Study of Prevalence and Antimicrobial Susceptibility Pattern of Enterococci Isolated from Clinically Relevant Samples with Special Reference to High Level Aminoglycoside Resistance (HLAR) in a Rural Tertiary Care Hospital

Deeksha Rana¹, Suvarna Sande²

¹Jawaharlal Nehru Medical College, Datta Meghe Institute of Medical Sciences (Deemed to Be University) Sawangi (Meghe), Wardha, Maharashtra, India. ²Department of Microbiology, Jawaharlal Nehru Medical College, Datta Meghe Institute of Medical Sciences (Deemed to Be University), Sawangi (Meghe), Wardha, Maharashtra, India.

ABSTRACT

BACKGROUND

Enterococci are important human pathogens that cause many infections including nosocomial infections. Some important clinical infections caused by *Enterococcus* species are urinary tract infections, bacterial endocarditis, genital tract infections, surgical wound infections, bacteraemia and meningitis. Around, 80 - 90% of infections are commonly caused by *E. faecalis* followed by *E. faecium* with a contribution of about 10 - 15% along with emergence of multi-drug resistance (MDR) including to vancomycin. Enterococci have developed both intrinsic and acquired resistance towards many antibiotics including to high level aminoglycosides. This short term project was undertaken to study the prevalence and antibiotic susceptibility (AST) pattern of *Enterococcus* species isolated from clinical specimen with special reference to high level aminoglycoside resistance (HLAR) in a rural tertiary care hospital.

METHODS

100 Enterococci isolated from clinically relevant samples were identified according to standard procedures and AST was carried out according to CLSI guidelines.

RESULTS

Out of 100 enterococci, 70 *E. faecalis*, 21 *E. faecium* and 09 other *Enterococcus* species were isolated. The results showed that majority of enterococci was isolated from >60 age group (37%), from male patients (59%), from urine samples (59%) and from medicine department (36%). AST showed overall high resistance to Penicillin (98%) Ampicillin (86%), Gentamicin (85%), Ciprofloxacin (60%), Vancomycin (12%) (VRE), high level gentamicin (42%) (HLGR) and high level streptomycin (34%) (HLSR) and 15% isolates showed resistance to HLGR + HLSR. Multi drug resistance was seen in 40 (57.1%) *E. faecalis* isolates and 11 (52.3%) *E. faecium* isolates. Minimum resistance was observed with Linezolid (3%).

CONCLUSIONS

The present study showed high prevalence of antibiotic resistance in Enterococci. Hence, *Enterococcus* species isolated from samples should be routinely screened for HLAR, MDR and VRE so as to prevent the spread of multi drug resistant Enterococci and for proper selection of antibiotics.

KEY WORDS

Enterococci, HLGR, HLSR

Corresponding Author:

Dr. Suvarna Sande,

Professor,

Jawaharlal Nehru Medical College,

DMIMS (DU) Sawangi (Meghe),

Wardha, Maharashtra, India.

E-mail: suvarnasande@yahoo.co.in

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BACKGROUND

Enterococci are gram positive, ovoid, facultative anaerobic bacteria arranged in short chains or pairs which can survive harsh temperatures and grow in high salt concentrations.^{[1],[2]} Enterococci possess several proteins secreted in the extracellular medium such as haemolysin-cytolysin, extracellular serine protease and protease gelatinase which contribute towards its virulence.^[3] Enterococci are part of normal intestinal flora of humans and have been recognized as important human pathogens causing various infections including nosocomial infections.^{[1],[2],[4]} The Enterococci have the ability to acquire, accumulate and share extra chromosomal elements which help in encoding antibiotic resistance genes expressing virulence traits thus explaining their increasing importance as nosocomial pathogens.^{[5],[6]} Some important clinical infections caused by *Enterococcus* species are urinary tract infections, bacterial endocarditis, genital tract infections, surgical wound infections, bacteraemia and meningitis.^{[2],[7],[8]} Around, 80-90% of infections are commonly caused by *E. faecalis* followed by *E. faecium* with a contribution of about 10-15% along with its predominant emergence as a multi-drug resistance (MDR) towards vancomycin.^{[7],[9]}

The Centre for Disease Control and Prevention in a survey indicated *Enterococcus* as the second most common causative agent of hospital acquired UTIs (13.9%) next to *E. coli*.^[10] Also, in an ICU setting, Enterococci can cause up to 15% of nosocomial urinary tract infections.^[1]

