

Prevalence of multidrug-resistant enterococci in a tertiary care hospital in Mumbai, India

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Introduction

Enterococci are one of the major causes of nosocomial and community-acquired infections. In recent years, the evolution of antimicrobial resistance in enterococci has posed enormous challenges for clinicians. The antimicrobial therapy 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 and perhaps more importantly, because of their acquired resistance to all currently available antibiotics, that leaves limited medicative options and results in the selection and spreading of multidrug-resistant (MDR) strains in hospitals [1,2]. Empirical use of antibiotics, absence of national guidelines for screening patients for MDR bacteria and lack of sufficient information and programs to control rapid spread of enterococci has led to increased mortality caused by enterococcal infections [3-5]. Knowledge of the antibiogram is essential to formulate therapeutic strategies for treating enterococcal infections [1]. This prospective study aimed to investigate species prevalence and extent of antimicrobial resistance among clinical isolates of enterococci in a tertiary care hospital in India.

The study

Clinical isolates of enterococci from KEM Hospital, Mumbai, India, were studied at the Department of Microbiology. Enterococci were identified to the species level by standard biochemical tests [6]. Species confirmation was accomplished by the use of species-specific PCR assays [7]. Antibiograms were performed by the breakpoint broth

microdilution method as standardised by the Clinical and Laboratory Standards Institute (CLSI) [8,9]. The antibiotics tested were ampicillin, penicillin, high-level gentamicin (HLG), high-level streptomycin (HLS), vancomycin, teicoplanin, erythromycin, tetracycline, ciprofloxacin, nitrofurantoin, rifampin, chloramphenicol, and linezolid. Minimum inhibitory concentrations (MICs) of the most clinically significant antibiotics (ampicillin, penicillin, gentamicin, streptomycin, vancomycin, teicoplanin, and linezolid) were determined by the broth microdilution method in accordance with the CLSI guidelines [2,8]. *E. faecalis* ATCC 29212 and *E. faecalis* ATCC 51299 were used for quality control. Statistical analysis was performed employing Fisher's exact test ($p < 0.05$).

In this study, 291 (11.6%) enterococci were isolated from 2,500 clinical specimens. *Enterococcus faecalis* (70.1%; 204/291) was the predominant species identified, followed by *Enterococcus faecium* (29.9%; 87/291). Predominance of *E. faecalis* in the endogenous flora of the body could be the reason behind its high proportion among the hospital isolates.

Since beta-lactams remain the drug of choice for most enterococcal infections, increasing resistance to these antibiotics is of concern. The rate of resistance to ampicillin (64.9%) and penicillin (75.9%) among isolates in this study was higher than the resistance rates reported recently from India (Table 1) [4,10,11]. High-level resistance to ampicillin (MIC $\geq 64\mu\text{g/ml}$) and penicillin (HLPR) (MIC $\geq 128\mu\text{g/ml}$) was observed in 28.9% (84/291) and 34.0% (99/291) of the isolates, respectively, with MICs raised up to 512 $\mu\text{g/ml}$ (Table 2) [9]. The rate of HLPR reported in this

Table 1. Antimicrobial resistance rates of enterococcal isolates of the study

Antibiotic	Resistance [#]		
	<i>E. faecalis</i> (n = 204)	<i>E. faecium</i> (n = 87)	Total (n = 291)
	n (%)	n (%)	n (%)
Ampicillin	125 (61.3)	64 (73.6)	189 (64.9)
Penicillin G	148 (72.5)	73 (83.9)	221 (75.9)
HLG	147 (72.1)	67 (77.0)	214 (73.5)
HLS	132 (64.7)	74 (85.1)	206 (70.8)
Vancomycin ^a	38 (18.6)	19 (21.8)	57 (19.6)
Teicoplanin ^b	9 (4.4)	24 (27.6)	33 (11.3)
Erythromycin ^c	184 (90.2)	84 (96.5)	268 (92.1)
Tetracycline ^d	165 (80.9)	81 (93.1)	246 (84.5)
Ciprofloxacin	194 (95.1)	84 (96.5)	278 (95.5)
Nitrofurantoin	153 (75.0)	79 (90.8)	232 (79.7)
Rifampin	65 (31.9)	59 (67.8)	124 (42.6)
Chloramphenicol ^f	81 (39.7)	42 (48.3)	123 (42.3)
Linezolid ^g	1 (0.5)	6 (6.9)	7 (2.4)

[#]Resistance data inclusive of intermediate-level resistant isolates

^aHLG, high-level gentamicin; HLS, high-level streptomycin

Intermediate isolate: ^aVancomycin, 2.1% (6/291); ^bTeicoplanin, 3.8% (11/291); ^cErythromycin, 1.4% (4/291); ^dTetracycline, 2.1 (6/291);

^eRifampin, 1.0% (3/291); ^fChloramphenicol, 0.3% (1/291); ^gLinezolid, 0.3% (1/291)

study was slightly lower than that reported in the literature [4,12]. This finding could be due to the limited use of penicillin in the management of infections in our hospital. To the best of our knowledge, this is the first report on high-level ampicillin resistant enterococci from India.

