Prevalence of Resistance to Ampicillin, Gentamicin, Streptomycin and Vancomycin in Enterococcus Species Isolated from Clinical Specimens

Priyanka Paul Biswas[1], Anamika Singh[2], Mohammad Intekhab Alam Chand[3], Sangeeta Dey[4], Aninda Sen[5]

[1]Associate Professor, Department of Microbiology, Katihar Medical College, Al-Karim University, Katihar, Bihar, India. [2]Assistant Professor, Department of Microbiology, Katihar Medical College, Al-Karim University, Katihar, Bihar, India. [3]Assistant Professor, Department of Community Medicine, Katihar Medical College, Al-Karim University, Katihar, Bihar, India. [4]Professor, Department of Microbiology, Katihar Medical College, Al-Karim University, Katihar, Bihar, India. [5]Professor, Department of Microbiology, Katihar Medical College, Al-Karim University, Katihar, Bihar, India.

ABSTRACT

BACKGROUND

We wanted to determine the frequency of occurrence of HLAR, HLGR, and HLSR phenotypes among Enterococcus species isolated from clinical specimens and determine their susceptibility to different groups of antibiotics.

METHODS

Enterococci were isolated from various clinical samples, and identification to species level was done using standard methods. High level resistance to gentamicin and streptomycin was done by high potency disc diffusion method [HPDDM]. Biofilm production was seen by microtiter assay and slime layer formation and gelatinase production. Minimum inhibitory concentrations [MIC] to vancomycin, ampicillin, gentamicin, streptomycin and teicoplanin were determined by agar dilution method. Multiplex PCR was used to detect the presence of AME genes.

RESULTS

Strains showing combined resistance [HLAR] were recovered from the age group 51 - 60 years (75%). *Enterococcus faecalis,* that carried aph (3″)-IIIa gene 7.6% [1/3] exhibited complete resistance to vancomycin with MIC of 32-64 µg/mL. 7.6% [1/6] unusual species that expressed both [aac (6′)-ie-aph (2″)-Ia + aph(3″)-IIIa] genes was also resistant to vancomycin. VR Enterococcus faecium that showed high level resistance to streptomycin [HLSR] 10.0% [1/10] also exhibited resistance to linezolid. The differences in antibiotic resistance pattern to ampicillin, penicillin, piperacillin, tetracycline, erythromycin, ciprofloxacin and imipenem amongst *Enterococcus faecalis*, *Enterococcus faecium* and unusual strains were statistically significant [P=0.05, P= 0.01, P=0.000]. Resistance to piperacillin amongst the HLGR strains was also significant [P= 0.001]. Biofilm formation was seen in 79.3% [23/29] of Enterococcus species resistant to streptomycin followed by 50.0% of HLAR strains and 45.7% of HLGR strains.

CONCLUSIONS

This study showed the slowly increasing prevalence of HLAR and resistance to other antibiotics. Much more extensive synergism studies of cell wall–active agents combined with various aminoglycosides against enterococci that possess different combinations of aminoglycoside resistance genes must be conducted.

KEYWORDS

High Level Gentamicin Resistance, High Level Streptomycin Resistance, High Level Aminoglycoside Resistance

Corresponding Author:
Dr. Aninda Sen,
Department of Microbiology,
Katihar Medical College,
Al-Karim University, Katihar-854106,
Bihar, India.
E-mail: aninda_0428@yahoo.com

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BACKGROUND

The genetic plasticity of enterococci has given rise to strains showing resistance to cell wall active agents, aminoglycosides, penicillins, ampicillin and vancomycin. Enterococci show intrinsic resistance to most β-lactam antibiotics because they contain Penicillin Binding Proteins [PBPs], especially low molecular weight PBPs such as PBP5, enabling them to synthesize cell wall components even in the presence of modest concentrations of most β-lactam antibiotics.[1,2,3,4]

The aim of this study was to determine enterococcal susceptibility to different groups of antibiotics and the frequency of occurrence of High level aminoglycoside resistance [HLAR] regulated by AME genes such as aac [6′]-Ie and aph [2″]-Ia, High level gentamicin resistance [HLGR], and High level streptomycin resistance [HLSR] phenotypes in our setup.

