Original Research Article

Study of prevalence of multidrug resistant (MDR) Enterococci at a tertiary care hospital of Rajasthan

Surbhi Mathur*

Assistant Professor, Department of Microbiology, JLN Medical College, Ajmer, Rajasthan, India
*Corresponding author email: **surbhimathur11111@gmail.com**

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Abstract

Background: Enterococci, though commensals in adult faeces are important nosocomial pathogens. Their emergence in past two decades is in many respects attributable to their resistance to many commonly used antimicrobial agents (aminoglycosides, cephalosporins, aztreonam, semisynthetic penicillin, trimethoprim-sulphamethoxazole).

Objectives: To study the prevalence of Multidrug resistant (MDR) Enterococci plus Vancomycin resistance and High Level Gentamicin Resistance (HLGR) in different enterococcal isolates.

Materials and methods: Total 125 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. Enterococci resistant to more than three drugs plus high level Gentamicin (120 µg) resistance were labelled as multidrug resistant (MDR). HLGR was determined by disc diffusion method using high level Gentamicin disc (120 µg). Minimum inhibitory concentration (MIC) determination for detecting Vancomycin resistance was done by HiComb MIC Test strips and microbroth dilution method.

Results: Total 125 entetococcal isolates were studied. In this study the multiple drug resistance was verified in 44 (35.20%) isolates of Enterococcus species and only 2 isolates (1.72%) were found to be VRE but HLGR was detected in 53.6% of the isolates.

Conclusion: During past two decades, enterococci resistant to multiple antimicrobial agents have been recognized, including strains resistant to vancomycin, β-lactams and aminoglycosides, making it a formidable nosocomial pathogen. Such strains pose therapeutic dilemmas for clinicians. 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.

Key words

Enterococci, HLGR, VRE.

Introduction

Enterococci are members of the healthy human intestinal flora, but are also leading causes of highly antibiotic resistant, hospital-acquired infection [1]. Enterococci exhibit low level resistance to all aminoglycosides (MIC 8 to 256 µgm/mL) which appears to be due to low uptake of these agents. However, aminoglycoside uptake is enhanced when enterococci are exposed to β-lactams. This synergy underlies the long standing practise of combining both classes of antibiotics to treat serious enterococcal infections as combination overcomes the intrinsic resistance exhibited by enterococci and a synergistic effect is usually achieved since the intracellular penetration of aminoglycoside is facilitated by cell wall active agent. [2].

Vancomycin resistance in nosocomial isolates of enterococci is usually mediated by the resistance genes *vanA* or *vanB* [3]. High-level vancomycin resistance (MIC >64 mg/L) is mediated by the *vanA* gene cluster, located on the transferable genetic element transposon Tn*1546* [4]. Variable levels of vancomycin resistance (MIC 4–1000 mg/L) characterise the *vanB* genotype and the gene cluster is located on another mobile genetic element, Tn*1547* [5]. Some enterococci (including *E. gallinarum*) may posses intrinsic, but not transferable, resistance against vancomycin, coded by *vanC* (MIC 2–32 mg/L.

This organism is considered as second leading cause of hospital acquired infections [6, 7]. Therefore we conducted the study to find out prevalence of drug resistance in Enterococcal isolates with regards to HLAR (HLGR) and Vancomycin resistance in our set up.

Materials and methods

The present prospective study was conducted on 125 pure isolates (1 per patient) of enterococci isolated consecutively from various clinical samples like Pus, Blood; wound Swab, Sputum, urine, etc. received at Department of Microbiology of JLN Medical and AG of Hospitals, Ajmer for bacteriological culture and sensitivity.

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 and 45˚C and other biochemical reactions [6, 8, 9] .

Antibiotic susceptibility testing for ampicillin, amoxyclav, 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, 7, 12].

Enterococcus feacalis ATCC 29212 was used as quality control strain. Screening for Vancomycin resistance was performed by using Vancomycin screen agar (BHI agar) with 6 µg/ml Vancomycin. One or more colony indicated resistance to Vancomycin. Minimum inhibitory concentration (MIC) determination was done by HiComb MIC Test strips as this test is convenient to perform and microbroth dilution method [6, 13, 14, 15]. 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, 14, 15].

