streptomycin (HLSR) individually. HLAR is executed by aminoglycoside modifying enzymes coded by plasmid and are transferable. Although no single enzyme can inactivate all available aminoglycosides, 30% of VRE can produce multiple enzyme types and thus highly resistant to all known aminoglycosides.

Imipenem, the first widely used carbapenem antimicrobial agent, was shown in early studies to have good in vitro activity against *Enterococcus faecalis* but little activity against *Enterococcus faecium*. Imipenem combined with Aminoglycoside is the treatment of choice in serious *Enterococcus* infections. Imipenem resistance has also been noted widely among Enterococci. Resistance to Ampicillin has been proved as a good indicator of Imipenem resistance for *E. faecalis*. This study will focus on occurrence of these two resistance phenotypes in Enterococci from different sources.

Nosocomial acquisition of microorganisms resistant to multiple antibiotics represents a threat to patient safety and Enterococci is one such Nosocomial pathogen. In view of the increasing importance of Enterococcal infections and the current scarcity of pertinent clinical data in the medical
literature, the purpose of the present study was to analyse infections caused by *Enterococcus* spp. from UTI and various other specimens in patients followed up at a tertiary care hospital.

**AIM AND OBJECTIVES:**

To isolate, identify and speciate the *Enterococci* isolated from different specimens (urine, swabs, drain, blood cultures and other sterile body fluids). To determine their anti microbial resistance pattern to various antibiotics with special reference to high level Aminoglycoside and Imipenem resistance. To compare anti microbial resistance pattern of different *Enterococcal* species isolated from urinary tract infections with those isolated from other sources.

**MATERIALS & METHODS:**

The present study was carried out at a tertiary care hospital, Chennai. A total of 100 non repetitive *Enterococcal* isolates, 50 consecutively from urine and 50 consecutively from various other sources (which includes pus, wound swab, sterile fluids, blood etc.) isolated between June 2011 to September 2011 were included in this study. Mono/polymicrobial growth of *Enterococci* with colony count >10⁵ Colony forming units (CFU)/ml in urinary tract infections and any pure/predominant growth of *Enterococci* from other specimens like pus, drain, blood and other sterile body fluids was included in the study. *Enterococci* isolated with a colony count <10⁵ CFU/ml in UTI and *Enterococci* isolated only once in a blood culture were excluded from the study. Ethical clearance for the study has been obtained from the Institutional Ethical committee.

Speciation was based on conventional method described by Koneman et al, key identifying features being Gram stain, esculin hydrolysis, heat tolerance, salt tolerance for *Enterococci* spp., tolerance to tellurite for *E. faecalis*, fermentation of Arabinose and Mannitol for *E. faecium* and inability to utilise sugars and to grow in pyruvate broth for *E. durans*.

Antimicrobial susceptibility to Penicillin (10 U), Ampicillin (10 µg), High level Gentamicin (HLG 120µg), High level Streptomycin (HLS 300 µg), Tetracycline (30 µg), Erythromycin (15 µg), Norfloxacin (10 µg) & Nitrofurantoin (300 µg) (for urine isolates only), Chloramphenicol (30 µg) (except for urine), Linezolid (30 µg) and Rifampin (5 µg) (All disks obtained from Hi Media, Mumbai ISO 13485-2003 certified) was determined by Kirby Bauer’s disk diffusion method. Vancomycin MIC was determined by Broth macrodilution method as per CLSI guidelines. Appropriate Quality control strain *E. faecalis* ATCC 29212 and *S. aureus* ATCC 25923 were used.

**KEY BIOCHEMICAL REACTIONS OF DIFFERENT SPECIES OF ENTEROCOCCI.**
RESULTS: Table 1: DISTRIBUTION OF SPECIES OF ENTEROCOCCI FROM VARIOUS CLINICAL SPECIMENS.

E. faecalis was the predominant isolate constituting 63% of the total isolates followed by E. faecium (29%) and E. durans (8%). A significant increase in E. faecium isolates was observed in blood where 6 out of 10 isolates were E. faecium (60%) (p<0.05).

DISTRIBUTION OF SPECIES OF ENTEROCOCCI FROM VARIOUS CLINICAL SPECIMENS

Resistance rates of Enterococcus faecalis isolates from urine (42.8%) to Ampicillin and Imipenem was comparable to those isolated from other sources (40%). Resistance of Enterococcus faecium isolated from urine (63%) was higher than the resistance noted in isolates from other sources (52%). Resistance to Streptomycin was slightly lower than the resistance to Gentamicin in both the categories. When comparing Enterococci from both sources, the Enterococcal isolates from other sources showed increased resistance to high level Aminoglycosides (70%) when compared to isolates from urine (56%).