METHODS

This study was done on 300 various clinical samples recovered from the patients with different clinical conditions from a tertiary care centre in eastern India, after obtaining approval of the Institutional Ethics Committee. 112 Enterococcal strains were isolated from the 300 samples were identified to species level using standard procedures.[5,6] Antibiotic susceptibility test was done by Kirby-Bauer disc diffusion method on Mueller-Hinton agar, using antibiotic discs obtained from HiMedia Laboratories, Mumbai, India.[7] HLAR in enterococci was detected by Disc Diffusion Method and Agar Screening Method as per standard protocol.[7,8]

Determination of MIC by Agar Dilution Method [ADM]

ADM was used to determine MIC of vancomycin, ampicillin, gentamicin, streptomycin and teicoplanin as per standard protocol. *Enterococcus faecalis* ATCC 29212 was used as negative control strain. Resistance and susceptibility of Enterococci was measured using standard protocols.[7,8]

DNA Extraction Method

Genomic DNA was prepared using conventional phenol-chloroform DNA extraction method.[8,9]

PCR Assay for AMEs

Amplification was performed with PCR system, using primers obtained from Merck Specialities, Lucknow, India, and products were resolved by electrophoresis as per standard protocol, stained with ethidium bromide and visualized under gel documentation system. [8,9] Genes identified were aac [6″]-Ie- aph [2″]-Ia, aph [2″]-Ib, aph [2″]-Ic, aph [2″]-Id, aph [3″]-IIIa.

Statistical Analysis

Statistical analysis was done using chi-square test. $p<0.05$ was considered significant and $P<0.001$ as highly significant.

RESULTS

Of the 112/300 [37.3%] *Enterococcus* that was isolated, 102 [95.5%] were obtained in pure culture while 10 [8.9%] were in mixed culture. Of these, 42/112 [37.5%] were identified as *Enterococcus faecalis*. 24/112 [21.4%], 29/112 [25.8%], 5/112 [4.5%] and 6/112 [5.4%] strains carried the aac [6′]-Ie-aph [2′′]-Ia, aph [3′]-IIIa, aph [2′′]-Ic and aph [2′′]-Id genes, respectively. Table 1 shows the breakdown of the types of infections caused by the 35 HLGR and the 29 HLSR isolates.

Resistance to penicillin, imipenem and ciprofloxacin was exhibited by 92.9%, 73.8% and 69.1% of *Enterococcus faecalis* whereas 90.0% strains of *Enterococcus faecium* showed resistance equally to both penicillin & ciprofloxacin, while 81.8% strains were resistant to piperacillin. Penicillin resistance was shown by all [100%] strains of both *Enterococcus solitarius* & *Enterococcus pseudoavium*, and by 60.0% strains of *Enterococcus gallinarum*. 18.2% and 15.1% strains of *Enterococcus faecium* were resistant to vancomycin and teicoplanin, respectively, and by 16.7% & 4.8% of *Enterococcus faecalis*. *Enterococcus faecalis* [7.1%] and *Enterococcus faecium* [3.0%] showed complete resistance to linezolid [Table 2].

Table 3 shows the statistical analysis of differences in the prevalence of antibiotic resistance between different groups of Enterococcus. The differences in antibiotic resistance pattern to ampicillin, piperacillin, tetracycline, erythromycin, ciprofloxacin, imipenem [P=0.000] and penicillin [P=0.01] amongst *Enterococcus faecalis*, *Enterococcus faecium* and unusual strains were statistically significant. Resistance to piperacillin and imipenem amongst the HLGR *Enterococcus faecalis*, HLGR *Enterococcus faecium* [P= 0.01; P= 0.040], and HLGR unusual strains were statistically significant.

