Original article:

Prevalence of High Level Aminoglycoside Resistance Among Enterococcal Isolates with special reference to Multidrug Resistance : A one year study

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Abstract:

Introduction: A study of epidemiological profile of infections caused by *Enterococci* and their antibiotic resistance pattern with relation to high level aminoglycosides and multidrug resistance was carried out over a period of one year at a tertiary care teaching hospital.

Methodology: The study comprised of 9522 routine clinical specimens of which 140 enterococcal isolates were recovered and speciated as per the scheme of Facklam and Collins. Antibiotic susceptibility was determined for various drugs including high level aminoglycoside and vancomycin by Kirby–Bauer disc diffusion method and results interpreted as per CLSI guidelines.

Observation: Amongst 140 enterococcal strains, *E. faecalis* (47.85%) was the commonest species isolated followed by *E.faecium*. Majority of isolates were recovered from urine and pus. In present study overall highest resistance was observed against erythromycin (86.42%) and ciprofloxacin (80.71%) while only 2.85% strains showed vancomycin resistance. Most resistant species was *E.faecium* , with more than 80% resistance against erythromycin, ciprofloxacin & high level gentamicin. Multidrug resistance was observed in 28.35% of *E. faecalis* and 58.52% of *E. faecium*, commonest phenotype being ampicillin, ciprofloxacin , high level gentamicin resistance.

Conclusion: Results of this study provides benchmark activity of three most traditionally used antibiotics i.e. ampicillin, ciprofloxacin and gentamicin (high level) against local enterococcal isolates and will serve as baseline to monitor future changes in resistance pattern. Furthermore, the knowledge of resistance pattern helps in empirical treatment and to initiate preventive measures.

Keywords: multidrug resistance , enterococi

Introduction:

Enterococi usually inhabit the alimentary tract, genital tract of humans in addition to being isolated from environmental and animal source.They have been implicated in variety of clinical infections like urinary tract infections, hepatobiliary sepsis, endocarditis, surgical wound infection, bacteraemia and neonatal sepsis.[1] Furthermore enterococci have assumed greater importance because of their increasing resistance to many commonly used antimicrobial agents.[2] They have a remarkable ability to adapt to the environment and acquire antibiotic resistance determinants. The evolution of antibiotic resistance in these organisms poses enormous challenges for clinicians when faced with patients having severe infections. The increased frequency of multidrug resistance in clinical isolates has been reported. The emergence of vancomycin

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resistance in enterococci in addition to the increasing incidence of resistance to penicillins and high level aminoglycosides presents a serious therapeutic challenge for physicians treating patients with enterococcal infections. [3] Central to antibiotic resistant enterococcal prevention are timely antimicrobial resistance screening of all enterococcal isolates, especially vancomycin resistance & prompt reporting by clinical laboratory using accurate & reliable methods.

The study was undertaken to determine the antibiotic susceptibility of enterococci isolated from various clinical samples and to know the prevalence of High Level Aminoglycosides Resistance (HLAR) and multi drug resistance pattern among the isolates. With knowledge of local HLAR prevalence , clinicians can prescribe the various drug combination (cell wall inhibitor + aminoglycoside) at the very beginning of treatment thus avoiding the unnecessary usage of other antimicrobials.

Materials and methods:

Out of all the routine clinical specimens received in the microbiology laboratory, a total of 140 isolates of enterococci were recovered over a period of one year. The isolate was confirmed as enterococcus by-gram stain,catalase test, bile esculin hydrolysis, PYR test and salt tolerance test. Each enterococcal isolate was identified upto species level as per Facklam & Collins scheme.[4]

Antimicrobial susceptibility test for different classes of antimicrobials was performed by Kirby Bauer disk diffusion method and result was interpreted according to Clinical Laboratory Standards Institute guidelines 2014. Isolates were also screened for high level aminoglycoside resistance with respect to high content gentamicin disk (120 µg) and streptomycin disk (300µg). Those isolates which showed resistance to three or more antibiotics of different class were considered as Multidrug resistant.

