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Research Article

PREVALENCE OF HIGH-LEVEL AMINOGLYCOSIDE AND VANCOMYCIN-RESISTANT ENTEROCOCCUS SPECIES AT A TERTIARY CARE HOSPITAL IN GREATER NOIDA, U.P

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A R T I C L E I N F O

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High-level Gentamicin (HLG), High-Level Streptomycin (HLS), Vancomycin-Resistant Enterococci, High-Level Aminoglycoside Resistance (HLAR)

A B S T R A C T

Background: Enterococcal infection has emerged as a major therapeutic challenge. Exposure of High- Level Aminoglycoside Resistance (HLAR) and Vancomycin-Resistant among Enterococcus species has further limited the drug in Enterococcal infections.
Objective: To estimate the prevalence of High- Level Aminoglycoside Resistant (HLAR), i.e HLG and HLS resistance and Vancomycin-Resistant among Enterococcus species at a tertiary care hospital in Greater Noida.
Material & Methods: A total of 80 isolates identified by morphological and biochemical characteristics were tested for antibiotic susceptibility using Kirby- Bauer disc diffusion method and the Epsilometer test (E- test) and Vitek automated as per standard protocol.
Results: Eighty out of 13,639 culture-positive clinical samples comprising of 48 urine, 15 from pus, 6 blood, 5 vaginal swabs, 2 ETT, 1 BAL, and 1 tissue fluid(Pleural fluid) isolates were identified as *Enterococcus faecalis* (57.5%), *Enterococcus faecium* (35%), *Enterococcus durans* (5%), and *Enterococcus gallinarum* (2.5%). The majority of the isolates (61.25%) were from a urine specimen. While High-Level Aminoglycoside Resistance was seen in 71.62% of the isolates. Vancomycin-Resistant Enterococci were found to be majorly in urine isolates.
Conclusions: The petrifying rise in the prevalence of Vancomycin and multidrug resistance strains authorize immediate, sufficient, and efficient surveillance programs to prevent and control its spread.

INTRODUCTION

Enterococci are gram-positive oval cocci, Catalase-negative, non-spore-forming, and facultatively anaerobic organisms that associate with the Lancefield group D Streptococci[(1)]. Enterococci have been reported as the foremost cause of urinary tract infection (Cystitis, Urethritis, and Pyelonephritis), bacteremia, and few more clinical problems, mostly in hospital settings[(2)].

Enterococci can harbor intrinsic low-level antibiotic resistance. Moreover, in recent years, Enterococci have developed an escalating process of acquiring high-level antibiotic resistance to Aminoglycosides, β- lactams, and Glycopeptides, Cephalosporins. However, the resistance to Enterococci to multiple antibiotics allows them to proliferate, especially in patients receiving multiple antimicrobials, causing superinfection. These microorganisms are competent in acquiring and interchanging genes encoding resistance to antimicrobial agents either by mutation or accession of Extra chromosomal DNA such as transposons or plasmids[(3)].

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This genus is an introductory clinically suitable group of gram-positive cocci to gain resistance to Vancomycin, thus the title Vancomycin-Resistant Enterococci (VRE), and now it becomes a significant public health interest. No research study has been done in Greater Noida, U.P to determine the prevalence of VRE. So, this study on the prevalence of Vancomycin and High- level Aminoglycoside Resistance among Enterococci seems essential, a report of which could be highly beneficial for infection control and formulation of antibiotic policies in a hospital set up in this region.

MATERIAL AND METHODS

Study Design and Area

It was a cross-sectional study conducted from December 2019-November 2020 in Sharda Hospital, Greater Noida. A total number of 80 Enterococcal Isolates were collected from different clinical samples from outpatient and in patients.

Inclusion Criteria: All Enterococcus strains isolated from a clinical sample received in the central laboratory.

Exclusive criteria: All isolated strains other than Enterococcus.