Traditionally, infections caused by Enterococci species were treated with combinations of cell wall active agents (e.g., penicillin) with aminoglycoside (streptomycin), yet the synergistic effects of such combination therapy failed because some strains developed high level resistance to aminoglycosides, β lactam antibiotics and vancomycin thus forming an alliance of HLAR with multi drug resistance (MDR).^{[4],[9]} Enterococci have developed both intrinsic and acquired resistance towards antibiotics. Intrinsic resistance is chromosomally mediated and is the characteristic feature of species whereas, acquired resistance results from either DNA mutation or acquisition of new DNA.^[9] Intrinsically, Enterococci express its tolerance against penicillinase-resistant penicillin, cephalosporins and low levels of aminoglycosides and clindamycin. Enterococci also have acquired resistance, which includes resistance to chloramphenicol, erythromycin, high level of clindamycin, tetracycline, high level of aminoglycosides, penicillin, fluoroquinolones; vancomycin etc., resistance to high level aminoglycosides occurs because of the release of various aminoglycoside modifying enzymes.^{[11],[12]} Interestingly, Enterococci are susceptible to co-trimoxazole in-vitro but not in-vivo, because of its ability to incorporate preformed folic acid which helps them to bypass the inhibition of folate synthesis produced by co-trimoxazole.^[9]

Till 1970, Streptomycin was clinically regarded as the drug of choice but soon, tolerance to the drug was observed in more than half of the Enterococci strains. In 1979, resistance to high level gentamicin was first noted in a strain of *E. faecalis*.^[1] Until recently, the drug that could be considered for the treatment of Multidrug resistant Enterococci infections was Vancomycin. However, emergence and worldwide prevalence of Vancomycin

resistant Enterococci (VREs) has made treatment of serious Enterococcal infections cumbersome. Strains of VRE along with ampicillin resistance and HLAR are most difficult to treat.^[13] *Enterococcus* posing resistance to three important antimicrobial agents (penicillin, aminoglycosides and vancomycin) is a serious challenge for both clinicians and healthcare care institutions as it leads to imprudent antibiotic use, failure of treatment, colonization pressure and spread of resistant strains of isolates.^[14]

Hence, this study has been undertaken to study the prevalence and antibiotic susceptibility (AST) pattern of Enterococci species isolated from clinical specimen with special reference to high level aminoglycoside resistance (HLAR) from a rural tertiary care hospital.

METHODS

This cross-sectional study was conducted in the Department of Microbiology in a rural tertiary care hospital for 3 months [June–August 2019, ICMR–STS] after obtaining approval from Institutional Ethics Committee. Sample collection and processing duration was of 2 months (ICMR–STS). The sample size was calculated using open EPI software. Based on findings from previous study^[15] about HLAR Enterococci isolates - expecting 5% margin of error and confidence interval of 90%, the sample size was calculated as 96 which was rounded off to 100. So, 100 Enterococci isolated from various clinically significant samples [urine, blood, in-dwelling catheter, pus, surgical site and body fluids etc] were included in the study.

Enterococci isolated from samples of OPD patients and samples from various environmental sources were excluded.

The details of each sample were recorded carefully in each case like details of the patients' name, age, sex, registration number, ward, diagnosis and site from which sample was collected etc. Direct microscopy which includes gram staining was carried out on appropriate samples. Samples were cultured on Nutrient Agar, Blood Agar and MacConkey Agar and incubated overnight at 37°C.

Enterococci isolated were identified according to the guidelines of Koneman (1975)^[16] on the basis of colony characters, gram staining and biochemical test like Catalase Test, Bile Esculin Test, Arabinose and Sorbitol fermentation test, growth on 6.5% NaCl agar and black coloured colonies on Potassium tellurite medium etc.

Antibiotic susceptibility profile of 100 Enterococci strains isolated from clinical samples was studied by Kirby – Bauer Disc Diffusion method as per Clinical Laboratory Standard Institute (CLSI) guidelines^[17]

Colonies from pure culture of *Enterococcus* were taken from the plate with the help of a sterile loop and transferred to a test tube containing sterile peptone water and incubated to 37 °C for 3–4 hours (turbidity adjusted to 0.5 McFarland standard). Lawn culture was done on Muller – Hinton agar plate by using sterile cotton swabs dipped in the broth culture of Enterococci strains. Following commercially available antibiotic discs (HiMedia) were placed aseptically on the surface of Muller - Hinton agar plate using sterile forceps and gently pressed. The plates were incubated at 37 °C for 16 -18 hours. Next day susceptibility profile of

Enterococci to different antibiotics was noted. The area of inhibitory zone was measured and recorded.