46% of the total isolates showed Ampicillin/Imipenem resistance. Imipenem resistance was noted highest in E. durans isolates from urine (75%). 70% of the Enterococci showed HLAR (either of HLGR and HLSR) and combined HLGR and HLSR was higher in E. faecium, 14 of 29 (48%) than E. faecalis, 27 of 63 (42%). A/I-Ampicillin/Imipenem. HLGR-High level gentamicin resistance. HLSR-High level Streptomycin resistance.

CSF and Ascitic fluid isolates were not compared because of less number of isolates.

Concomitant resistance of HLGR and HLSR strains to the two lactam antibiotics, Penicillin and Ampicillin was quite high for both the species (p<0.05) and it was higher to Penicillin (81%) than Ampicillin (55%) in E. faecalis. Chloramphenicol resistance was found to be significantly higher in HLAR isolates (14.2% in E. faecalis and 12.7% in E. fecium) than isolates with no HLAR (4.7% in E. faecium). (p<0.05)

DISCUSSION:

Isolates of Enterococci were obtained from various clinical specimens. 50 isolates from urine, 18 isolates from pus/aspirate, 16 isolates from wound swabs, 10 isolates from blood, 5 isolates from ascitic fluid and 1 from CSF. 82% was pure growth of Enterococci spp. 10% of Enterococcal infections were polymicrobial, other organisms that were isolated along with Enterococci being Escherichia...
coli, Klebsiella pneumoniae, Citrobacter koseri, Pseudomonas aeruginosa and Candida spp. E. faecalis was the predominant isolate constituting 63% of the total isolates followed by E. faecium (29%) and E. durans (8%)(Table 1). The ratio of E. faecalis to E. faecium isolates in urine is 4.7:1 whereas the ratio of E. faecalis to E. faecium isolates in isolates from other sites is 1.19:1. An increase in E. faecium isolates was observed in blood where 6 out of 10 isolates were E. faecium (60%). An increase in the occurrence of E. faecium in blood is a significant observation that has been noted in our study. These observations are similar to the study done by Seema Sood et al, 2008, and Edwards et al, 2000 who have recorded that while E. faecalis remains the predominant species, the E. faecium isolates are increasing in number. They have observed a ratio of 1.9:1 for E. faecalis to E. faecium. 9, 10 Srujana Mohanty et al, 2005, Gray et al, 1991 and Simonson et al, 2003 have also found a greater proportion of E. faecium in blood cultures and E. faecalis in cultures of samples from other sites. 11, 12, 13 Changes in the hospital population and antimicrobial use patterns coupled with a greater antibiotic resistant nature of E. faecium probably confers a greater selective survival advantage compared to E. faecalis and explains the emergence of E. faecium bloodstream infections. 20 Thus, the traditional outnumbering of E. faecium by E. faecalis in clinical specimens no longer seems to be valid. This further supports the fact that we should study the distribution of Enterococcal species among various specimens to reflect the local prevalence of E. faecium isolates. E. durans was also noted in 8% of the isolates. This was compared to the study by Lata Kapoor et al 14 and Srujana et al 11 (8% and 1.9% respectively) and was found to be similar to our study.

Antimicrobial profile of Enterococci from urine and other specimens were determined and compared.(Table 2&3). In our study, Enterococci responded poorly to Penicillin, Erythromycin and Tetracycline making their therapeutic use in empirical therapy practically irrational in our hospital setup. Resistance to Imipenem can be predicted by Ampicillin resistance for E. faecalis. 15, 16 Resistance rates of Enterococcus faecalis isolates from urine (42.8%) to Ampicillin and Imipenem was comparable to those isolated from other sources (40%) while resistance of Enterococcus faecium isolated from urine (63%) was higher than the resistance noted in isolates from other sources (52%). Resistance to Streptomycin was lower than the resistance to Gentamicin in both the categories. When comparing Enterococci from both sources, the Enterococcal isolates from other sources showed increased resistance to high level Aminoglycosides (70%) when compared to isolates from urine (56%). All the urinary isolates were sensitive to Nitrofurantoin and Rifampin making them available for the treatment of uncomplicated urinary tract infections. These results are comparable with the results of Baragundi MC et al who has noted the resistance of urinary isolates to be more than 70% for Ampicillin, Erythromycin and Tetracycline, HLGR in 77.69% of isolates and HLSR in 61.63% of isolates. They have also noted that E. faecium was more resistant to antimicrobials than E. faecalis which is comparable with the results of our study. 17 In our study, the overall resistance to Chloramphenicol and Rifampin was found to be 24% and 20% and they still remain as a treatment option of Enterococcal infections excluding serious infections like
blood stream infections and meningitis. Similar results have been observed by Lata Kapoor et al\textsuperscript{14} who has observed Chloramphenicol resistance to be 0%, Ampicillin resistance to be 70% and Penicillin resistance to be 100% in blood isolates of Enterococci. Vancomycin resistant Enterococci (VRE) was isolated in 2% samples, one each from blood and pus sample. The patient with Enterococci bacteremia succumbed due to multiorgan failure whereas the other patient with wound infection by VRE responded well to Chloramphenicol. Indian studies have reported Vancomycin resistance in 0-5% of enterococci\textsuperscript{21,22,23}

