

Study Of Antibiotic Resistance Pattern Of Enterococci With Special Reference To Newer Antibiotic

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Abstracts: **Background:** Multidrug-resistant Enterococci are major problem and are increasingly reported worldwide. Enterococcal infections have been associated with higher hospitalization costs and a higher number of related deaths. Treatment of multidrug-resistant enterococci has become a challenge in hospitals around the world due to the lack of reliable therapeutic options. So the present study was undertaken with the aim to know the antimicrobial resistant pattern of enterococcus to newer antibiotic. **Methodology:** 22 isolates of enterococcus resistant to the entire routinely tested antibiotic were further tested for newer antimicrobial agent linezolid, daptomycin, rifampin, synergid, teicoplanin, telavancin, vancomycin, gentamicin (HLG). **Results:** Among these 22 enterococci isolated none of our isolates showed resistance to vancomycin, teicoplanin, telavancin, tigecycline and linezolid. But one isolate of *E.faecalis* showed resistance to daptomycin. This daptomycin non-susceptible isolate was found susceptible to ceftaroline. *E. faecalis* showed higher resistance to Gentamicin (HLG) and Synergid as compared to *E.faecium*. Specimen-wise higher distribution of enterococci (resistant to routinely tested antimicrobial agents) was observed in Urine 11(50%) followed by Pus 4(18.18%) and Miscellaneous 4(18.18%) and from Blood 3(13.64%). **Conclusion:** In our study none of the isolates showed resistance to vancomycin, tigecycline, telavancin, linezolid and ceftaroline but the presence of daptomycin non-susceptible enterococci 1(4.55%) in our rural set-up is cause of concern. [Kaur D NJIRM 2016; 7(1):50-54]

Key Words: Enterococci, Newer antibiotic- ceftaroline, telavancin, linezolid, daptomycin.

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Introduction: Nosocomial infections with enterococci are major concern and are increasingly reported worldwide. Enterococci are the gram-positive bacteria inhabiting the gastrointestinal tracts of humans and many animals. The most common nosocomial infections produced by these organisms are urinary tract infections, surgical wound infections, bacteraemia, endocarditis, neonatal sepsis and rarely meningitis.^{1,2} *E.faecalis* is the most common cause (80-90%) of infection followed by *E.faecium*(10-15%)² Enterococcal infections are the third most common cause of nosocomial infection in intensive care units (ICUs), and multidrug-resistant enterococcal infections have been associated with higher hospitalization costs and a higher number of related deaths.³

Over the past three decades, enterococci have shown resistance to many commonly used antimicrobial agents.⁴ The resistance may be intrinsic or acquired via gene transfer.⁵ The antimicrobial treatment of Enterococcal infections is complicated because of the inherent resistance shown by enterococci to several commonly used antibiotics such as cephalosporins, low-level aminoglycosides, and low-level clindamycin. As this organism shows acquired resistance to all currently available antibiotics, tigecycline, daptomycin, linezolid, ceftaroline and telavancin are now the drugs of choice for the treatment of infections.

Recently few cases of linezolid (LZD)-resistant enterococci and also the emergence of daptomycin resistance in *Enterococcus faecium* during daptomycin therapy are observed.^{6, 7, 8} Daptomycin-resistant *Enterococcus faecium* in a patient with no prior exposure to daptomycin.⁹ Daptomycin-nonsusceptible vancomycin-resistant *Enterococcus faecium* (VRE) strains are a formidable emerging threat to patients with comorbidities, leaving few therapeutic options in cases of severe invasive infections. So the present study was undertaken with the aim to know the antimicrobial resistant pattern of enterococcus to newer antibiotic

Material and Methods: A Prospective study was carried out in the department of Microbiology during the period of January 2012 to August 2014. Enterococcus isolated from various clinical specimens was tested for routine antibiotic by Kirby-Bauer disc diffusion method on Mueller-Hinton agar as per CLSI.¹⁰ The antibiotics tested were Amikacin (Ak) 30µg, ciprofloxacin (CIP) 5µg, gentamicin (G) 30µg, calithromycin (CLR) 15µg, cefotaxime (CF) 30µg, sparfloxacin (SF) 5µg, cefuroxime (CR) 30µg, cefoperazone (CFP) 30µg, ampiclox (ACX) 20µg, azithromycin (AZ) 15µg, cefadroxil (CD) 30µg, roxythromycin (RX) 30µg, tetracycline, erythromycin (E) 15µg. Antibiotic disc was obtained from Hi-media Laboratories Pvt. Ltd, Mumbai, India.

