

Prevalence of Multidrug Resistant Enterococci in a Tertiary Care Hospital in India: A Growing Threat

Nita Gangurde¹, Manisha Mane², Sunita Phatale¹

¹Dr. V. P. Medical College, Hospital & RC, Nashik, India

²ESIC-Medical College, PGIMSR, Chennai, India

Email: nitagangurde@gmail.com

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Abstract

Introduction: Enterococci are members of the healthy human intestinal flora, but are also leading causes of highly antibiotic-resistant infections. Serious enterococcal infections are often difficult to treat since the organisms have a tremendous capacity to acquire resistance to penicillin, high concentration of aminoglycoside & vancomycin. Careful review of *in vitro* susceptibility data is required to treat infections caused by MDR Enterococci. Therefore we conducted the study to find out prevalence of MDR Enterococci. **Aims & Objectives:** To study the prevalence of Vancomycin resistance, High Level Streptomycin Resistance (HLSR) & High Level Gentamicin Resistance (HLGR) in different enterococcal isolates. **Materials & Methods:** Total 180 enterococcal isolates were studied. Identification was done by conventional biochemical methods. Antibiotic susceptibility testing was done by Kirby-Bauer disc diffusion method on Mueller-Hinton agar and results were interpreted as per CLSI guidelines. HLSR & HLGR was determined by disc diffusion method using high level Gentamicin disc (120 µg) & Streptomycin (300 µg) discs. Minimum inhibitory concentration (MIC) determination for Vancomycin was done by vancomycin E test strips. **Results:** Total 180 enterococcal isolates were studied. *E. faecalis* was 60%, *E. faecium* was 32.2%, *E. durans* and *E. raffinosus* were 4.4% & 3.3% respectively. *Enterococcus fecium* showed resistance in high percentage as compared to *E. faecalis*. 15 isolates were found to be vancomycin resistant. **Conclusion:** Resistance to aminoglycoside is of great concern. Regular screening of enterococcal isolates for vancomycin resistance detection should be implemented. It is very important to implement infection control measures, screening of health care workers, surveillance cultures in intensive care units which can control spread of multidrug resistant enterococci.

Keywords

Enterococci; HLGR; HLSR; VRE

1. Introduction

Enterococci are members of the healthy human intestinal flora, but are also leading causes of highly antibiotic-resistant, hospital-acquired infection [1]. There is growing evidence that these bacteria frequently possess several specific traits that enable them to survive in the hospital environment, colonize patients, and cause infections such as bacteraemia, peritonitis, endocarditis and urinary tract, wound, and device-related infections [2]. Serious enterococcal infections are often difficult to treat since the organisms have a tremendous capacity to acquire resistance to penicillin, high concentration of aminoglycoside & vancomycin [3]. Enterococci with high level resistance to aminoglycosides (HLAR), beta lactamase production & glycopeptide resistance including vancomycin resistance are posing a great therapeutic challenge, not only for clinicians but also for healthcare institutions [4]. Multidrug resistance complicates treatment of enterococcal infections and the therapeutic spectrum of these cases is limited. Careful review of *in vitro* susceptibility data is required to treat infections caused by MDR *E. faecium*, the most commonly found group of VRE. Empiric therapy of enterococcal infections should be guided by local patterns of drug resistance. Nowadays emergence of MDR enterococci is thought to be due to antibiotic selective pressure. This organism is considered as second leading cause of hospital acquired infections [5]-[7]. Therefore we conducted the study to find out prevalence of drug resistance in Enterococcal isolates with regards to HLAR (HLSR & HLGR) and Vancomycin resistance in our set up.

2. Aims & Objectives

To study the prevalence of

- 1) Vancomycin resistance in different Enterococcal isolates;
- 2) High Level Streptomycin Resistance (HLSR) & High Level Gentamicin Resistance (HLGR) in different enterococcal isolates.

3. Materials & Methods

Total 180 enterococcal isolates obtained from clinical samples received in the Department of Microbiology, Dr. Vasantrao Pawar Medical College, Hospital & Research Centre, Nashik, India. The clinical specimens were received for routine culture and sensitivity during August 2012 to July 2013 was included in the study.