In the present study, 46% of the total isolates showed Ampicillin/Imipenem resistance. Imipenem resistance was noted highest in \textit{E. durans} isolates from urine (75%). 70% of the enterococci showed High level Aminoglycoside resistance (either HLSR or HLGR) and combined HLGR and HLSR was higher in \textit{E. faecium}, 14 of 29 (48%) than \textit{E. faecalis}, 27 of 63 (42%). (Table 4) Resistance to lactam antibiotics was higher in Enterococci isolated from urine than those isolated from other sources, where as resistance to High level Aminoglycoside was found more in Enterococci isolated from other sources than those isolated from urine. This could be explained by the increased usage of lactam antibiotics for Urinary tract infections in our hospital.

Significantly high levels of resistance was noted in \textit{E. faecium} strains isolated from non urinary samples. (Table 5) In pus, Aminoglycoside resistance was found to predominate (66.67% in \textit{E. faecalis} and 83.33% in \textit{E. faecium}). Aminoglycoside and Imipenem/Ampicillin resistance was comparable in isolates from wound swab. High level Aminoglycoside resistance was found to predominate in \textit{E. faecium} isolates of pus samples (83.33%). Ampicillin/Imipenam resistance was found maximum in \textit{E. durans} of wound swab (100%) followed by \textit{E. faecium} of pus (66.67%).

Resistance to Aminoglycosides in Enterococci is often associated with multidrug resistance\textsuperscript{18}. Concomitant resistance of HLGR and HLSR strains to the two lactam antibiotics, Penicillin and Ampicillin was quite high for both the species and it was higher to Penicillin (81%) than Ampicillin (55%) in \textit{E. faecalis} (Table 5). Resistance to Penicillin and Ampicillin is primarily due to low affinity of penicillin binding proteins (PBPs) and it results in loss of synergistic effect between lactams and Aminoglycosides leading to treatment failures. HLAR has also been linked to lactamase production, resistance to Quinolones and Chloramphenicol. Even in our study, Chloramphenicol resistance was found to be significantly higher in HLAR isolates (14.2% and 12.7% in \textit{E. faecalis} and \textit{E. fecium} isolates) than isolates with no HLAR (4.7% in \textit{E. faecium}). This multidrug resistance in addition to the intrinsic resistance already seen in Enterococci will be a potential threat in near future. These findings are supported by the study done by Mendiratta et al\textsuperscript{20} who reports 91.4% resistance of HLAR isolates to Penicillin and 87.2% to Ampicillin.

Vancomycin resistance was seen in 2 cases of \textit{E. faecium}. Both showed HLAR and Ampicillin/Imipenem resistance (4.8% of \textit{E. faecium}). Concomitant HLAR, high level Penicillin resistance and resistance to Vancomycin has been reported in 16% isolates by Agarwal et al\textsuperscript{19}. The overwhelming majority of clinically Vancomycin resistant strains are \textit{E. faecium} which is inherently more resistant to
multiple drugs making therapy more problematic. The vast majority of E. faecium than those isolated from urine. HLAR strains that are resistant to Vancomycin also Enterococci were found to be resistant to multiple antibiotics. The overall resistance was found to be 24% and 20% respectively in our study and still remains as a treatment option of Enterococcal infections excluding serious infections like blood stream infections and meningitis. VRE was noted in 2% of cases in this study. This cautions us of the impending threat of serious consequences when these organisms are allowed to spread in a tertiary care hospital.

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