22 isolates of enterococcus resistant to the entire routinely tested antibiotic were further tested for newer antimicrobial agent linezolid, daptomycin, rifampin, synergid, teicoplanin, telavancin, vancomycin, gentamicin (HLG). Susceptibility breakpoint for daptomycin was considered as $<4 \mu\text{g/ml}$ for enterococci and linezolid susceptible breakpoint of $<2 \mu\text{g/ml}$ for enterococci.¹⁰ daptomycin non-susceptible isolate was tested for ceftaroline. A p value of <0.05 was taken as statistically significant.

Results: 22 (11.89%) isolates of Enterococci isolated from various clinical specimens were resistant to the routinely tested antibiotic. Specimen-wise higher distribution of enterococci (resistant to routinely tested antimicrobial agents) was observed in Urine 11(50%) followed by Pus 4(18.18%) and Miscellaneous 4(18.18%) and from Blood 3(13.64%). (Table 1) No statistical significance was noted gender-wise.

Table 1: Distribution of 22 Enterococcus among the specimen

Specimen	E.faecalis (n=11)	E.faecium (n=11)	Total 22
Pus	1	3	4(18.18%)
Blood	1	2	3(13.64%)
Miscellaneous	3	1	4(18.18%)
Urine	6	5	11 (50%)
Total	11	11	22

Table 2: Antibiotic resistant pattern of 22 enterococcus isolates (resistant to the routinely tested antibiotic)

Antimicrobial agent	E.faecalis (n=11)	E.faecium (n=11)
gentamicin (HLG)	4	1
rifampin/rifampicin	3R/2I	5R/3I
vancomycin	0	0
Telavancin	0	0
Teicoplanin	0	0
Tigecycline	0	0
Daptomycin	1	0
Linezolid	0	0
ampicillin	1	11
synergid	11	5I

R- Resistance; I-Intermediate Resistance

Table 1 depicts 50% of Enterococcus from urine

Among these 22 enterococci isolated none of our isolates showed resistance to vancomycin,

teicoplanin, telavancin, tigecycline and linezolid. But one isolate of *E.faecalis* showed resistance to daptomycin. This daptomycin non-susceptible isolate was found susceptible to ceftaroline. *E.faecalis* showed higher resistance to Gentamicin (HLG) and Synergid as compared to *E.faecium*. (Table 2)

Table 2 depicts none of our isolates showed resistance to vancomycin, teicoplanin, telavancin, tigecycline and linezolid. But one isolate of *E.faecalis* showed resistance to daptomycin. *E.faecalis* showed resistance to Gentamicin (HLG) and Synergid as compared to *E.faecium*.

Discussion: Treatment of multidrug-resistant enterococci has become a challenging clinical problem in hospitals around the world due to the lack of reliable therapeutic options. These infections are recognized by 3 ts - tough, tenacious and oftentimes troublesome.