The isolates were identified by colony morphology, Gram's staining, catalase production, growth in nutrient broth containing 6.5% NaCl, aesculin hydrolysis in presence of 40% bile salts, growth at 10°C, 37°C & 45°C & other biochemical reactions [6] [8] [9]. Antibiotic susceptibility testing for ampicillin, amoxycylav, chloramphenicol, erythromycin, cotrimoxazole, ciprofloxacin, teicoplanin was done by Kirby-Bauer disc diffusion method [10] [11] on Mueller-Hinton agar and results were interpreted as per CLSI guidelines [6] [12] [13]. *Enterococcus faecalis* ATCC 29212 [Hi Media Laboratories, Mumbai] was used as quality control strain. HLSR & HLGR was determined by disc diffusion method using high level Gentamicin disc (120 µg) & Streptomycin (300 µg) discs [Hi Media Laboratories, Mumbai]. A diameter of the zone of inhibition <6 mm indicated resistance, 7 - 9 mm as intermediate and ≥10 mm as susceptible [10] [14].

Screening for Vancomycin resistance was performed by using Vancomycin screen agar [BHI agar with 6 µg/ml Vancomycin] (Hi-media Laboratory, Mumbai). One or more colony indicated resistance to Vancomycin. Control used were *E. faecalis* ATCC 51299 [Hi-media Laboratory, Mumbai] as positive control and *E. faecalis* ATCC 29212 (Hi-media Laboratory, Mumbai) as negative control. Minimum inhibitory concentration (MIC) determination was done by Vancomycin E test strips (Hi-media Laboratory, Mumbai). Since E test is convenient to perform, MIC determination of Vancomycin against VRE were done by E test (Hi-media Laboratory, Mumbai) [6] [15] [16]. Test procedure was performed as per the manufacturer's instructions. MIC values ≤4 µg/ml was taken as susceptible and ≥32 µg/ml as resistant [6] [15] [16]. *Enterococcus faecalis* ATCC 29212 and *S. aureus* ATCC 25923 were used as control strains [6] [17].

4. Results

Total 180 enterococcal isolates obtained from clinical samples over a period of one year were studied. We found *E. faecalis* as commonest (60%) species. *E. faecium* was 32.2% and other enterococci included *E. durans* (n = 8) and *E. raffinosus* (n = 6) (Table 1).

Enterococcus faecium showed resistance in high percentage as compared to *E. faecalis*. Total 63 enterococcal isolates showed High level streptomycin resistance (HLSR). Out of those 63 isolates 23 were *E. faecalis*, 34 were *E. faecium* and other enterococci were 6 in numbers. Also High level Gentamicin resistance (HLGR) was shown by 54 enterococcal isolates. Out of which 21 isolates were *E. faecalis*, 29 were *E. faecium* and 4 were other enterococci. 15 isolates were found to be Vancomycin resistant, of which 4 had MIC between 8 - 16 µg/ml. VRE included 5 isolates of *E. faecalis*, 8 isolates of *E. faecium*, one isolate of *E. durans* and one isolate of *E. raffinosus*. all isolates were sensitive to teicoplanin (Table 2).

5. Discussion

In our study, *E. faecalis* was the predominant species. This findings of our study are similar with findings of S. Farnandes et al. and S. Bose et al. [6] [10]. But there are few studies from north India (S. Jain et al.) [18], Mumbai (Karmarkar et al.) [7] and South India (Telkar et al.) [14] found *E. faecium* as predominant species. Also our these findings for *E. faecalis* as predominant species are similar to other studies from different parts of India [4] [17] [19] [20].

Enterococci are widely distributed in nature and are usually part of mixed flora commonly found in gastrointestinal tract and remains difficult to differentiate colonization from true infection [19]. Intensive use of broad spectrum antibiotics is responsible for conversion of enterococci to opportunistic nosocomial pathogens (Huycke et al., 1998) [1], Mohanty et al., 2005) [3]. Enterococci demonstrate both intrinsic as well as extrinsic types of resistance to antibiotics [6]. Our study revealed presence of multidrug resistant enterococcal species. Our findings are similar with other studies (Mathur et al., A. Telkar et al., Senal et al.) [10] [14] [21]. In our study we found *E. faecium* to be more drug resistant than *E. faecalis*. similar findings have been reported by other studies [10] [14] [18]. Resistance to ampicillin was found to be higher in *E. faecium* in our study as compared to *E. faecalis*. Similar findings have been reported by Kalyan et al. [22]. Erythromycin resistance in our study was quite high in accordance with findings reported by Sanal et al., Mathur et al., Agarwal J et al. [10] [21] [23]. Also almost 51% isolates were resistant to chloramphenicol. Cotrimoxazole resistance was almost equal in both the species. Frequent use of these antimicrobials for empirical treatment of enterococcal infections could be

Table 1. Number and percentage (%) of different species of Enterococci isolated in our study.