Ampicillin resistant strains, 35/57 [61.4%] were mostly isolated from IPD. Vancomycin resistance was seen commonly with 3/5 [60.0%] *Enterococcus faecalis* isolated from OPD. Likewise, 44.4% [4/9] VR *Enterococcus faecium* and 11.1% [1/9] VR *Enterococcus gallinarum* were isolated from IPD. Among the HLGR isolates, 1/3 [33.3%] were *Enterococcus faecium*. *Enterococcus mundtii* & *Enterococcus solitarius*, each, and were isolated from OPD. 3/10 [30.0%] HLSR *Enterococcus faecalis* isolates were from IPD as compared to 2/6 [33.3%] from OPD. All [100%] *Enterococcus faecalis* that were HLAR were recovered from IPD [Table 4].

Interestingly, the comparison between strains carrying genes like aac [6′]-Ie-aph [2′′]-Ia, aph [2′′]-Ic & aph [2′′]-Id showed high resistance to vancomycin [27.2%] with MIC [32 – 64 µg/mL] in *Enterococcus faecium*. All the unusual strains [100%] followed by *Enterococcus faecium* [63.6%] and *Enterococcus faecalis* [41.2%] had ampicillin resistance [MIC 16 – 64 µg/mL] with the simultaneous expression of gentamicin resistant gene. HLGR *Enterococcus faecium* were resistant to imipenem [90.9%].

Among the strains that expressed aph [3′]-IIIa gene, 33.3% were unusual species which were also resistant to vancomycin [MIC 32 – 64 µg/mL]. In comparison to HLSR strains, 100% *Enterococcus faecium* were resistant to ampicillin [MIC 16 – 64 µg/mL]. 83.3% unusual and 69.2% strains were resistant to imipenem and *Enterococcus faecalis*, respectively.

HLGR	Infection Type				Total [%]
	UTI	Wound Infection	BSI	Catheter Induced Infection	
Enterococcus faecalis	3 [30.0%]	4 [44.4%]	5 [45.4%]	5 [100%]	17 [48.5%]
Enterococcus faecium	4 [40.0%]	4 [44.4%]	3 [27.2%]	0	11 [31.4%]
Enterococcus gallinarum	2 [20.0%]	0	0	0	2 [5.7%]
Enterococcus mundtii	0	0	0	0	0
Enterococcus pseudoavium	0	1 [11.1%]	1 [9.0%]	0	2 [5.7%]
Enterococcus solitarius	1 [10.0%]	0	1 [9.0%]	0	2 [5.7%]
Enterococcus dispar	0	0	1 [9.0%]	0	1 [2.7%]
Total	10	9	11	5	35
HLSR	**Infection Type**				
	UTI	**Wound Infection**	**BSI**	**Catheter Induced Infection**	**Total [%]**
Enterococcus faecalis	6 [60.0%]	5 [55.5%]	1 [14.2%]	1 [33.3]	13 [44.8%]
Enterococcus faecium	4 [40.0%]	4 [44.4%]	2 [28.5%]	0	10 [34.4%]
Enterococcus gallinarum	0	0	1 [14.2%]	1 [33.3]	2 [6.8%]
Enterococcus mundtii	0	0	1 [14.2%]	0	1 [3.4%]
Enterococcus pseudoavium	0	0	1 [14.2%]	0	1 [3.4%]
Enterococcus solitarius	0	0	1 [14.2%]	0	1 [3.4%]
Enterococcus dispar	0	0	0	1 [33.3]	1 [3.4%]
Total	**10**	**9**	**7**	**3**	**29**
Non-HLAR Isolates	**Infection Types**				
	UTI	**Wound Infection**	**BSI**	**Catheter Induced Infection**	**Total [%]**
Enterococcus faecalis	9 [56.2%]	1 [14.2%]	0	2 [33.3%]	12 [27.2%]
Enterococcus faecium	5 [31.2%]	3 [42.8%]	2 [13.3%]	0	10 [22.7%]
Enterococcus gallinarum	0	3 [42.8%]	3 [20.0%]	2 [33.3%]	8 [16.3%]
Enterococcus mundtii	1 [6.2%]	0	2 [13.3%]	1 [16.6%]	4 [8.1%]
Enterococcus pseudoavium	0	0	4 [26.6%]	0	4 [8.1%]
Enterococcus solitarius	1 [6.2%]	0	2 [13.3%]	0	3 [6.1%]
Enterococcus dispar	0	0	2 [13.3%]	1 [16.6%]	3 [6.1%]
Total	**16**	**7**	**15**	**6**	**44**
HLAR Isolates	**Infection Types**				
	UTI	**Wound infection**	**BSI**	**Catheter induced infection**	**Total [%]**
Enterococcus faecalis	0	3 [75.0%]	0	0	3 [75.0%]
Enterococcus gallinarum	0	1 [25.0%]	0	0	1 [25.0%]
Total	**0**	**4**	**0**	**0**	**4**