Isolation and Detection of various Enterococcus species Isolates

Several specimens including Urine, Blood, Pus, Tissue fluids, and Vaginal swab were received to the Microbiology laboratory from IPD and OPD patients from the bacteriological examinations were contained in this study. All the specimens that were inoculated on the Blood agar and MacConkey agar & Urine specimens on the CLED agar (Cysteine- lactose electrolyte deficient) were mentioned. Isolates of Enterococci were identified by colony morphology, Gram staining, Catalase test, and growth on Bile Esculin agar, and the L-pyrrolidonyl-β-napthylamide(PYR) test, Arginine hydrolysis, and 6.5% NaCl tolerance were used to identify the isolated strains. Then speciation was performed by sugar fermentation test (Mannitol, Mannose, Arabinose, Raffinose, Sorbitol, Fructose), Mannitol media for motility testing, growth on Tellurite Agar. All the tests were carried out and interpreted as describe by Facklam and Collins[4]. All isolates were detected to the species level by using Vitek 2 Automated system[5].

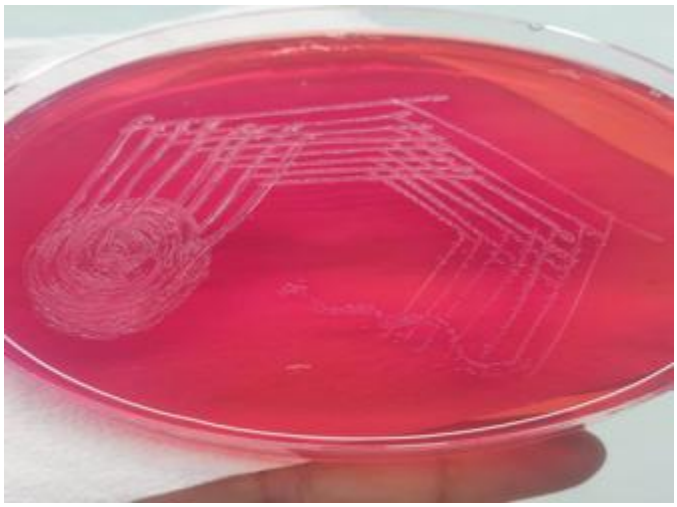

Fig 1 MacConkey agar showing minute lactose fermenting colonies by Enterococcus.

Fig 2 Bile esculin hydrolysis test (left- negative, right-positive result, black color due to esculin hydrolysis).

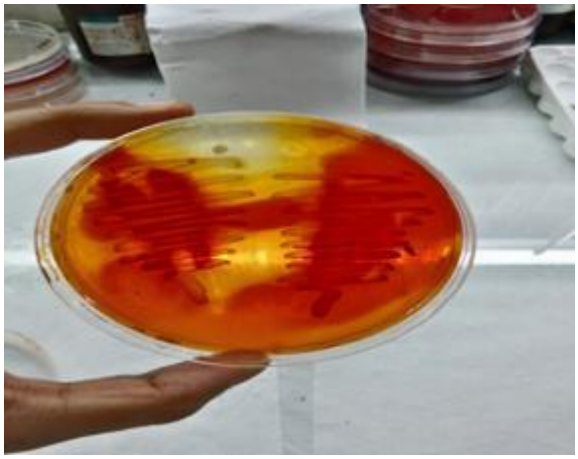

Fig 3 PYR test: Pyrrolidonyl-β-Napthylamide. Development of deep cherry red color within a minute of addition of the reagent (N, N-dimethylaminocinnamaldehyde)