Observation & Results:

Table1: Distribution of enterococci in various clinical specimens

Clinical specimens	**Number of strains (n= 140)**	**Percentage (%)**
Urine	97	69.29
Pus	22	15.71
Body fluid	6	4.29
Blood	10	7.14
Vaginal Swab	5	3.57
Total	140	100

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Table 2: Speciation of enterococcal isolates

Species	Number of strains (n=140)	Percentage (%)
E.faecalis	67	47.85
E.faecium	51	36.42
E.raffinosus	7	5.00
E.casseliflavus	8	5.71
E.durans	7	5.00
Total	140	100

Species	Urine n=97	Pus n=22	Fluid n=6	Blood n=10	Vaginal Swab n=5	Total n=140
	no (%)	**no (%)**	**no (%)**	no (%)	**no (%)**	**no (%)**
E.faecalis	46(47.42)	13(59.09)	3(50.0)	3(30.0)	2(40.0)	67(47.85)
E.faecium	33(34.02)	7(31.81)	1(16.66)	7(70.0)	3(60.0)	51(36.42)
E.casseliflavus	8(8.24)	0(0)	0(0)	0(0)	0(0)	8(5.71)
E.raffinosus	5(5.15)	0(0)	2(33.33)	0(0)	0(0)	7(5.0)
E.durans	5(5.15)	2(9.09)	0(0)	0(0)	0(0)	7(5.0)
Total	97	22	6(100)	10(100)	5(100)	140(100)

Table 3: Specimen wise distribution of enterococcal species

Table 4: Antimicrobial resistance pattern of various enterococci.

Antibiotic	*E.faecalis n=67*	*E. faecium n=51*	*E.raffinosus n=7*	*E. casseliflavus n=8*	*E. durans n=7*	*Total n=140*
	no (%)	**no (%)**	**no (%)**	**no (%)**	**no (%)**	**no(%)**
Penicillin	32(47.76)	38(74.50)	2(33.33)	5(62.50)	0 (0)	77(55.0)
Ampicillin	20(29.85)	38(74.50)	1(16.66)	5(62.50)	0 (0)	64(45.71)
Erythromycin	54(80.59)	45(88.23)	7(100)	8(100)	7(100)	121(86.42)
Ciprofloxacin	48(71.64)	45(88.23)	7(100)	6(75.0)	7(100)	113(80.71)
Tetracycline	20(29.85)	32(62.74)	5(71.42)	5(62.50)	5(71.42)	67(47.85)
Linezolid	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

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Vancomycin	2(2.98)	2(3.92)	0 (0)	0 (0)	0 (0)	4(2.85)
Teicoplanin	2(2.98)	2(3.92)	0(0)	0(0)	0(0)	4(2.85)
Genta(High level)	32(47.76)	42(82.35)	4(57.14)	7(87.5)	5(71.42)	90(64.28)
Norfloxacin	35(52.23)	38(74.50)	6(85.71)	6(75.0)	5(71.42)	90(64.28)
Nitrofurantoin	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0(0)
Fosfomycin	0 (0)	0(0)	0 (0)	0 (0)	0 (0)	0(0)
Quinpristin-Dalfopristin	48(71.64)	45(88.23)	7(100)	6(75.0)	7(100)	113(80.71)

Table 5: Distribution of high level aminoglycoside resistance (HLAR) in enterococcal species.

Resistance pattern	***E.faecalis n=67***	***E. faecium n=51***	***E.raffinosus n=7***	***E. casseliflavus n=8***	***E. durans n=7***	***Total n=140***
	no (%)	**no (%)**	**no (%)**	**no (%)**	**no (%)**	**no (%)**
Only HLGR	12(17.91)	38(74.50)	1(14.28)	5(62.5)	0(0)	56(40.0)
Only HLSR	2(2.98)	0(0)	2(28.57)	0(0)	0(0)	4(2.85)
HLGR + HLSR	20(29.85)	4(7.84)	3(42.85)	2(25.0)	5(71.42)	34(24.28)
Total	34(50.74)	42(82.35)	6(85.71)	7(87.50)	5(71.42)	94(67.14)

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Table 6: Species wise distribution of Multidrug resistant enterococci.