Table 1. Distribution of HLGR, HLSR, Non HLAR and HLAR [Clinical] in Different Infection Types

Antibiotics	Susceptibility pattern	*Enterococcus faecalis* n=42	*Enterococcus faecium* n=33	*Enterococcus gallinarum* n=15	*Enterococcus mundtii* n=5	*Enterococcus Solitarius* n=5	*Enterococcus Dispar* n=5	*Enterococcus pseudoavium* n=7
Ampicillin	Sensitive	4 [9.5%]	5 [15.1%]	7 [46.6%]	2 [40.0%]	2 [40.0%]	2 [40.0%]	2 [28.5%]
	Resistant	31 [73.8%]	18 [54.6%]	3 [20.0%]	1 [20.0%]	1 [20.0%]	1 [20.0%]	2 [28.6%]
	Intermediate	7 [16.6%]	10 [30.3%]	5 [33.3%]	2 [40.0%]	2 [40.0%]	2 [40.0%]	3 [42.8%]
Penicillin	Sensitive	3 [71.4%]	3 [9.0%]	3 [20.0%]	1 [20.0%]	0	3 [60.0%]	0
	Resistant	39 [92.9%]	30 [90.0%]	9 [60.0%]	4 [80.0%]	5 [100%]	1 [20.0%]	7 [100%]
	Intermediate	0	0	3 [20.0%]	0	0	1 [20.0%]	0
Piperacillin	Sensitive	4 [9.5%]	3 [9.0%]	8 [53.3%]	3 [60.0%]	3 [60.0%]	1 [20.0%]	4 [80.0%]
	Resistant	38 [41.8%]	27 [81.8%]	5 [33.3%]	1 [20.0%]	1 [20.0%]	1 [20.0%]	1 [14.3%]
	Intermediate	0	3 [9.0%]	2 [13.3%]	1 [20.0%]	1 [20.0%]	3 [60.0%]	2 [28.5%]
Tetracycline	Sensitive	10 [23.8%]	9 [27.2%]	9 [60.0%]	4 [80.0%]	5 [100%]	5 [100%]	4 [80.0%]
	Resistant	26 [61.9%]	21 [63.6%]	4 [26.7%]	0	0	0	2 [28.6%]
	Intermediate	6 [14.2%]	3 [9.0%]	2 [13.3%]	1 [20.0%]	0	0	1 [14.3%]
Erythromycin	Sensitive	15 [35.7%]	6 [18.1%]	6 [40.0%]	4 [80.0%]	3 [60.0%]	3 [60.0%]	4 [80.0%]
	Resistant	22 [52.4%]	19 [57.6%]	2 [13.3%]	0	0	0	2 [28.6%]
	Intermediate	5 [11.9%]	8 [24.2%]	7 [46.6%]	1 [20.0%]	2 [40.0%]	2 [40.0%]	1 [14.3%]