In our study out of the 22 isolates resistant to routinely tested, none of them were resistant to vancomycin, teicoplanin, telavancin, tigecycline and linezolid but one isolate of *E.faecalis* showed daptomycin non-susceptible ($<4 \mu\text{g/ml}$). The prevalence of VRE is reported to the tune of 5.5% to 23%.^{11,12}

S. Chitnis et al in their study observed out of 144 clinical isolates of enterococci, vancomycin resistance in 14.29% and 100% were susceptible to daptomycin.¹³ India Daptomycin Study Group reported daptomycin MIC in the range of 0.047- $1 \mu\text{g/ml}$.¹⁴ In a first study from India, Dhawan B et al reported 100% susceptible to daptomycin against vancomycin-resistant *Enterococcus faecium* (VREF), this isolates were recovered from hospitalized patients with skin and soft tissue infections.¹⁵ Lewis JS et al observed emergence of daptomycin resistance in *Enterococcus faecium* during daptomycin therapy.⁸

We found 100% susceptible to vancomycin and teicoplanin. S.Chitnis observed enterococci isolates with high MIC for vancomycin also had high MIC for teicoplanin suggesting cross-resistance between the two drugs.¹³

Daptomycin possesses a unique mechanism of action that involves interactions with the cell membrane in a calcium-dependent manner, mainly at the level of the bacterial septum. Development of daptomycin resistance is associated with mutations in genes

encoding proteins with two main functions, (i) control of the cell envelope stress response to antibiotics and antimicrobial peptides (LiaFSR system) and (ii) cell membrane phospholipid metabolism.¹⁶ No cross-resistance with other classes of antimicrobial agents has been documented, making it an option for the treatment of infections caused by multidrug-resistant gram-positive organisms.

There are only 2 reports of the development of spontaneous daptomycin susceptibility in clinical *Enterococcus* isolates from patients with no documented prior exposure to the agent.^{9,17}

In our study daptomycin susceptible isolate was tested for susceptible to ceftaroline. Ceftaroline, is a new, broad-spectrum cephalosporin recently approved in the USA for the treatment of acute bacterial skin and skin structure infections (ABSSSIs) and community-acquired bacterial pneumonia (CABP). George Sakoulas et al in their study observed ceftaroline restores daptomycin activity against daptomycin-nonsusceptible vancomycin-resistant *Enterococcus faecium*. ceftaroline showed synergy against the daptomycin-nonsusceptible VRE strain (~2 log₁₀ CFU reduction at 24 h). Ceftaroline cotreatment increased daptomycin surface binding with an associated increase in membrane fluidity and an increase in the net negative surface charge of the bacteria as evidenced by increased poly-L-lysine binding. ceftaroline resulted in increased binding and killing of daptomycin-nonsusceptible VRE by human cathelicidin LL37.¹⁸ George ME et al in their literature study found the prevalence of daptomycin susceptibility was 0.2% (10 of 4051; range 0.1%–1.1%) for VSE isolates 1.0% (34/3445; range 0.0%–9.9%) for VRE isolates, 2.6% (54 of 2092; range 0.6%–19.5%) for *E faecium*, and 0.1% (5/3398; range 0.0%–1.3%) for *E faecalis* isolates. They found the overall prevalence of daptomycin susceptibility for all *Enterococcus* isolates was 0.6% (111 of 17084; range 0.0%–19.1%).¹⁹

Linezolid is a member of a class oxazolidinones. As it does not require testing for adequate serum drug concentrations or dose adjustment in renal or hepatic failure, it is a valuable drug in the hands of clinicians and can be used in situations where vancomycin use is either contraindicated or ineffective. Linezolid was licensed for clinical use in the United States in 2000. It was approved for use in United Kingdom a year later. The first isolates

of *Enterococcus* resistant to Linezolid were reported from the United Kingdom in 2002.⁶

In our study we observed all our enterococci isolates were 100% susceptible to linezolid. The reason may be these drugs are reserved in our institute. Recurrent linezolid-resistant *Enterococcus faecalis* infection was reported in a patient with pneumonia by Zhi-jian Yu et al.⁷ Their study indicated that LZD-resistant *E. faecalis* strains may colonize persistently in vivo, leading to recurrent infection and resistance is usually associated with prior and prolonged exposure. Linezolid-resistant and vancomycin-resistant enterococci have also been isolated from patients without any prior therapy with linezolid.²⁰ Linezolid exposure and patient-to-patient transmission appear to be responsible for LRE infections by Marion AK et al.²¹ In a clonal outbreak study by Dobbs et al, 6 (15%) of the patients had received linezolid before contracting LRVRE, and 17 (42.5%) were in a particular ICU before acquiring LRVRE.²²