MDR type	***E.faecalis n=67***	***E. faecium n=51***	***E.raffinosus n=7***	***E. casseliflavus n=8***	***E. durans n=7***	***Total n=140***	
AMP, CIP, VAN	2(2.98)	2(3.92)	0	0	0	4(2.85)	
Amp, cip hlgr	16(23.88)	26(50.98)	0	3(37.5)	0	45(32.14)	
Amp, cip VAN, hlgr	1(1.49)	2(3.92)	0	0	0	3(2.14)	
Total	19(28.35)	30(58.82)	0	3	0	52(37.14)	

Results:

The study comprised of 9522 samples which included urine, pus ,blood, body fluids, vaginal swabs and other samples like aspirate, endotracheal tube tip, etc. received in the microbiology laboratory. A total of 140 enterococcal isolates were recovered from various clinical samples. Among these, majority of isolates were from urine (97) followed by pus (22) blood (10), body fluid (6) and vaginal swab(5) **(Table 1)**.

Table 2 shows species distribution of enterococcal isolates. In this study, out of total 140 isolates, *E.faecalis* 67 (47.85%) was the commonest species followed by *E.faecium* 51 (36.42%).

Antimicrobial resistance pattern of various enterococci is shown in **Table 4**. In present study overall highest resistance was observed against erythromycin (86.42%) and ciprofloxacin (80.71%) while only 4.82% strains showed vancomycin and teicoplanin resistance. Most resistant species was *E.faecium* , with more than 80% resistance against erythromycin, ciprofloxacin & high level gentamicin. Percentage positivity of the isolates having HLGR (40%) was higher in comparison to HLSR (2.85%) **(Table 5)**

Multidrug resistance defined as concurrent resistance to three / more antimicrobials of different chemical classes, was observed in 28.35% of *E. faecalis* and 58.82% of *E. faecium*, commonest phenotype being ampicillin, ciprofloxacin , vancomycin and high level gentamicin resistance **(Table 6).**

Discussion:

Enterococci have assumed greater importance because of their increasing resistance to many commonly used antimicrobial agents.[2] Over the decades, the occurrence of acquired antimicrobial resistance to high level aminoglycosides & glycopeptides (esp vancomycin) has been

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increasingly reported.[5] Since the knowledge of antibiotic resistance pattern is useful to formulate treatment guidelines the present study was undertaken with the aim to determine the profile of enterococcal species in various clinical specimens and to determine their antimicrobial susceptibility pattern with reference to high level aminoglyosides vancomycin and multi drug resistance.

In our study, majority (69.29%) of enterococci were isolated from urine (Table 1) similar to other studies. Luna adhikari obtained 67.22% [6], Padmasini et al obtained 60.11%[7] while M.G. Karmarkar et al obtained 50%[8] enterococcal isolates from urine samples. We recovered 22 (15.71%) isolates from pus samples, a finding similar to M.G Karmarkar et al who obtained 8 (19.04%) isolates from pus and wound swab.[8]Overall, the isolation rate from pus was lower in other studies also like Luna Adhikari obtained 13.89% of isolates from wound infection and Padmasini et al isolated only 5.05% of enterococci from pus samples. In our study, only 7.14% of enterococci were isolated from blood which was less as compared to other studies (table 3). Luna Adhikari obtained 17.22%,[6] Padmasini et al obtained 24.71%[7], Mathur et al obtained 38%,[9]and Edet et al obtained 10.4% of enterococci from blood samples.[10] This may have occurred because of the fact that almost all blood samples in our study were from sepsis cases and none was from endocarditis patients.

E.faecalis & *E.faecium* are responsible for the majority of human enterococcal infections. *E.faecalis* has been reported as the most common species in many studies and as shown in (table 2) we have also isolated 47.85% of *E.faecalis,* a finding similar to Padmasini et al (48.3%)[7]. Reported isolation rate for *E.faecium* in reviewed literature was observed to have a wide range , i.e 7.7% to 87.77%[10,8,11] . In our study, *E.faecium* isolates were 36.42% (table 2) while in M.G.Karamkar et al study it was 87.77%. This may be because of the fact M.G.Karamkar et al studied samples only from hospitalized patients while our study included both IPD & OPD patients' samples.