HLGR was determined by disc diffusion method using high level Gentamicin disc (120 µg). A diameter of the zone of inhibition <6 mm indicated resistance, 7 - 9 mm as intermediate and ≥10 mm as susceptible [10, 13]. Enterococci resistant to more than three drugs plus high level Gentamicin (120 µg) resistance were labelled as multidrug resistant (MDR).

Results

Out of 3534 various clinical samples (1 per patient), 125 (3.53%) were identified as

enterococci. Urine yielded the maximum number 79 (6.04%) of enterococcal isolates. Isolation rate of enterococcus was 5.53% from Pus and Wound swabs and 1.94 % from Blood and 0.82% from samples of lower respiratory tract No. enterococcal isolate was recovered from body fluids and cereberospinal fluids (CSF).

60 (53.6%) and 56 (44.80%) of the isolates were resistant to penicillin–G and Ampicillin respectively. Resistance to Penicillin–G and Ampicillin among E. faecium isolates was significantly higher (P value < 0.05) than E. faecalis isolates. The rates of resistance to Penicillin–G and Ampicillin were different in 11 (8.80%) enterococcal isolates including 7 E. faecalis and 4 E. faecium. All such 11 (8.80%) isolates were resistant to Penicillin but susceptible to Ampicillin (**Table – 1**).

67 (53.60%) of total enterococcal isolates expressed high–level resistance to Gentamicin (HLGR). E. faecium showed higher resistance rate to high level Gentamicin (72.09%) than E. faecalis (49.29%). Other enterococci accounted only 33.33%. Almost all of the isolates were resistant to Erythromicin (98.40% maximum resistance) followed by Ciprofloxicin (76%) and Quinupristin / Dalfopristin (55.2%). Only 20 % of the isolates were resitant to Tetracycline (**Table – 1**).

Out of 125 enterococcal strains tested, 2 (1.60%) were resistant to vancomycin (VRE) in the disc diffusion method No isolate was found resistant to linezolid. All of the 125 enterococcal isolates were tested on the vancomycin screen agar. Out of 125 strains tested 2 (1.60%) were resistant to vancomycin (VRE) and 123 (98.4%) were vancomycin susceptible (**Table – 1**).

Table - 1: Anti microbial Resistance pattern of Enterococcus species tested by Kirby Bauer disc diffusion method.

Anti Microbial agents	**No. (%) of resistant strains**			**Total (n=125)**
	E. faecalis (n= 79)	**E. faecium (n = 43)**	**Other enterococci (n = 3)**	
Penicillin – G (10 units)	25 (31.64)	41 (95.34)	1 (33.33)	67 (53.6)
Ampicillin (10µg)	18 (22.78)	37 (86.04)	1 (33.33)	56 (44.8)
Gentamicin (HLGR) (120 µg)	35 (44.30)	31 (72.89)	1 (33.33)	67 (53.6)
Erythromycin (15 µg)	77 (97.46)	43 (100)	3 (100)	123 (98.40)
Vancomycin (30 µg)	1 (1.26)	1 (2.32)	0	2 (1.60)
Teicoplanin (30 µg)	1 (1.26)	1 (2.32)	0	2 (1.60)
Quinupristin/ Dalfopristin (15 µg)	65 (82.27)	1 (2.32)	3 (100)	69 (55.2)
Linezolid (30 µg)	0	0	0	0
Ciprofloxacin (5 µg)	57 (72.15)	35 (81.39)	3 (100)	95 (76.00)
Tetracycline (30 µg)	23 (29.11)	1 (2.32)	1 (33.33)	25 (20%)

HLGR = High Level Gentamicisn resistance Include E – hirae (2) and E – Durans (1)

The VRE strains showed high degree of resistance to most of the antibiotics tested. All VRE strains were resistant to Penicillin-G, Ampicillin, Teicoplanin, Linezolid, Quinu pristin/ Dalfopristin, Erythromycin, Gentamiun (HLGR) and Ciprofloxacin. Least resistance was seen for Tetracycline (50%) none of the strains shoved resistance to Linezolid (**Table – 2**).

Characteristics of vancomycin resistant enterococci isolated in the present study was as per **Table – 3**. Out of 125 isolates tested HLGR was detected in 67 (53.60%) of the isolates. E.faecium showed higher resistance rate to high level gentamicin 31 (72.09%) than E-faecalis 35 (44.30%). Other enterococci accounted for 1 (33.33%) as per **Table – 4**.