Ciprofloxacin	Sensitive	8 [19.0%]	3 [9.0%]	3 [20.0%]	1 [20.0%]	1 [20.0%]	1 [20.0%]	3 [42.8%]
	Resistant	29 [69.1%]	30 [90.0%]	6 [40.0%]	2 [20.0%]	2 [20.0%]	2 [40.0%]	2 [28.6%]
	Intermediate	5 [11.9%]	0	6 [40.0%]	2 [40.0%]	2 [40.0%]	2 [40.0%]	2 [28.6%]
Imipenem	Sensitive	4 [9.5%]	8 [24.2%]	10 [66.6%]	4 [80.0%]	4 [80.0%]	4 [80.0%]	5 [71.4%]
	Resistant	31 [73.8%]	18 [54.6%]	3 [20.0%]	1 [20.0%]	1 [20.0%]	1 [20.0%]	2 [28.6%]
	Intermediate	7 [16.6%]	7 [32.0%]	2 [13.3%]	0	0	0	0
Teicoplanin	Sensitive	37 [88.0%]	27 [81.8%]	13 [86.6%]	4 [80.0%]	5 [100%]	4 [80.0%]	6 [85.7%]
	Resistant	2 [4.8%]	5 [15.1%]	2 [13.3%]	0	0	2 [40.0%]	1 [14.2%]
	Intermediate	3 [7.1%]	1 [3.0%]	0	1 [20.0%]	0	0	0
Vancomycin	Sensitive	28 [66.6%]	20 [60.6%]	13 [86.6%]	3 [60.0%]	2 [40.0%]	4 [80.0%]	6 [85.7%]
	Resistant	7 [16.7%]	6 [18.2%]	1 [6.7%]	0	0	0	0
	Intermediate	7 [16.6%]	7 [32.0%]	1	2 [40.0%]	3 [60.0%]	1 [20.0%]	1 [14.2%]
Linezolid	Sensitive	37 [88.0%]	29 [87.8%]	15 [100%]	5 [100%]	5 [100%]	5 [100%]	7 [100%]
	Resistant	3 [7.1%]	1 [3.0%]	0	0	0	0	0
	Intermediate	2 [4.7%]	3 [9.0%]	0	0	0	0	0
Gentamicin	Sensitive	36 [85.7%]	29 [87.8%]	14 [93.3%]	4 [80.0%]	4 [80.0%]	3 [60.0%]	6 [85.7%]
	Resistant	6 [14.2%]	4 [12.1%]	2 [13.3%]	1 [20.0%]	1 [20.0%]	2 [40.0%]	1 [14.2%]
	Intermediate	0	0	0	0	0	0	0
Streptomicin	Sensitive	37 [88.0%]	28 [84.8%]	13 [86.6%]	4 [80.0%]	4 [80.0%]	4 [80.0%]	6 [85.7%]
	Resistant	5 [11.9%]	5 [15.1%]	2 [13.3%]	1 [20.0%]	1 [20.0%]	1 [20.0%]	1 [14.2%]
	Intermediate	0	0	0	0	0	0	0