Antimicrobial susceptibility testing, AST

These Enterococcus isolates were additionally further tested for the antimicrobial susceptibility to different antimicrobial agents such as Penicillin(10units), Ampicillin(10µg), Linezolid(30µg), Vancomycin(30µg), Teicoplanin(30µg), High-level Gentamicin HLG (120µg), High-level Streptomycin HLS (300µg), Erythromycin(15µg), Tetracycline(30µg), Levofloxacin(5µg), Ciprofloxacin(5µg), Chloramphenicol (30µg), Nitrofurantoin(300µg), Norfloxacin (10µg) and Fosfomycin(200µg) by Kirby Bauer disc diffusion method after meeting bacterial suspension with Mac Farland's 0.5 standards according to the CLSI guidelines 2020[6] using standard microbiological methodologies on the Mueller Hinton, MHA agar plates zone of inhibition equal to or less than 6mm for both high-level Aminoglycoside (HLA), i.e, HLG and HLS were outlined as HLA resistant. Further, Minimum inhibitory concentration (MIC) for Vancomycin was identified by E- strip test, MIC less than 4µg/ml was considered as sensitive, 8-16 µg/ml as intermediate, and greater than 32µg/ml as resistant. *Enterococcus faecalis* ATCC 29212 was used as a control strain.

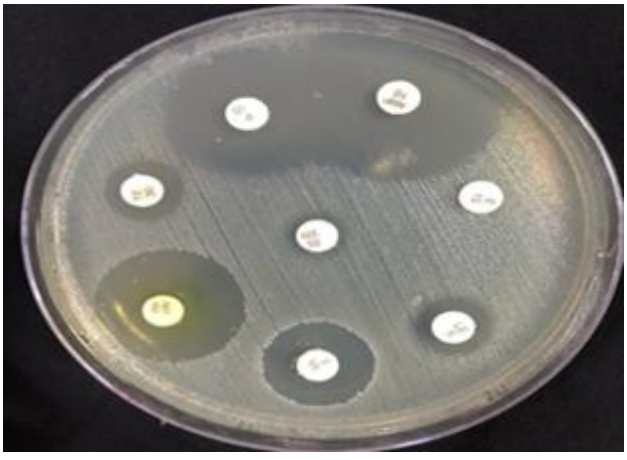

Fig 4 Antibiotic susceptibility test by Kirby- Bauer disk diffusion method.

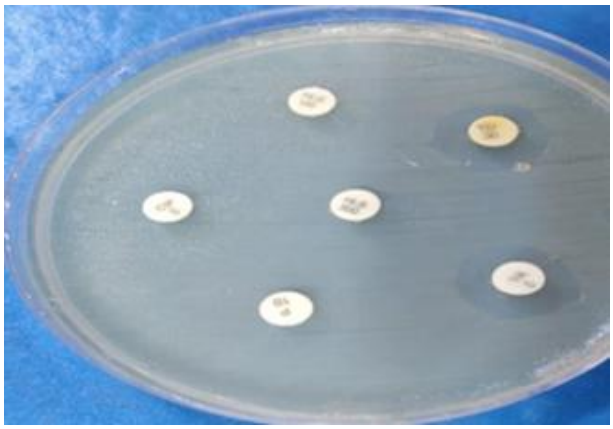

Fig 5 Antibiotic susceptibility test showing HLAR (High-level Aminoglycoside resistant)

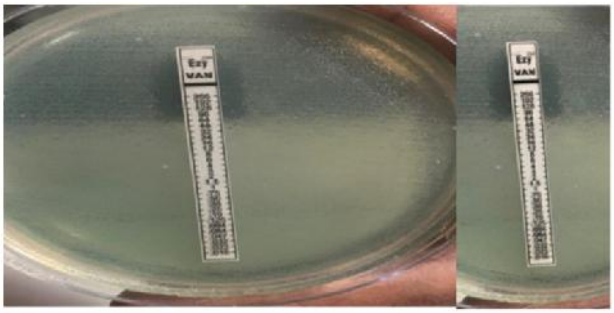

Fig 6 Vancomycin E-strip showing 32µg/ml against Enterococci

OBSERVATIONS & RESULTS

A total of about 13,639 samples were received in which 10,727 were Urine specimens, 603 Pus, 1332 Blood, 68 ETT, 58 CSF, 394 Sputum, 53 Stool, 129 Swabs, 127 Body fluids, 21 Bronchoalveolar lavage (BAL), 65 Catheter & tips, 13 Tissue, 32 Tracheal secretion, 4 Bronchial aspirates, and 6 Bile.