Table - 2: Species specific antibiotic resistance pattern of VRE isolates.x

Antimicrobial Agents	No. (%) of VRE strains			Total (n=2)
	E. faecalis (n=1)	E. faecium (N=1)	Other enterococci (N=0)	
Penicillin-G	1 (100)	1 (100)	0	2 (100)
Ampicillin	1 (100)	1 (100)	0	2 (100)
Teracycline	1 (100)	0	0	1 (50)
Teicoplanin	1 (100)	1 (100)	0	2 (100)
Linezolid	0	0	0	0
Quinu pristin/ Dalfopristin	1 (100)	1 (100)	0	2 (100)
Erthromycin	1 (100)	1 (100)	0	2 (100)
Gentamiun (HLGR)	1 (100)	1 (100)	0	2 (100)
Ciprofloxacin	1 (100)	1 (100)	0	2 (100)

Table - 3: Characteristics of vancomycin resistant enterococci isolated in the present study.

Isolate No.	Source	Zone diameter (mm) (Interpretation)		Vancomycin Screen agar	MIC (ug/ml)		PYR Phenotype
		Vancomycin	Teicoplanin		Hi comb MIC Test	Broth Dilution	
(1)	Blood	N (R)	N (R)	R	> 256	256	Van – A
(2)	Urine	N (R)	N (R)	R	> 256	512	Van – A
(3) E. faecalis ATCC 29212	----	22 (S)	18 (S)	S	4	4	--------
(4) E. faecalis ATCC 51299	----	N (R)	10 (R)	R	> 256	---	Van - A

Table - 4: High Level Gentamicin resistant (HLRG) enterococcal Strains.

Total No of Isolates tested	No (%) of Resistant Strains			Total no (%) of HLGR (Out of total)
	E.Faecalis	E.faecium	Other enterococci *	
125	35 (44.30)	31 (72.09)	1 (33.33)	67 (53.60)

The resistance patterns of HLGR strains were shown in Table - Out of 67 high level gentamicin resistant strains 74.62% and 64.17% were found to be while E. faecalis isolates showed higher resistance to Tetracycline and Quiniupristrin /Dalfopristin than E. faecium all the HLGR isolates were resistant to tetracycline. Least resistance was shown by vancomycin (2.98%) and Teicoplanin (2.98%) None of the HLGR isolates were resistant to Linezolid (**Table – 5**).

Resistance of vancomycin Resistant strains (VRE) and high level Gentamicin Resistant (HLGR) strains to B-lactams (both Penicillin and Ampicillin) was as per **Table - 6**. Out of 125, 35.2% entercoccal strains were resistant to more than three drugs plus high level Gentamicin (120ug) and hence were labelled multidrug resistant (MDR) Both E. faecalis and E. faecium showed multi drug resistance, the former being more resistant to multiple drugs, than later (**Table – 7**).

Table - 5: Resistance of HLGR isolates to various antibiotics.

Antimicrobial Agents	No (%) of HLGR Strains			Total (n=67)
	E. faecalis (N=35)	E. faecium (n=31)	Other Enterococci (n=1)	
Penicillin-G	18 (51.42)	31 (100.0)	1 (100)	50 (74.62)
Ampicillin	12 (34.28)	30 (96.77)	1 (100)	43 (64.17)
Tetracycline	10 (28.57)	1 (3.22%)	1 (100)	12 (17.91)
Erythromycin	35 (100.0)	31 (100.0)	1 (100.0)	67 (100.0)
Vanco mycin	1 (2.85)	1 (3.22)	0	2 (2.98)
Teicoplanin	1 (2.85)	1 (3.22)	0	2 (2.98)
Linezolid	0	0	0	0
Quinupristin/ Dalfopristin	21 (60)	3 (9.67)	1 (100)	25 (37.31)
Ciprofloxacin	17 (48.57)	26 (83.87)	1 (100)	44 (65.67)

Table - 6: Resistance of vancomycin Resistant strains (VRE) and high level Gentamicin Resistant (HLGR) strains to B-lactams (both Penicillin and Ampicillin).

Type of Enterococcal Strain	Total Tested	Resistance to β-lactams(both Penicillin and Ampicillin)			Total
		E. faecalis	E. faecium	Other enterococci	
VRE	2	1 (50)	1 (50)	0	2 (100%)
HLGR	67	13 (19.40)	29 (43.28)	1 (1.49)	43 (64.17%)

Table - 7: Multiple drug Resistance (MDR) in Enterococci.