The results were interpreted using CLSI guidelines.^[17] Multi – drug resistance (MDR) is defined as resistance to at least three drugs from a variety of antibiotic classes.

The antibiotic discs used were Penicillin (10 U), Ampicillin (10 µg), Gentamicin (10 µg), High Level Gentamicin (120 µg), High Level Streptomycin (300 µg), Vancomycin (30 µg), Teicoplanin (30 µg), Linezolid (30 µg), Ciprofloxacin (5 µg) and Nitrofurantoin (300 µg) [only in urine samples].

High level aminoglycoside resistance was detected as per CLSI^[17] guidelines using High Level Gentamicin [HLG] disc (120 µg) and High-Level Streptomycin [HLS] disc (300 µg). Inhibition zone of 6 mm was taken as resistant, 7 – 9 mm as inconclusive and ≥ 10 mm as susceptible.

Staphylococcus aureus ATCC 25923 and *Enterococcus faecalis* ATCC 29212 were used as control strains.

Statistical Analysis

The statistical analysis was carried out by relevant statistical methods like percentage method (%).

RESULTS

Age Wise Distribution					
S.no	Age Group	<i>E. faecalis</i>	<i>E. faecium</i>	Other sp. of Enterococci	Total Enterococci in percentage (%)
1.	<20	12	04	-	16%
2.	21 - 30	06	-	02	08%
3.	31 - 40	08	03	01	12%
4.	41 - 50	07	03	01	11%
5.	51 - 60	08	07	01	16%
6.	>60	29	04	04	37%
Total		70	21	09	100
Gender Wise Distribution					
	GENDER	<i>E. faecalis</i>	<i>E. faecium</i>	Other sp. of Enterococci	Total Enterococci in Percentage (%)
1.	Male	39	16	04	59%
2.	Female	31	05	05	41%
Total		70	21	09	100
Sample Wise Distribution					
	Sample	<i>E. faecalis</i>	<i>E. faecium</i>	Other sp. of Enterococci	Total Enterococci in percentage (%)
1.	Urine (U)	48	07	04	59%
2.	Pus (P)	07	09	03	19%
3.	Blood (BD)	10	-	01	11%
4.	Others	05	05	01	11%
Total		70	21	09	100
Table 1. Age, Gender, Sample and Department Wise Distribution of Enterococci (n = 100)					
Others include local surgical site, pleural fluid, ascitic fluid, vaginal swab, sputum and Foley's catheter tip.					

Out of 100 isolates, 37 enterococci were isolated from age group of greater than 60 years followed by 16 Enterococci isolates each from age group of below 20 years and from age group between 51 – 60 years. Gender wise distribution showed 59 isolates were from male patient and 41 isolates were from female patients. Sample wise distribution showed that 59 isolates were from urine sample followed by 19 from pus sample and 11 from blood sample.

Among 59 urine isolates, 24 (40.6%) of Enterococcal isolates were from catheterized patients and among 11 blood isolates, 06 (45.4%) isolates were from the paediatric age group.

Department wise distribution showed that out of 100 Enterococcal isolates, 36 Enterococci were isolated from Medicine department followed by 35 isolates from Surgery department, 13 isolates from Paediatrics department, 6 isolates from Obstetrics/Gynaecology department, 5 isolates from Neuro - Surgery department, 3 isolates from Orthopaedics department and 2 isolates from Respiratory Medicine department.