In our study, *E.faecalis* outnumbered other enterococci in all clinical samples except in blood cultures in which *E.faecium* was more common.i.e 70% followed by *E.faecalis* 30% as shown in (table 3). Similarly, Padmasini et al observed *E.faecium* as the predominat species (79.54%) followed by *E.faecalis* (15.90%) from blood samples[7]. Likewise, M.G.Karamkar et al reported the distribution of *E.faecium* & *E.faecalis* from blood isolates as 78.22% & 22.22% respectively [8] .Though the blood enterococcal isolates were few in our study, the proportion was almost similar to others.

All the 140 strains of enterococci were subjected to the antimicrobial drug susceptibility test by disk diffusion method and the results were analysed as per CLSI 2014[12]. Table 4 shows antimicrobial resistance pattern of various enterococci. Our study shows that overall highest resistance was observed against erythromycin (86.42%) and ciprofloxacin (80.71%).Our findings are as per the ones reported by an indian study i.e. Mathur et al who reported 85% of strains resistant to erythromycin and 88% resistant to ciprofloxacin[9]. Only 2.85% of strains in our study showed vancomycin & teicoplanin resistance.

It has been observed in various studies that *E.faecium* is comparartively more resistant than *E.faecalis* with respect to β lactams, erythromycin, tetracycline and fluoroquinolones. The observation of our study (table 4) is in accordance with the studies by M.G.Karmarkar et al[8]and Edet et al[10]. The much

higher resistance of *E.faecium* in our study might be due to more use of broad spectrum antibiotics in patients from whom *E.faecium* has been isolated.

We screened all the 140 enterococcal strains for high level aminoglycoside resistance by disk diffusion. As per CLSI guidelines, high content disks of gentamicin (120 µg) and streptomycin (300 µg) were used to detect high level aminoglycoside resistance[12]. The prevalence of HLAR is stated between 15% and 55%, and glycopeptides resistance has become widespread in various geographical areas[3]. In our study, as shown in (table 5) there was a higher percentage positivity of the isolates having high level gentamicin resistance (HLGR 40%) in comparison to the isolates having high level streptomycin resistance (HLSR 2.85%).Amongst *E.faecalis & E.faecium*, 17.91% and 74.50% of strains were found to be HLGR respectively (Table 5). However, M.G Karmarkar et al observed 100% of both the species to be high level gentamicin resistant.[8] This may be due to that all their study isolates were from hospitalized patients who usually have various risk factors. But our figures matched with those of Edet et al (14%)[10], Mendiratta et al (14.8%)[13] and Jose Arellano et al (14.4%)[11]who observed *E.faecalis* to be high level gentamicin resistant.

Also, it was observed that high level gentamicin resistance (HLGR) was more commom in urine samples (41.5%) followed by blood (36%) samples, while high level streptomycin resistance (HLSR) was more common in pus samples (52.6%) followed by blood samples(36%).

We observed 2.85% of enterococcal isolates to be only HLSR in our study. Out of total *E.faecalis*, 2.98% were HLSR but none of the *E.faecium* strain showed HLSR (Table 5). However, Jose Arellano et al observed 33.7% of *E.faecalis* & 12.5% *E.faecium* to be resistant to high level streptomycin.[11]

Combined resistance to both the aminoglycosides (HLGR + HLSR) was observed in 24.28% of total enterococcal isolates which was much higher in *E.faecalis* (29.85%) as compared to *E.faecium* (7.84%) as shown in Table 5. While, in Mendiratta et al study 15.3% of isolates showed combined resistance and it was much higher in *E.faecium* (59.1%) as compared to *E.faecalis* (7.8%)[13]. Jose Arellano observed combined resistance in 48.1% of *E.faecalis* and 37.5% *E.faecium* strains[11]. With respect to *E.faecalis,* resistance to high level gentamicin alone and combined aminoglycosides was 17.91% and 29.85% respectively, however in *E.faecium* resistance to gentamicin alone was much higher (74.50%) as compared to the combined aminoglycosides (7.84%) (Table 5). Resistance of *E.faecalis* to streptomycin alone and combined aminoglycosides was 2.98% and 29.85%. Although none of the *E.faecium* strains showed only HLSR, combined resistance to aminoglycosides was observed in 7.84% of *E.faecium* strains (Table 5). None of these differences were however statistically significant. Similarly, in Mendiratta et al study, there was no difference in resistance of *E.faecalis* to either gentamicin or streptomycin (14.8% each alone and 22.6% each overall) however in *E.faecium* it was higher to gentamicin (22.7% alone and 81.8% overall) than to streptomycin (13.6% alone and 72.7% overall).[13]This shows that combined resistance to aminoglycosides varies from place to place . The reason may be due to difference in practice of prescribing antibiotics by the clinicians.