Table 2. Antibiotic Susceptibility Profile of Enterococcus Species

Antibiotic	susceptibility	All strains				HLGR				HLSR			
		E faecalis, n=42	E *faecium*, n=33	Unusual strains, n=37	P Value	E faecalis, n=17	E *faecium*, n=11	Unusual Strains, n=7	P value	E faecalis, n=13	E *faecium*, n=10	Unusual Strains, n=6	P value
	S	4 [9.5%]	5 [15.1%]	15 [40.5%]		1 [5.8%]	5 [45.4%]	3 [42.8%]		1 [7.6%]	0	3 [50.0%]	
Amp	I	7 [16.6%]	10 [30.3%]	14 [37.8%]	0.000	4 [23.5%]	1 [9.0%]	0	0.409	0	0	0	-
	R	31 [73.8%]	18 [54.6%]	8 [21.6%]		12 [70.5%]	5 [45.4%]	4 [57.1%]		12 [92.3%]	10 [100%]	3 [50.0%]	
	S	3 [7.1%]	3 [9.0%]	7 [18.9%]		1 [5.8%]	1 [9.0%]	1 [14.2%]		0	0	4 [66.6%]	
P	I	0	0	4 [10.8%]	0.01	0	0	1 [14.2%]	0.271	0	0	0	-
	R	39 [92.9%]	30 [90.0%]	26 [70.2%]		16 [94.1%]	10 [90.9%]	5 [71.4%]		13 [100%]	10 [100%]	2 [33.3%]	
	S	4 [9.5%]	3 [9.0%]	19 [51.3%]		2 [11.7%]	2 [15.1%]	5 [71.4%]		0	0	4 [66.6%]	
Pi	I	0	3 [9.0%]	9 [24.3%]	0.00	0	0	1 [14.2%]	0.001	0	1 [10.0%]	0	-
	R	38 [90.4%]	27 [81.8%]	9 [24.3%]		15 [88.2%]	9 [81.8%]	1 [14.2%]		10 [76.9%]	9 [90.0%]	2 [33.3%]	
	S	10 [23.8%]	9 [27.2%]	27 [72.9%]		2 [11.7%]	9 [81.8%]	7 [100%]		5 [38.4%]	6 [60.0%]	4 [66.6%]	
Te	I	6 [14.2%]	3 [9.0%]	4 [10.8%]	0.000	3 [17.6%]	0	0		2 [15.3%]	0	0	0.86
	R	26 [61.9%]	21 [63.6%]	6 [16.2%]		12 [70.5%]	2 [15.1%]	0		6 [46.1%]	4 [40.0%]	2 [33.3%]	
	S	15 [35.7%]	6 [18.1%]	20 [54.0%]		8 [35.2%]	6 [54.5%]	0		3 [23.0%]	2 [20.0%]	2 [33.3%]	
E	I	5 [11.9%]	8 [24.2%]	13 [35.1%]	0.000	0	2 [15.1%]	4 [57.1%]	0.407	3 [23.0%]	2 [20.0%]	4 [66.6%]	0.42
	R	22 [52.4%]	19 [57.6%]	4 [10.8%]		9 [52.9%]	3 [27.2%]	3 [42.8%]		7 [53.8%]	6 [60.0%]	0	
	S	8 [19.0%]	3 [9.0%]	9 [24.3%]		8 [35.2%]	1 [9.0%]	1 [14.2%]		0	0	3 [50.0%]	
Cip	I	5 [11.9%]	0	14 [37.8%]	0.000	0	0	3 [42.8%]	0.060	323.0%]	0	0	0.80
	R	29 [69.1%]	30 [90.0%]	14 [37.8%]		9 [52.9%]	10 [90.5%]	3 [42.8%]		10 [76.9%]	10 [100%]	3 [50.0%]	
	S	4 [9.5%]	8 [24.2%]	25 [67.5%]		4 [23.5%]	0	0		0	0	5 [83.3%]	
I	I	7 [16.6%]	7 [32.0%]	4 [10.8%]	0.00	5 [13.5%]	1 [9.0%]	2 [28.5%]	0.060	4 [30.7%]	6 [60.0%]	1 [16.6%]	-
	R	31 [73.8%]	18 [54.6%]	8 [21.6%]		8 [35.2%]	10 [90.5%]	4 [57.1%]		9 [69.2%]	4 [40.0%]	0	
	S	37 [88.0%]	27 [81.8%]	32 [86.4%]		14 [82.3%]	9 [81.8%]	5 [71.4%]		13 [100%]	10 [100%]	6 [100%]	
Tei	I	3 [7.1%]	1 [3.0%]	1 [2.7%]	-	1 [5.8%]	0	1 [14.2%]	-	0	0	0	-
	R	2 [4.8%]	5 [15.1%]	5 [13.5%]		2 [11.7%]	2 [15.1%]	1 [14.2%]		0	0	0	
	S	28	20 [60.6%]	28 [75.6%]		13 [76.4%]	8 [72.7%]	6 [85.7%]		12 [92.3%]	9 [90.0%]	5 [83.3%]	
Van	I	7 [16.6%]	7 [32.0%]	8 [21.6%]	0.827	4 [23.5%]	0	0	-	1 [7.6%]	1 [10.0%]	1 [16.6%]	
	R	7 [16.7%]	6 [18.2%]	1 [2.7%]		0	3 [27.2%]	1 [14.2%]		0	0	0	
	S	37 [88.0%]	29 [87.8%]	37 [100%]		17 [100%]	10 [90.5%]	7 [100%]		10 [76.9%]	8 [80.0%]	6 [100%]	
Lz	I	2 [4.7%]	3 [9.0%]	0		0	0	0	-	1 [7.6%]	1 [10.0%]	0	-
	R	3 [7.1%]	1 [3.0%]	0		0	1 [9.0%]	0		2 [15.3%]	1 [10.0%]	0	