Antibiotics n = (10)	<i>E. faecalis</i> n = (70)		<i>E. faecium</i> n = (21)		Other sp. of Enterococci n = (09)		Total Resistant Enterococci in Percentage (%)
	S	R	S	R	S	R	
Penicillin (10 U)	02 (2.9%)	68 (97.1%)	-	21 (100%)	-	09 (100%)	98%
Ampicillin (10 µg)	09 (12.9%)	61 (87.1%)	04 (19.1%)	17 (80.9%)	01 (11.2%)	08 (88.8%)	86%
Gentamicin (10 µg)	08 (11.5%)	62 (88.5%)	05 (23.9%)	16 (76.1%)	02 (22.3%)	07 (77.7%)	85%
High Level Gentamicin (120 µg)	40 (57.2%)	30 (42.8%)	09 (42.9%)	12 (57.1%)	09 (100%)	-	42%
High Level Streptomycin (300 µg)	43 (61.5%)	27 (38.5%)	14 (66.7%)	07 (33.3%)	09 (100%)	-	34%
Vancomycin (30 µg)	62 (88.6%)	08 (11.4%)	18 (85.8%)	03 (14.2%)	08 (88.9%)	01 (11.1%)	12%
Teicoplanin (30 µg)	64 (91.5%)	06 (8.5%)	18 (85.8%)	03 (14.2%)	08 (88.9%)	01 (11.1%)	10%
Linezolid (30 µg)	68 (97.2%)	02 (2.8%)	20 (95.3%)	01 (4.7%)	09 (100%)	-	03%
Ciprofloxacin (5 µg)	27 (38.6%)	43 (61.4%)	07 (33.4%)	14 (66.6%)	06 (66.7%)	03 (33.3%)	60%
Nitrofurantoin (300 µg) [only in Urine Sample] n = (59)	<i>E. faecalis</i> n = (48)		<i>E. faecium</i> n = (07)		Other sp. of Enterococci n = (04)		Total Resistant Enterococci in percentage (%)
	S	R	S	R	S	R	
	23 (48.0%)	25 (52.0%)	05 (71.5%)	02 (28.5%)	04 (100%)	-	46%

*S - Susceptible *R - Resistance

Table 2. Antibiotic Resistance Pattern of Enterococci Isolates (n = 100)

From total 100 Enterococci species, total 98% Enterococci isolates showed resistance to Penicillin followed by 86% isolates showed resistance to Ampicillin, 85% isolates showed resistance to Gentamicin, 60% isolates showed resistance to Ciprofloxacin, 42% isolates showed resistance to High Level Gentamicin (HLG), 34% isolates showed resistance to High Level Streptomycin (HLS), 12% isolates showed resistance to Vancomycin, 10% isolates showed resistance to Teicoplanin and out of total 59 Enterococci species isolated from urine sample; about 46% isolates showed resistance to Nitrofurantoin.

Out of total 100 Enterococci isolates, we found 12 (12%) Enterococci strains resistant to Vancomycin by Kirby – Bauer disc diffusion method. We were unable to confirm the Vancomycin resistance (VRE) strains because of the non – availability of E – strips and Vitek AST cards during study period. Table 2 also depicts Species wise antibiotic resistance which showed that *E. faecalis* was more resistant to antibiotics than *E. faecium*.

Sl. No.	Antibiotics	Samples			
		Urine	Pus	Blood	Others
1.	High Level Gentamycin (HLGR) (120 µg) n = (42)	27 (64.2%)	07 (16.6%)	06 (14.2%)	02 (4.7%)
2.	High Level Streptomycin (HLSR) (300 µg) n = (34)	21 (61.7%)	06 (17.6%)	05 (14.7%)	02 (5.8%)

Table 3. Sample Wise Prevalence of High-Level Aminoglycoside Resistance (HLAR) in Enterococci Species Isolates (n = 76)

Sl. No.		HLGR	HLSR	HLGR + HLSR
1.	<i>E. faecalis</i> n = (70)	30 (42.8%)	27 (38.5%)	13 (18.5%)
2.	<i>E. faecium</i> n = (21)	12 (57.1%)	07 (33.3%)	02 (9.5%)
3.	Other sp. of <i>Enterococcus</i> = (09)	-	-	-
Total		42%	34%	15%

Table 4. Prevalence of High-Level Aminoglycoside Resistance (HLAR) among Enterococci isolates (n = 100)

Antibiotics	Penicillin	Ampicillin	Vancomycin	Total Resistant Enterococci in Percentage (%) [Avg.]
HLGRn = (42)	40 (95.2%)	37 (88.0%)	09 (21.4%)	68.2%
HLSRn = (34)	33 (97.0%)	28 (82.3%)	03 (8.8%)	62.7%
Total <i>Enterococcus</i> isolates n = (76)	73 (96.0%)	65 (85.5%)	12 (15.7%)	65.7%