A total of 80 Enterococcal isolates were isolated from the above samples, of which the majority were from Urine specimens- 48,15 from Pus, 6 Blood, 5 Vaginal swabs, 2ETT, 1 BAL, and 1 Tissue fluid (pleural fluid). The majority of the specimens were inpatients (77.5%) than from outpatients (22.5%). Maximum Enterococcal isolates were from urine specimens 49(61.25%), followed by Pus 15(18.7%), Blood 7(8.7%), High vaginal swab 5(6.25%), ETT 2(2.5%), BAL, and Pleural fluid (1.25%) as shown in Table 1.

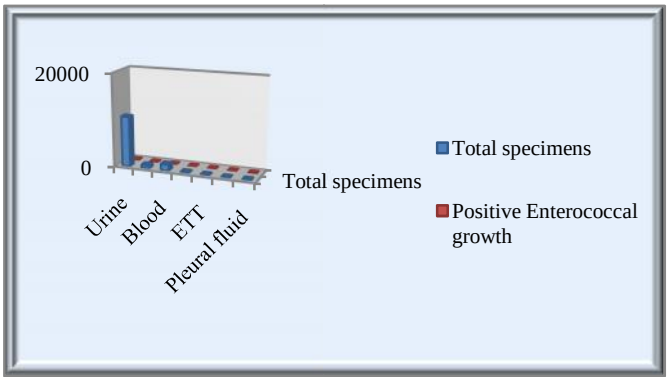

Figure 7 Distribution of Specimens Positive for the Growth of Enterococcus Species

The majority of isolates were from various specialties were 19(23.7%) from the General surgery 13(16.25%) and ICU 13(16.25%) as shown in Table 2.

Table 1 Distribution of Samples Showing the Growth of Enterococci

Specimen type	OPD		IPD		Total	
	Number of samples analyzed	Positive for Enterococcal growth	Number of samples analyzed	Positive for Enterococcal growth	Number of samples analyzed	Positive for Enterococcal growth
Urine	9368	17	1359	32	10,727	49(61.25%)
Pus	282	1	321	14	652	15(18.75%)
Blood	52	-	1280	7	1332	7(8.75%)
HVS	56	-	73	5	129	5(6.25%)
ETT	-	-	68	2	68	2(2.5%)
BAL	-	-	21	1	21	1(1.25%)
Pleural fluid	-	-	50	1	50	1(1.25%)
Total	9758	18(22.5%)	3172	62(77.5%)	12,979	80

*****HVS = High vaginal swab, ETT = Endotracheal tube, BAL= Bronchoalveolar lavage, OPD= Outpatient department, IPD = Inpatient department

Table 2 Distribution of Enterococcal Isolates in Different Wards

Location	Urine	Pus	Blood	HVS	ETT	BAL	Pleural fluid	Total
NICU	-	-	1	-	1	-	-	2(2.5%)
PICU	1	-	-	-	-	-	-	1(1.25%)
SICU	-	1	-	-	1	-	-	2(2.5%)
Neuro ICU	3	1	1	-	-	1	1	7(8.75%)
ICCU	-	-	1	-	-	-	-	1(1.25%)
Pediatric	1	-	1	-	-	-	-	2(2.5%)
General surgery	7	10	1	1	-	-	-	19(23.7%)
Gynecology	11	-	-	3	-	-	-	14(17.5%)
Respiratory medicine	1	-	-	-	-	-	-	1(1.25%)
General medicine	8	2	2	1	-	-	-	13(16.25%)
OPD	17	1	-	-	-	-	-	18(21.25%)
Total	49(61.2%)	15(18.7%)	7(8.7%)	5(6.25%)	2(2.25%)	1(1.25%)	1(1.25%)	80