Table 3. Statistical Analysis of Differences in the Prevalence of Antibiotic Resistance between Different Groups of Enterococcus

Antibiotics MIC [µg/mL] & the no. of Strains	*Enterococcus faecalis* with aac [6′]-Ie-aph [2′′]-Ia, aph [2′′]-Ic & aph [2′′]-Id [n= 17]	*Enterococcus Faecium* with aac [6′]-Ie-aph [2′′]-Ia, aph [2′′]-Ic & aph [2′′]-Id [n=11]	Unusual Strains with aac [6′]-Ie-aph [2′′]-Ia, aph [2′′]-Ic & aph [2′′]-Id [n= 7]	Total
Vancomycin Intermediate [8-16] = 6	4 [23.5%]	-	-	4
Vancomycin Resistant [32 - 64] = 10	-	3 [27.2%]	1 [14.2%]	4
Ampicillin Resistant [16 – 64] = 70	12 [70.5%]	5 [45.5%]	4 [57.1%]	21
Teicoplanin Resistant [16 - 64] = 15	2 [11.7%]	2 [18.1%]	1 [14.2%]	5
Antibiotics Disc diffusion method				
Imipenem Intermediate = 18	5 [29.4%]	1 [9.0%]	2 [28.5%]	8
Imipenem Resistant = 57	8 [47.0%]	10 [90. 4%]	4 [57.1%]	22
Antibiotics MIC [µg/mL] & the no. of strains	*Enterococcus faecalis* with aph [3′]-IIIa [n =13]	*Enterococcus faecium* with aph [3′]-IIIa [n = 10]	Unusual strains with aph [3′]-IIIa [n = 6]	**TOTAL**
Vancomycin Intermediate [8-16] =6	1 [7.6%]	1 [10.0%]	-	2
Vancomycin Resistant [32 - 64] = 10	1 [7.6%]	1 [10.0%]	1 [16.6%]	3
Ampicillin Resistant [16 - 64] = 70	12 [92.3%]	10 [100%]	3 [50.0%]	25
Teicoplanin Resistant [16 - 64] = 15	-	-	-	0
Antibiotics Disc diffusion method				
Imipenem Intermediate = 18	2 [15.3%]	6 [60.0%]	1 [16.6%]	9
Imipenem Resistant = 57	9 [69.2%]	4 [40.0%]	0	13

Table 4. Antibiotic Resistance Pattern of Enterococcus Species Carrying AME Genes

DISCUSSION

Treatment of enterococcal endocarditis should comprise a bactericidal synergic combination of an aminoglycoside and a cell-wall active agent.[10,11] Ampicillin is usually preferred to glycopeptides, unless the strain is ampicillin resistant. With the increased prevalence of aminoglycoside resistant genes, viz. aac [6′]-Ie-aph [2′]-Ia, among clinical enterococcal isolates, the choice of aminoglycoside for synergistic combination therapy has been very limited. Both ampicillin resistance and HLGR are a matter of concern.[12]

Dissemination of the chromosomally encoded PBP5 gene by enterococcal conjugative plasmids among clinical enterococci isolates might contribute to a global spread.[13] Nonetheless, the synergistic combination of penicillin/ampicillin [β-lactam drugs] and aminoglycoside can be useful in treating serious enterococcal infections.

In contrast to our study results, other authors have reported that *Enterococcus faecium* leading to bacteraemia [53%] was higher in prevalence than *Enterococcus faecalis* [33%].[15] These differences could be attributed to variable host dynamism imposed by gender, diet, age or other environmental conditions.[14]

The present study shows that 65.7% & 11.4% HLGR isolates, and 65.5% & 10.3% HLSR isolates were obtained from the Medicine department, and from MICU, respectively. In other studies, the HLAR strains were isolated most frequently from Surgery ward, followed by ICU. Such variation in results signifies the diverse possibilities of the infection.[15]

Interestingly, it is clear that there is a great variation among the various study results regarding the prevalence of HLGR and HLSR enterococcal isolates in various types of infections including urinary tract infection [UTI], wound infection [WI], blood stream infection [BSI] and Catheter related infections [CI]. Such contrasting reports from different research locations clearly indicate that variation in isolation rate depends on the location of study and the sample type chosen.[16]