Table 5. Prevalence of Enterococci Resistance to Penicillin, Ampicillin and Vancomycin among HLGR and HLSR Isolates (n = 76)

Out of 76 isolates showing HLAR, 42 isolates showed High Level Gentamicin Resistance (HLGR) from which 27 (64.2%) were isolated from urine sample, 07 (16.6%) from pus sample and 06 (14.2%) from blood sample. 34 isolates showed High Level Streptomycin Resistance (HLSR) from which 21 (61.7%) were isolated from urine sample, 06 (17.6%) from pus sample and 05 (14.7%) from blood sample.

Out of 70 isolates of *E. faecalis*, 30 (42.8%) isolates showed High Level Gentamicin Resistance (HLGR) followed by 27 (38.5%) isolates showed High Level Streptomycin Resistance (HLSR) and 13 (18.5%) isolates showed combined resistance to High Level Gentamicin and High-Level Streptomycin (HLGR + HLSR). Out of 21 isolates of *E. faecium*, 12 (57.1%) isolates showed High Level Gentamicin Resistance (HLGR) followed by 07 (33.3%) isolates showed High Level Streptomycin Resistance (HLSR) and 02 (9.5%) isolates showed combined resistance to High Level Gentamicin and High-Level Streptomycin (HLGR + HLSR).

Out of total 42 isolates of High-Level Gentamicin Resistance (HLGR), 95.2% isolates showed resistance to Penicillin, 88% isolates showed resistance to Ampicillin and 21.4% isolates showed resistance to Vancomycin. Out of 34 isolates of High-Level Streptomycin Resistance (HLSR), 97% isolates showed resistance to Penicillin, 82.3% isolates showed resistance to Ampicillin and 8.8% isolates showed resistance to Vancomycin.

Multi - Drug Resistance (MDR) Profile among Enterococci Isolates

Out of 70 *E. faecalis* strains, 40 (57.1%) isolates showed multidrug resistance i.e. resistance to at least three drugs from a variety of antibiotic classes. Out of 21 *E. faecium* strains, 11 (52.3%) isolates showed MDR. So, total 51 (51%) Enterococcal isolates showed Multidrug Resistance. Among the samples, the MDR strains were mostly isolated from urine samples (12%) and among the wards, MDR strains were

mostly isolated from Paediatric wards (53.8%) and Medicine wards (33.3%).

DISCUSSION

Enterococcus is a species of substantial medical importance and is a prototypical multidrug resistance pathogen and is recognized for its ubiquity and is notorious for being intrinsically advanced antibiotic resistance. Although a wide variety of antimicrobial agents with antibacterial activities are available, life threatening conditions caused by Enterococci continue to be prevalent. It is the reason for high mortality rate as it has arisen as a vital pathogen.

In present study, total 100 Enterococci species were isolated from various clinically significant samples, from the hospitalized patients and their antimicrobial susceptibility pattern was determined. Most of the isolates belonged to elderly age group of >60 years (37%) followed by younger age group of <20 years (16%) and older age group of 51 – 60 years (16%). The least number of isolates were found in the age group of 21 – 30 years (8%). (TABLE 1) Decreased immunity, prolonged hospitalization and other associated co – morbidities can be relatable cause in the age group > 60 years.

In present study, prevalence of Enterococci infection was found to be more in males (59%). (Table 1) This result was in accordance with the study of Abdulhakim Abamecha et al^[18] which reported Enterococcal infection in 50.7% males and Varun Goel et al^[19] also reported maximum cases in males (53.9%).

Present study showed that maximum Enterococci were isolated from urine sample (59%) followed by pus sample (19%) and blood sample (11%). (Table 1) Study by Deepika Atray et al^[20] reported maximum Enterococci isolates from urine (50%) followed by pus (20%) and blood (10%).