* ICU- Intensive care unit, ICCU- Intensive coronary unit, NICU- Neonatal ICU, PICU-Pediatric ICU, SICU- Surgical ICU

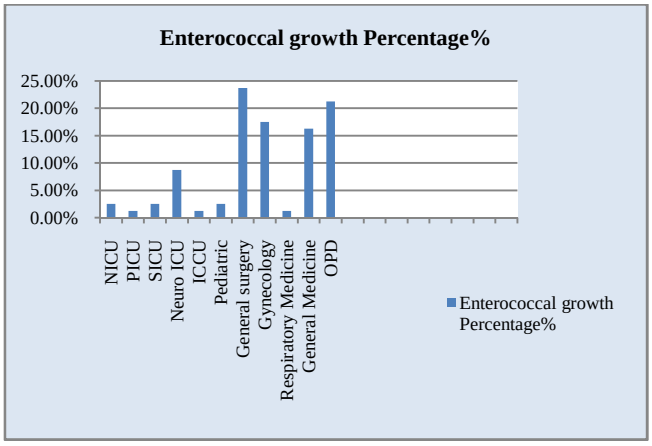

Figure 8 Distribution of Enterococcal Growth in Different Wards

Table 3 Age and Sex Distribution of Enterococcal Isolates (N= 80)

Age	Sex		Total
	Male	Female	
Adult(≥13years)	32(40%)	40(50%)	72(90%)
Pediatric(≤12 years)	3(3.75%)	5(6.25%)	8(10%)
Total	35(41.25%)	45(56.25%)	80

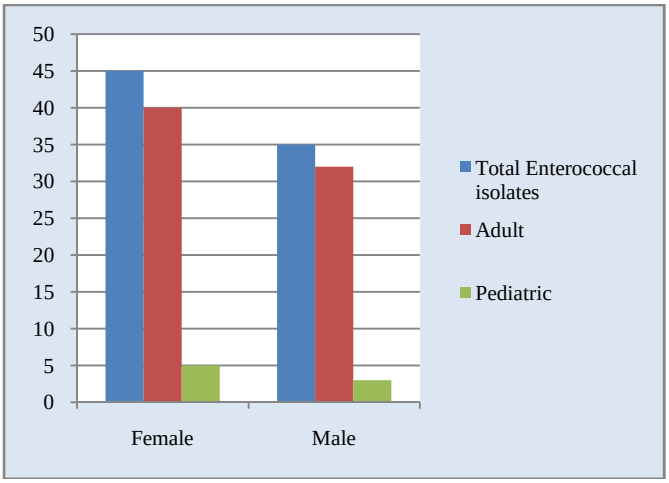

Figure 9 Distribution of Enterococcal Isolates Among Male And Female Patients

Out of the total 80 Enterococcal isolates, the majority were isolated from adult patients 72(90%), however, around 8(10%) isolates were from pediatric patients (Figure 9).

Table 4 Distribution of Different Enterococcal Species among The Specimens

Enterococcal species	Urine	Pus	Blood	HVS	ETT	BAL	Body fluid	Total
E. faecalis	37	4	2	1	2	-	-	46
E. faecium	12	7	3	4	-	1	1	28
E. durans	-	4	-	-	-	-	-	4
E. gallinarum	-	-	2	-	-	-	-	2
Total	49	15	7	5	2	1	1	80

E. faecalis (n=46) was the predominant species followed by *E. faecium* (n=28). Other Enterococcal species such as *E. durans* (n=4) were isolated from pus samples and *E. gallinarum* (n=2) from blood depicted in Table 4.