<i>E. faecalis</i>	<i>E. faecium</i>	<i>E. durans</i>	<i>E. raffinosus</i>
n = 108	n = 58	n = 8	n = 6
60%	32.2%	4.4%	3.3%

Table 2. Antibiotic resistance (%) among enterococcal isolates.

ANTIBIOTIC	<i>E. faecalis</i> % (n = 108)	<i>E. faecium</i> % (n = 58)	<i>E. durans</i> % (n = 8)	<i>E. raffinosus</i> % (n = 6)
Ampicillin	41.6 (45)	55.1 (32)	37.5 (3)	33.5 (2)
Amoxyclav	81.4 (88)	81.0 (47)	75 (6)	66.6 (4)
Erythromycin	85.1 (92)	91.3 (53)	25 (2)	33.5 (2)
Chloramphenicol	50.9 (55)	48.2 (28)	37.5 (3)	0
Cotrimoxazole	70.3 (76)	72.4 (42)	0	0
Ciprofloxacin	80.5 (87)	86.2 (50)	87.5 (7)	83.3 (5)
HLSR	42.5 (46)	55.1 (32)	12.5 (1)	33.3 (2)
HLGR	44.4 (48)	53.4 (31)	12.5(1)	50 (2)
Teicoplanin	0	0	0	0
Vancomycin	4.6 (5)	13.7 (8)	12.5 (1)	16.6 (1)

the reason for this antibiotic resistance. All the isolates were sensitive to teicoplanin. Therefore indicating teicoplanin as a reserve drug for therapy against these notorious organisms. These findings are in congruence with other similar studies [20].

According to our study High level Gentamicin resistance (HLGR) was 44.4% in *E. faecalis* and 55.1% in *E. faecium*. We found HLGR in higher percentage in *E. faecium* than in *E. faecalis*. This finding matches with some studies. Sanal Fernandes *et al.* have reported similar findings. Also study by Loveena *et al.* [4] reports HLGR higher in *E. faecium* than *E. faecalis*. A Telkar *et al.* reports HLGR of 71.4% in *E. faecium* and 57% in *E. faecalis*. Also Mendiratta *et al.* have reported greater resistance to HLG among *E. faecium* as compared to *E. faecalis*. Studies from New Delhi and Mumbai have reported HLGR prevalence to be as high as 70% and 100% respectively [7] [24]. High level streptomycin resistance was 42.5% in *E. faecalis* and 55.1% in *E. faecium*. Our this finding of HLSR being greater in *E. faecium* than *E. faecalis* is in accordance with other studies (Sanal *et al.*, Loveena *et al.*, Telkar A *et al.*, Sarika Jain *et al.*). But Ramalingam S *et al.* [25] found HLSR higher in *E. faecalis* than *E. faecium*.

The emergence of Vancomycin resistant enterococci poses a serious threat to hospitalized patients with impaired host defenses [3]. In India, the prevalence of VRE has been reported to be between 0 - 30 percent [21] [23] [26]-[28]. In our study, 15 isolates were found to be resistant to Vancomycin with *E. faecium* (13.7%) showing higher resistance than *E. faecalis* (4.6%). Similar studies by Sanal *et al.*, Telkar *et al.* & Karmarkar *et al.* had also reported greater resistance among *E. faecium* isolates [7] [10] [14]. Agarwal *et al.* found Vancomycin resistance to be greater among *E. faecalis* isolates. Thus the present study indicated an increase in Vancomycin resistance of the enterococcal isolates. The Vancomycin resistance in enterococci not only leaves fewer options for the disease management, but it is also important due to the potential risk of the Vancomycin resistance gene transfer from the enterococci to *Staphylococcus aureus* [29]. Also emergence of Vancomycin resistant enterococci has been attributed to imprudent use of cephalosporins, Vancomycin, colonization pressure and non-compliance with infection control measures [7]. All the VRE isolates in our study found to be sensitive to teicoplanin, leaving teicoplanin as treatment options.

6. Conclusions

Resistance to aminoglycoside is of great concern, since it eliminates the synergy of aminoglycosides with beta lactam antibiotics, which is the therapy of choice for most of enterococcal infection which can limit the therapeutic options. Also now a day there is emergence of Vancomycin resistance, therefore regular screening of enterococcal isolates for Vancomycin resistance detection should be implemented to limit the spread of MDR enterococci.

Also since enterococci can survive in hospital environment due to their intrinsic resistance to several commonly used antibiotics and their ability to acquire resistance to all currently available antibiotics by mutation or through plasmid, it is very important to implement infection control measures, screening of health care workers, surveillance cultures in intensive care units which can control spread of multidrug resistant enterococci.

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