Resistance to aminoglycosides in enterococci is often associated with multidrug resistance.[13] Multidrug resistance (MDR) enterococci are those strains which

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show significant resistance to three or more antibiotics of different class often including but not limited to vancomycin.[14] The recommended therapy for serious enterococcal infections include a combination of a cell wall active agent such as a β-lactam (usually penicillin / ampicillin) or vancomycin combined with an aminoglycoside therefore, resistance against these antibiotics is important clinically effecting therapeutic prognosis. Multidrug resistance was observed in 28.35% of *E. faecalis* and 58.52% of *E. faecium*, commonest phenotype being ampicillin, ciprofloxacin , high level gentamicin resistance.

It was observed that *E.faecalis* and *E.faecium* strains were almost equally resistant to ampicillin, ciprofloxacin and vancomycin. While resistance to ampicillin, ciprofloxacin and HLGR was less in *E.faecalis* (23.88%) than *E.faecium* (50.98%) Table 6. Overall,there was higher percentage positivity observed with *E.faecium* being resistant to more commonly used antibiotics- ampicillin, ciprofloxacin, vancomycin and gentamicin (high level) as shown in (Table 6). Since HLAR is often associated with resistance to other antimicrobial drugs, this may theoretically result in group of organism for which there is no effective antimicrobial treatment. The synergistic bactericidal effect of aminoglycosides and β lactam or glycopeptides antibiotics is lost if there is high level resistance to aminoglycosides.[3] Considering this possible risk, all clinically significant isolates of enterococci should be examined for their antibiotic sensitivity pattern including HLAR before administration of β lactam or glycopeptide antibiotic in combination with an aminoglycoside.[3]

Though VRE has been reported from worldwide, the incidence of enterococcal infections and species prevalent in India is not thoroughly investigated. Few studies from India reported *E.faecalis* as the most prevalent species, with high-level resistance to aminoglycosides but no resistance to vancomycin.[8] In the present study, only 2.85% of the isolates were resistant to vancomycin, by disk diffusion method although HLGR was observed in 40 % and HLSR was observed in 2.85% of all the isolates.Various studies have shown that HLAR prevalence in enterococci is high in many countries which may pose a serious threat to the treatment of infections due to these bacteria. Furthermore, such resistance may spread to other gram positive bacteria.[3] Other authors have shown that HLAR can be two folds higher in VRE isolates than in isolates of VSE. Since vancomycin resistance was observed in only few of the isolates in our study, instead resistance for both aminoglycosides was observed. Therefore, it is important to detect HLGR and HLSR because this would also help to limit the intrahospitalary dissemination of resistance and establish a surveillance program about the use of vancomycin and aminoglycosides for management of enterococcal infections[11].

Conclusion:

Deficiency of effective antimicrobial therapy and control measures for prevention of dissemination for multiple drug resistant enterococci are among the major factors for increasing prevalence of VRE and HLAR. These drug resistant enterococci present a challenge for the clinician and the clinical microbiologist because of their increased occurrence in nosocomial infections. Since antibiotic resistance is observed with most frequent species i.e. *E.faecalis & E.faecium* hence ,characterization of enterococcal isolates should be done. Identifying a strain with HLAR is of utmost importance from therapeutic

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point of view especially in systemic enterococcal infections. The occurrence of high level aminoglycoside resistance in enterococcal isolates in our setup was high. Though the prevalence of glycopeptide resistance was found only among few isolates studied, multidrug resistance together with high level aminoglycosides resistance suggests that regular surveillance of antimicrobial susceptibility should be undertaken to detect emerging resistance and to prevent the establishment and its spread. Thus, it is crucial for laboratories to provide accurate antimicrobial resistance patterns for enterococci so that effective therapy and infection control measures can be initiated.

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