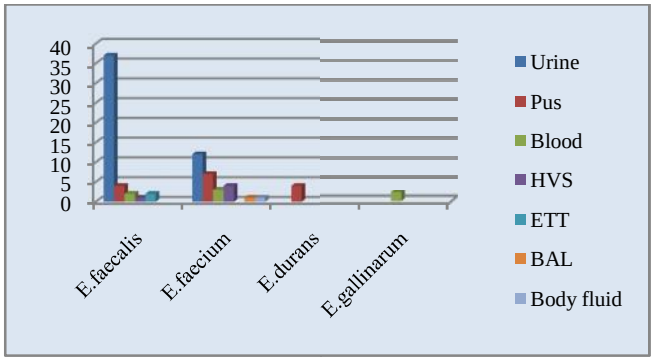

Figure 10 Distribution of Different Enterococcal Species Among The Specimen

Table 5 Susceptibility Pattern of Predominant Enterococcal Species By Kirby-Bauer Disc Diffusion Method

Antimicrobial agents	Susceptibility of Enterococcal isolates (%)											
	E.faecalis (n=46)			*E.faecium*(n=28)			*E.durans*(n=4)			*E. gallinarum*(n=2)		
	R	I	S	R	I	S	R	I	S	R	I	S
Penicillin-G	28	-	18	20	-	8	4	-	-	0	-	2
Ampicillin	15	-	31	19	-	9	0	-	4	0	-	2
Linezolid	0	-	46	0	-	28	0	-	4	0	-	2
Vancomycin	1	-	45	4	-	24	0	-	4	0	-	2
Erythromycin	40	-	6	24	-	4	3	-	1	2	-	0
Tetracycline	36	-	10	14	-	14	3	-	1	0	-	2
Ciprofloxacin	43	-	3	25	-	3	4	-	0	2	-	0
Levofloxacin	43	-	3	24	-	4	4	-	0	2	-	0
Chloramphenicol	24	-	22	13	-	15	2	-	2	0	-	2
High-level Gentamicin	19	-	27	11	-	17	0	-	4	0	-	2
High-level Streptomycin	26	-	20	16	-	12	0	-	4	0	-	2
Teicoplanin	1	-	45	4	-	24	0	-	4	0	-	2
*Nitrofurantoin	2	-	35	0	-	12	-	-	-	-	-	-
*Norfloxacin	34	-	3	10	-	2	-	-	-	-	-	-
**Fosfomycin	0	-	37	0	3	9	-	-	-	-	-	-

* Total urine isolates were 37 in *E. faecalis* and 12 in *E. faecium*.
** Fosfomycin was effective only in *E. faecalis* and *E. gallinarum* is intrinsically resistant to Vancomycin.

Erythromycin was found to be highly resistant in all four species of Enterococcus species comprises 86.25% and the rates of Ciprofloxacin, Levofloxacin, Penicillin, and Ampicillin resistance were determined as 72%, 71%, 65%, 42.5% respectively as shown in Figure 11.

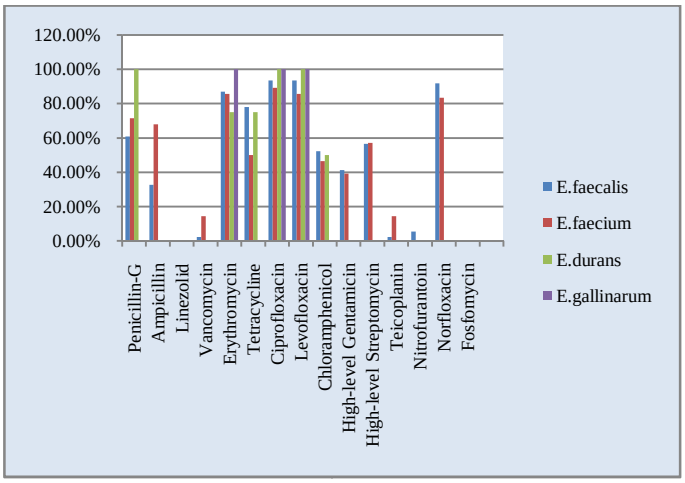

Figure 11 Antimicrobial Resistance Pattern in Various Species of Enterococcus

HLAR has observed in *E. faecalis* isolates was 67.39% and about 78.57% in *E. faecium* while resistance to HLS only was observed in 24(32.4%) and resistant to HLG only 16(21.65%) as shown in Table 6.