In present study, out of total 59 Enterococcal isolates from urine sample, maximum number of isolations was of *E. faecalis* 48(81.3%) followed by 07(11.8%) isolates of *E. faecium*. However, out of total 19 enterococcal isolates from pus samples, maximum isolation was of *E. faecium* 09(47.3%) followed by 07 (36.8%) isolates of *E. faecalis*. Out of total 11 enterococcal isolates from blood samples, maximum isolation was of *E. faecalis* 10 (90.9%) and no *E. faecium* isolates were reported. Other species of *Enterococcus* reported were 04 (6.7%) isolates in urine sample, 03 (15.7%) isolates in pus sample and 01 (9%) isolates in blood sample. (Table 1)

Among 59 urine isolates, 24 (40.6%) Enterococcal isolates were from catheterized patients and among 11 blood isolates, 06 (45.4%) isolates were from the paediatric age group which is in accordance with the study by S. Sreeja et al^[21] who reported 38% of Enterococcal isolates from catheterized patients and 58% isolates from paediatric age group.

In present study, 36% of Enterococci isolates were from medicine department followed by surgery department (35%) and paediatrics department (13%) which was in accordance with the study done by A. Tripathi et al^[22] who reported Enterococci isolation from medicine department up to

52.33% and 39.26% of Enterococcal isolation from surgery department.

In present study, overall resistance pattern showed that 98% of isolates were resistant to Penicillin followed by 86% to Ampicillin, 85% to Gentamicin, 60% to Ciprofloxacin, 42% to High Level Gentamicin, 34% to High Level Streptomycin, 12% to Vancomycin, 10% to Teicoplanin and with lowest resistance of 3% to Linezolid. 46% of urine isolates show resistance to Nitrofurantoin. (Table 2)

Various Study	P	AMP	GEN	HLG	HLS	V	TE	LZ	CIP	NIT
Kalpna Devi Venkatesan et al ^[23]	49.7 %	21.7 %	-	37.2 %	-	5.7 %	-	0 %	56 %	13.1 %
Kheya Mukherjee et al ^[24]	-	70 %	91.4 %	-	-	3.8 %	-	0 %	92 %	-
Mitali R Maradia et al ^[25]	97.44 %	91.6 %	-	29.49 %	-	3.2 %	1.92 %	0 %	76.28 %	-
Seema Mittal et al ^[26]	66.67 %	-	-	29 %	35 %	5 %	-	1 %	25 %	24.3 %
VA Rahangdale et al ^[27]	89.43 %	43.9 %	-	49.59 %	47.96 %	11.38 %	-	0% %	-	22.76 %
Jahnabi Barman et al ^[28]	100 %	93.6 %	-	-	-	1 %	1.1 %	0 %	69.9 %	15.9 %
Aasish Karna et al ^[29]	40.7 %	39.5 %	-	18.7 %	-	20.9 %	4.4 %	2.2% %	61.5 %	17.9 %
Present Study	98 %	86 %	85 %	42 %	34 %	12 %	10 %	3 %	60 %	46 %

Table 6. Comparison of Overall Antibiotic Resistance Pattern of Enterococci in Different Studies with Present Study

Present study reports 42% of isolates resistant to HLG and 34% isolates resistant to HLS (Table 2) which was in accordance with the study by Smeeta Huidrom et al^[30] who reported that 33.3% and 36.2% of enterococci were resistant to HLGR and HLSR respectively.

In present study, 12% of isolates showed resistance to Vancomycin and 10% of isolates showed resistance to Teicoplanin. (Table 2) Study by VA Rahangdale et al^[27] reports 11.3% of Vancomycin resistance and study by Maninder Kaur Jagdev et al^[31] reports 5.7% of Teicoplanin resistance which is in accordance with the present study.

Present study reports 3% of Enterococcal isolates resistant to Linezolid (Table 2) which is accordance with the study by Aasish Karna et al^[29] who reported Linezolid resistance in 4.9% isolates.

In the present study, HLGR was shown by total 42 Enterococcal isolates in which 27 (64.2%) were isolated from urine samples followed by 07 (16.6%) from pus samples and 06 (14.2%) from blood samples (Table 3) which is higher than the study by Seema Mittal et al^[26] which reported HLGR more common in urine samples (17%) followed by pus samples (15.7%).

In present study, 42.8% *E. faecalis* isolates showed HLGR resistance (Table 4) which was in accordance with the study by S. Sreeja et al^[21] which showed HLGR resistance by 44.3% isolates of *E. faecalis*. However, the study by Maninder Kaur Jagdev et al^[31] showed HLGR resistance by 82.5% *E. faecalis* isolates. In present study, 38.5% *E. faecalis* isolates showed HLSR resistance which was in accordance with the study by Smeeta Huidrom et al^[30] which showed HLSR resistance by 33.3% isolates of *E. faecalis*.