In our study *Enterococci* isolates showed 100% resistance to tetracycline but no isolate showed resistance to tigecycline. Tigecycline is a broad-spectrum glycylcycline antimicrobial agent which was introduced in 2005. Esteban CN, in their finding reported tigecycline, showed good in vitro and in vivo in a mouse peritonitis model against several *E. faecalis* and *E. faecium* strains, including tetracycline-resistant strains, suggesting that this may be an attractive agent for the treatment of enterococcal infections.²³ Tigecycline-resistant *Enterococcus faecalis* isolates have been reported from surgical and intensive care unit patient.^{24,25}

In a study, Tigecycline in vitro surveillance conducted in Taiwan hospitals (TIST), during the period of 2006 to 2010, all isolates were susceptible to linezolid and daptomycin, and 98.6% were susceptible to tigecycline. Tsai HY et al observed there was a shift toward higher tigecycline MIC values (MIC (90) from 2006-2007 (0.06 µg/ml) to 2008-2010 (0.12 µg/ml). The MIC (90)s of daptomycin and linezolid remained stationary.²⁶ Deshpande et al. reported resistance to 2.4% linezolid, 11.3% teicoplanin, 11.3% vancomycin 73.5% HLG, 70.8% HLS.²⁷

Enterococcus shows inherent resistance to low-level aminoglycoside. The 22 isolates resistant to gentamicin was tested for HLG, we found 5 isolates were resistant to gentamicin (HLG). Four were *E. faecalis* and one

E. faecium. A study from Delhi, reported 81% of *E. faecium* and 72% of *E. faecalis* isolates exhibited HLAR.²⁸

In our study we found all our isolates were susceptible to telavancin. W. T. M. Jansen et al in their study observed telavancin showed high potency against vancomycin-susceptible enterococci, with (MICs ranging from ≤ 0.015 to $0.5 \mu\text{g/ml}$) as compared to VRE (MIC range from 0.12 to $8 \mu\text{g/ml}$). They found overall MIC₅₀ and MIC₉₀ for VRE were 4 times higher than those of non-VRE (1 and 2 $\mu\text{g/ml}$ versus 0.25 and 0.5 $\mu\text{g/ml}$). They also reported telavancin was the most active agent against vancomycin-resistant *E. faecium* (MIC₅₀ and MIC₉₀, 1 and 2 $\mu\text{g/ml}$, respectively), followed by daptomycin and linezolid. And against vancomycin-resistant *E. faecalis*, daptomycin showed the highest activity (MIC₅₀ and MIC₉₀, 0.5 and 2 $\mu\text{g/ml}$, respectively), followed by linezolid (MIC₅₀ and MIC₉₀, both 2 $\mu\text{g/ml}$).²⁹ In the resistance selection studies by Klaudia Kosowska-Shick et al, telavancin have a low potential for selection of spontaneous resistant mutants independently of bacterial species or resistance phenotype. The two enterococci developed resistance to daptomycin, and one developed resistance to linezolid. Single-step mutation frequencies for telavancin ($< 4.0 \times 10^{-11}$ to $< 2.9 \times 10^{-10}$ at 2x MIC) were lower than the spontaneous mutation frequencies.³⁰

Conclusion: In our study none of the isolates showed resistance to vancomycin, tigecycline, telavancin, linezolid and ceftaroline but the presence of daptomycin non-susceptible enterococci 1 (4.55%) in our rural set-up is cause of concern.

To prevent the emergence of resistance to newer antimicrobial agents, we recommend regular monitoring of antimicrobial resistance of the microorganism.

Antibiotic and infection control policy in hospitals to be strictly followed. Early detection of patients colonized or infected with VRE to prevent nosocomial transmission of VRE. Antimicrobial stewardship programmes can be implemented to reduce inappropriate use of antimicrobials, thereby controlling the development of resistance

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