Table 6 HLAR (High-Level Aminoglycoside Resistance) Among Various Enterococcus Species Isolated By Disc Diffusion Method

Enterococcal species	Total is olates	Resistant to Both HLG (120µg) And HLS(300µg)	Resistant to HLGonly	Resistantto HLS only	Total HLAR
Enterococcus faecalis	46	7	10	14	31(67.39%)
Enterococcus faecium	28	6	6	10	22(78.57%)
Total	74	13(17.56%)	16(21.6%)	24(32.4%)	53(71.62%)

* HLG = High –level Gentamicin, HLS= High-level Streptomycin

Table 7 MIC Values of Vancomycin for the VRE Isolates

VRE Isolates(n=5)	VancomycinMIC value(µg/ml)					Total
	Intermediate(8-16µg/ml)		Resistant(≥32µg/ml)			
	12µg/ml	16µg/ml	32µg/ml	48µg/ml	64µg/ml	
Enterococcus faecium	-	2	2	-	-	4
Enterococcus faecalis	-	1	-	-	-	1
Total	-	3	2	-	-	5

Out of a total of 5 VRE isolates, the MIC values of 3 isolates were within the intermediate range 8-16µg/ml and 2 isolates were within the resistant range 32µg/ml, interpreted as per CLSI guidelines[6] illustrated in Table 7.

Table 8 Demographic and Clinical Characteristics of Patients with Enterococcal Infections

Pre-disposing risk factor	Category	Vancomycin	
		Sensitive	Resistant
Diabetes	Yes	7	1
	No		
Catheterization	Yes	43	
	No		
Surgery	Yes	41	1
	No		
Thyroid	Yes	4	
	No		
Previous antibiotic treatment (>2 weeks)	Yes	39	2
	No		
Chronic Kidney disease	Yes	7	
	No		
Leukemia	Yes	2	
	No		
Urinary Tract Infection(UTI)	Yes	20	
	No		

DISCUSSION

Enterococcus is coming out as one of the most usual agents of nosocomial infections in the hospital and also leads to opportunistic infections in immunocompromised individuals. Their survival capability under unfavorable environmental conditions alongside the mechanism of intrinsic and acquired resistance to a diversity of antibiotics enables them a difficult pathogen to treat with notable mortality and morbidity [7]. In this background, our study has been trying to utilize the antibiogram for antibiotic stewardship in the hospital and evaluate the prevalence of High-level Aminoglycoside resistance (HLAR) and Vancomycin resistance among the clinical isolates of Enterococci recovered from the patients in this area.

In our study, the majority of the Enterococcal isolates were from urine specimen 49(61.25%), followed by pus 15(18.75%), blood7(8.75%), HVS 5(6.25%), ETT(2.5%), BAL, and Pleural fluid(1.25%), a similar study was reported by Karna *et al* in 2018, where urine 56(61.5%), pus 18(19.7%), blood 5(5.49%), HVS, ETT 1(1.25%)[8]. In all likelihood reason for the higher isolation rate of Enterococci was from Urinary tract infection, UTI patients, and this is credible due to Urinary catheterization.

In our study, ward wise distribution of the isolates showed that 62(77.5%) were admitted to various wards, 13% were comprised from ICU,19(23.7%) belonged to surgery likely due to catheterization, hospitalized for a longer period, and surgical procedures while only 18(21.25%) were from outpatients. The present study showed varying isolation rates with other studies such as Bhatt PM *et al*, who have reported the Enterococcal isolation rate of about 27% from ICU, 33% from OPD[9].

It was observed that 49(61.2%) Enterococcal isolates were from urine as compared to other specimens. The assumable reason for the higher isolation rate of Enterococci from UTI, such as the anatomical organization of a female which is situated close to the anal opening to the urethra. Similarly, Alotaibi FE *et al* study has also shown the predominantly isolation of 41.4% of a urine sample[10].