Type of sample	Total Tested	MDR Strains No (%)			Total MDR No (% out of total MDR)	Isolation rate from each Sample No (%)
		E. faecalis	E. faecium	Other Enterococci		
Urine	79	7 (8.86)	21 (26.58)	1 (1.26)	29 (65.90)	29 (36.70)
Pus & W-swab	17	5 (29.41)	0	0	5 (11.36)	5 (29.41)
Blood	26	4 (15.38)	6 (23.07)	0	10 (22.72)	10 (38.46)
Others	3	0	0	0	0	0
Total	125	16 (12.8)	27 (21.6)	1 (0.8)	44 (100%)	44 (35.2%)

Discussion

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 [16] Patient samples received at Department of Microbiology for bacteriological culture were screened for presence of enterococci. One hundred and twenty five such samples which yielded pure isolate of enterococci were included in the study. Out of 125, 79 (63.20%), 43 (34.40%), 2 (1.60%) and 1 (1.26%) were identified as E. faecalis, E. faecium, E. hirae and E. durans respectively. Thus the isolation rate of enterococci was 3.53% in this study Out of 125 strains of enterococci 53.6 %, 44.80%, 53.60%, 98.40%, 1.60%, 1.60%, 55.20%, 0, 76.00%, 20.00% showed resistant to Penicillin – G, Ampicillin, Gentamicin (HLGR), Erythromycin, vancomycin (VRE), Teicoplanin, Quinupristin/ Dalfopristin, Linezolid, Ciprofloxacin, and Tetracycline respectively on the modified Kirby Bauer disc diffusion test.

The incidence of VRE in the present study is 1.60%, which reflects the emergence of VRE in

J.L.N. hospital, Ajmer .Because of the limited therapeutic options for treating serious infections caused by VRE, it has emerged as one of the leading clinical challenge for physicians. Since this is the first such study carried out in our location, the trend of VRE over years cannot be deciphered. But the study results indicate the need for constant monitoring and surveillance of VRE in our hospital. Studies from different parts of India indicate differences in the incidence & prevalence of VRE between places. The incidence of VRE obtained in the present study (1.60%) is comparable to that obtained by P Mathur etal (2003) [17] from Delhi 1 % and from Aligarh 1.29%. Higher incidence of 25.5%, 8%, 16.22% and 23.07% has been reported by Taneja N, et al., (2004) [21]; kapoor L, et al., (2005) [20]; Randhawa V.S. et al. [18] and M.G Karmarker, et al., (2003) [10] respectively from Chandigarh New Delhi, Ludhiana and Mumbai. Sekar R., et al. reported no vancomycin resistance in enterococci [19].

In the present study a total of 67 (53.60%) isolates showed high level resistance to gentamicin (HLGR) by high content 120 µg disc diffusion method HLGR among E. faecium isolates (72.09%) was significantly higher than E. faecalis (44.30%). The higher rate of HLGR (53.60%) in the present study may be ascribed to the source of the isolates being from a tertiary care set up where chronic cases are prevalent and a wider usage of broad spectrum antibiotics occurs. The present study highlighted the importance of high occurence of HLGR enterococci in out setup. This would necessitate routine testing of the isolates for HLGR Alternative regimes in the management of enterococcal infection need to be evaluated. Of the 67 HLGR isolates 65 (97.01%) were vancomycin sensitive. The result of present study is consistent with the previous studies viz. V.S. Randhawa, et al., (2003) from New Delhi observed 34 (97%) Out of 35 HLGR isolates to be vancomycin sensitive [18].

All the HLGR strains were susceptible to linezolid. Out of 125, 35.2% entercoccal strains were resistant to more than three drugs plus high level Gentamicin (120 µg) and hence were labelled multidrug resistant (MDR) Both E. faecalis and E. faecium showed multi drug resistance, the former being more resistant to multiple drugs, than later.

Conclusion

During past two decades, enterococci resistant to multiple antimicrobial agents have been recognized, including strains resistant to vancomycin, β-lactams and aminoglycosides, making it a formidable nosocomial pathogen. Such strains pose therapeutic dilemmas for clinicians. 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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