Comparing the resistance pattern of isolated strains in our study, and those of others, isolation rates of HLSR and HLGR, as measured by HPDDT appears to be lower. Other studies have showed that HLSR and HLGR in VSE strains was 19.8% and 9.9%, respectively.[17] Another study on VRE has reported incidence of 87.6% in HLGR, and 95.2% in HLSR among *Enterococcus* isolates.[18] Subsequently, higher percentages of *Enterococcus faecalis* [72%] and *Enterococcus faecium* [81%] isolates have been reported among HLAR from Delhi.[19] Such findings have unfavourable consequences for a patient with serious enterococcal infections since the synergistic anti-enterococcal effect of cell-wall–active agents and aminoglycosides is abrogated by HLAR.

Resistance pattern of various Enterococcal species appear to be similar in different studies. As against our results, Other studies reported 70.83%, 38.8%, 58.3% and 57.7% resistance to ampicillin, ciprofloxacin, tetracycline and erythromycin respectively. [17] Also, it is clear that there is a gradual ominous chronological increase in development of resistance to these antibiotics and newer antimicrobials. One study has reported, 3.1% resistant *Enterococcus faecium* strains, as against all *Enterococcus faecalis* strains that were sensitive to vancomycin. [16] Contrasting resistant patterns have been reported where isolates were resistant to teicoplanin [3%] and vancomycin [9%], along with other drugs.[19,20] This shows that resistance varies from one institution to another, even within the same area. Hence, it is essential to know the antibiogram of the enterococcal isolates in every setup to formulate an effective institution-specific antibiotic policy.[20]

The statistical analysis showed that the differences in antibiotic resistance pattern to most antibiotics were statistically significant. Other studies have reported that differences in the prevalence of resistance between groups of *Enterococcus faecalis* and *Enterococcus faecium* were statistically significant only in the case of gentamicin [p=0.046], ampicillin [p<0.001], imipenem [p<0.001], vancomycin [P<0.001], and trimethoprim/sulfamethoxazole [p<0.001].[4]

The intrinsic robustness of *Enterococcus faecalis* allows it to survive for extended periods of time, leading to its persistence and spread, particularly in the indoor hospital environment. While in our study, a total of 75.0% HLAR strains were recovered from IPD. In another study 55.6%, 49.0% and 46.8% indoor isolates of *Enterococcus faecium* were HLGR, HLSR and HLAR, while a meagre 16.0% strains from OPD were resistant.[15] Our study results reported 27.2% of Enterococcus faecium strains exhibiting higher resistance to vancomycin, and the expression of aminoglycoside modifying genes. 14.2% of unusual Enterococcus species expressing gentamicin resistant genes were also streptomycin resistant. Similar results have been reported by other workers, where, 100% unusual strains followed by 63.6% of *Enterococcus faecium* and 41.2% of *Enterococcus faecalis* were resistant to ampicillin, and also carried the gentamicin resistant gene. Among HLSR strains, a majority of faecalis-faecium, and unusual Enterococcus were resistant to ampicillin. HLAR is caused by the secretions of various aminoglycoside-modifying enzymes.[20]

Among the HLSR 83.3%, 69.2% and 40.0% unusual strains of *Enterococcus*, *Enterococcus faecalis* and *Enterococcus faecium* were resistant to imipenem. However, reports in other publications points out that *Enterococcus faecalis* strains are largely susceptible to ampicillin and imipenem, while *Enterococcus faecium* are mostly resistant. *Enterococcus faecium* resistance is associated with changes in PBPs.

CONCLUSIONS

Familiarization with the characteristics of resistance in enterococci in our setup reveals the importance of accurate determination of the results of antibiotic susceptibility. This will provide the basis for appropriate treatment of infections produced by these pathogens. VRE continues to pose a threat in our hospital. A higher resistance rate to other antibiotics in VRE strains, particularly to aminoglycosides is also worrisome. Limited treatment options for serious infections caused by resistant enterococci make it necessary to intensify infection control procedures and a follow-up of resistance.

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