In Present study, 57.1% *E. faecium* isolates showed HLGR resistance (Table 4) which was in accordance with the study by S. Sreeja et al^[21] which showed HLGR resistance by 54.1%

isolates of *E. faecium*. However, the study by Maninder Kaur Jagdev et al^[31] showed HLGR resistance by 92.5% *E. faecium* isolates. In present study, 33.3% *E. faecium* isolates showed HLSR resistance which was in accordance with the study by Smeeta Huidrom et al^[30] which showed HLSR resistance by 37.3% isolates of *E. faecium*.

In present study, other Enterococci species showed no HLGR and HLSR resistance (Table 4) but in the study by Maninder Kaur Jagdev et al^[31] HLGR resistance was detected in 66.6% isolates of other Enterococci species and in the study by Varun Goel et al^[19] HLSR resistance was detected in 50% isolates of other Enterococci species.

In the present study, out of 100 Enterococcal isolates, 42% isolates showed HLGR, 34% isolates showed HLSR and 15% isolates showed HLGR + HLSR. The combined resistance to both the aminoglycosides was showed by 18.5% *E. faecalis* isolates and 9.5% *E. faecium* isolates (Table 4) while study by DK Mendiratta et al^[32] who reported combined resistance to both the aminoglycosides by 59.1% isolates of *E. faecium* and 7.8% isolates of *E. faecalis*. HLGR and HLSR in the present study is 42% and 34% respectively, which is alarmingly high percentage of resistance and is worthy of note.

Prevalence of HLAR in cell wall active agents like Penicillin, Ampicillin and Vancomycin was also studied. (Table 5) In the present study, out of 42 HLGR Enterococci isolates, resistance to Penicillin, Ampicillin and Vancomycin was shown by 95.2%, 88% and 21.4% of HLGR Enterococci isolates respectively. Also, out of 34% HLSR Enterococci isolates, 97% of isolates showed resistance to Penicillin, 82.3% of isolates showed resistance to Ampicillin and only 8.8% of isolates showed resistance to Vancomycin. Hence, resistance to the cell wall active agents was quite high in both the species and it was higher to Penicillin than Ampicillin.

Resistant to Penicillin and Ampicillin which is usually intrinsic is due to low affinity of the penicillin – binding proteins (PBP) and it results in loss of synergistic effect between β lactams and aminoglycosides leading to treatment failures.

The bacterial strains that show resistance to three or more categories of antibiotics are defined as multidrug resistant (MDR) strains. Multidrug resistant (MDR) Enterococci is a growing problem. Altered drug sites, bacterial efflux pumps, enzyme production or inhibition, loss of membrane protein etc. are different mechanisms mediated by MDR Enterococci.

In present study, 40 (57.1%) strains of *E. faecalis* and 11 (52.3%) strains of *E. faecium* showed MDR which was in accordance with the study by DK Mendiratta et al^[32] who reported multi drug resistance of *E. faecalis* strains higher than the strains of *E. faecium*. However, the study by Aasish Karna et al^[29] reported prevalence of MDR highest in *E. faecium* (62%) than in *E. faecalis* strains.

Limitations

Present study was of short duration (3 months ICMR STS) and sample size was 100 isolates, studies involving large sample size and more duration to know about prevalence and changing pattern of antibiogram of *Enterococcus* will be more

appropriate. Therefore, further studies should be multicentric and include a large sample size.

Vancomycin resistance (VRE) in this study was detected by Kirby – Bauer Disc Diffusion method only. We were unable to confirm the Vancomycin resistance (VRE) strains because of the non – availability of E–strips and Vitek AST cards during study period. Detection of VRE by E–strips and Vitek AST cards will be more appropriate as these methods are considered as Gold Standard methods.

CONCLUSIONS

The present study showed high prevalence of antibiotic resistance in Enterococci. Hence, *Enterococcus* species isolated from samples should be routinely screened by all Clinical Microbiology Laboratories for HLAR, MDR and VRE so as to prevent the spread of multiple antibiotic resistant enterococci. A regular surveillance of drug resistance can be considered for selection of antibiotics, changes in the antibiotic susceptibility patterns, and usage of antibiotics like vancomycin and linezolid in the treatment of life-threatening infections caused by Enterococci.

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