High-level resistance to aminoglycosides (HLAR) is of great clinical concern, since it eliminates synergy with cell wall active antibiotics, which renders treatment of serious enterococcal infections difficult [1]. In the present study, a high proportion of tested isolates exhibited resistance to HLG (73.5%) and HLS (70.8%), with MICs raised up to 4000 µg/ml and 8000 µg/ml, respectively (Tables 1 and 2). Combined resistance to HLG and HLS was found in 58.8% (171/291) of all isolates, indicating the total elimination of synergy between aminoglycosides and cell-wall active agents. Compared to previous reports, this study has found a higher incidence of HLAR especially HLSR [10-13]. This result could be attributed to the increased use of streptomycin in our

hospital setting alternatively to gentamicin for the treatment of HLGR enterococci infections [14].

With increasing resistance to high-level aminoglycosides and penicillins, glycopeptides can provide a valuable treatment option [2]. Glycopeptide-resistant enterococcal (VRE) infections can be fatal. In the present study, vancomycin resistance was detected in 19.6% of the isolates and high-level vancomycin resistance (MIC ≥ 64 µg/ml) was exhibited by 15.5% (45/291) of the isolates with MICs raised up to 2048 µg/ml (Tables 1 and 2) [9]. All (11.3%) teicoplanin-resistant isolates showed high-level resistance to teicoplanin (MIC ≥ 16 µg/ml) with MICs raised up to 256 µg/ml (Tables 1 and 2) [1]. The frequency and extent of glycopeptide resistance in this study were much higher compared to those of previous reports from India [4,10-12]. Although vancomycin resistance is low in India compared to that in western countries, its rising rate is concerning [13].

Treatment of infections caused by VRE is extremely problematic because of the

Table 2. MIC ranges, MIC_{50s}, MIC_{90s}, and high-level resistance (HLR) to the most clinically-significant antibiotics among clinical isolates of enterococci

Antibiotic	MIC Range (µg/ml)	<i>E. faecalis</i> (n = 204)			<i>E. faecium</i> (n = 87)		
		MIC ^a (µg/ml)		HLR	MIC (µg/ml)		HLR
		MIC ₅₀	MIC ₉₀	No. (%)	MIC ₅₀	MIC ₉₀	No. (%)
Ampicillin	2 - 512	16	64	45 (22.06)	32	128	39 (44.83)
Penicillin G	2 - 512	32	256	50 (24.51)	128	512	49 (56.32)
Gentamicin	125-4000	1000	2000	147 (72.06)	2000	4000	67 (77.01)
Streptomycin	125-8000	2000	4000	132 (64.71)	2000	8000	74 (85.06)
Vancomycin	< 0.5 - 2048	4	64	27 (13.24)	4	256	18 (20.69)
Teicoplanin	< 0.5 - 256	1	8	9 (4.41)	2	64	24 (27.59)
Linezolid	< 0.5 - 32	1	1	-	1	1	-

HLR, High-level resistance; MIC, minimum inhibitory concentration

^aMIC₅₀ and MIC₉₀: Minimum concentrations of antimicrobial that inhibits the growth of 50 and 90% of the isolates, respectively

limited therapeutic alternatives. If the infecting VRE is highly resistant to ampicillin and aminoglycosides, there are other suggested treatment options, including chloramphenicol, ciprofloxacin, erythromycin, linezolid, nitrofurantoin, rifampin, and tetracycline [1]. In this study, the magnitude of resistance was highest for ciprofloxacin (95.5%), followed by erythromycin, tetracycline, nitrofurantoin, chloramphenicol, and rifampin. Linezolid had the lowest resistance rate of 2.4% (Table 1). Even though linezolid has demonstrated good anti-enterococcal activity, the emergence of linezolid resistance in enterococci is an alarming problem in the treatment of VRE infections [14]. Resistance to at least one agent of the three antimicrobial classes defining multidrug resistance was found in 57.0% (166/291) of all isolates [3]. Six isolates exhibited resistance to all tested antibiotics. This finding is of particular concern since the high prevalence of colonization and/or infection with MDR enterococci has reduced treatment options for these bacteria.

Another significant finding was the concomitant high-level resistance to the penicillins, aminoglycosides, and glycopeptides tested seen in 8.2% (24/291) of the isolates. Concomitant high-level resistance to these antibiotics has not been often reported in India [12,15]. Penicillins, aminoglycosides, and glycopeptides have been the mainstays of therapy for enterococcal infections; therefore, the organism's development to concomitant high-level resistance to these drugs would have important clinical implications, as it considerably narrows the therapeutic options.

In this study, *E. faecium* demonstrated a significantly higher frequency and extent of resistance as compared to *E. faecalis* for all tested antibiotics ($P < 0.05$), which is consistent with the results of previous studies [4,10,11,15].

This study illustrates the preferential spread of multidrug-resistant enterococci with highly raised MICs to most clinically significant antibiotics posing, thereby, a serious therapeutic challenge. This situation demands the implementation of an efficient infection control program and regular surveillance of antimicrobial resistance of enterococci in order to establish a rational antibiotic policy for the better management of enterococcal infections.

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