In our study, we observed that *E. faecalis* was the predominant species 46(57.5%) followed by *E.faecium* 28(35%), *E. durans*(5%), *E.gallinarum* (2.5%). In other studies, Hathiwala R *et al* have reported 56% *E. faecalis* and 40% *E. faecium* which is nearly the same as the finding in our study[11] and 1.25% *E. durans* in Shanmukhappa *et al* study[12]. In another study, Purohit G *et al* have reported *E. gallinarum* (2.4%), similar to our study[13].

In our study the antibiotic susceptibility pattern we've found that most *E. faecalis* and *E. faecium* isolates were shown resistant to Penicillin and Ampicillin (60.8%,32.6%) and (71.4%, 67.85%) respectively which can be correlated with the study of Sumangala B *et al* 2020, who have reported significantly higher resistance to Penicillin and Ampicillin in *E. faecalis* and *E. faecium* i.e, (85.29%, 38.7%) and (67%,54%) respectively[14], which could be due to resistance mechanism either because of low-affinity of penicillin-binding protein (PBP) or production of β lactamase enzyme[18].

High-level Resistance to Aminoglycoside(HLAR) is now becoming a significant clinical concern as it gets rid of synergy with cell wall active antibiotics, which makes treatment of serious Enterococcal infections difficult[15]. As per our study, the total HLAR observed was 71.62%. Resistance to High-level Streptomycin, HLS (300µg) only observed in a total of 24(32.4%) and to High-level Gentamicin, HLG (120µg) only observed in 16 isolates (21.6%), which found to be lower than the report from Mohan S *et al* from Puducherry, 64.49% to HLGR and 58.88% to HLS[15].

Resistance to Erythromycin was shown by the higher number of *E. faecium* strains. It was tested just to notice the resistance pattern and not use it for the treatment.

Based on our findings, adequate antienterococcal activity was observed in 100% with Linezolid when compared with other studies[16].

Out of the 80 Enterococcal isolates, 5(6.25%) were Vancomycin-resistant with 3(3.7%) isolates showing Vancomycin MIC values 16µg/ml (intermediate) and 2(2.5%) isolates were showing 32µg/ml and Teicoplanin resistance

were 6.25%. Another study Gill JS *et al* 2020 from Pune, reported 3.03% Vancomycin MIC were intermediate and 4.95% were showing in resistant range[17].

In our study, Ciprofloxacin and Levofloxacin were found to be higher in both *E. faecalis* and *E. faecium* were (93.47% to both) and (89.28%,85.71%) respectively whereas, in another study Purohit G *et al* Ciprofloxacin resistant were reported 100% and 90.2% in *E. faecalis* and *E. faecium* respectively[13]. According to Sumangala B *et al* study Tetracycline, Nitrofurantoin and Norfloxacin resistance in *E. faecalis* and *E. faecium* were (80.64%,72.72%), (10.53%, 21.06%) and (16.67%,33.34%) respectively while in our study resistance to Tetracycline, Nitrofurantoin and Norfloxacin were noted in *E. faecalis* and *E. faecium* (78% and 50 %), (5.4% in *E. faecalis*) and (91.89%, 35.71%) respectively.

In our study, Fosfomycin was sensitive (100%) in E. faecalis and a similar study was conducted by Kiruthiga A *et al* [19]. Apart from Nitrofurantoin, Fosfomycin can also be used as a therapeutic alternative in Urinary tract infections, UTI caused by Enterococcus species.

CONCLUSION

The exposure of Vancomycin-resistant Enterococci worsens the matter because of the certainty of multidrug resistance expressed by these agents leaving a couple of therapeutic alternatives for the clinicians in treating the acute life-threatening VRE infections. Fundamentally, phenotyping of VRE isolates carry out by detection of Minimum Inhibitory Concentration, MIC of Vancomycin becomes crucial. Consequently, this method is frequently embraced in resource-limited settings (where the genotyping ought not to be available) for the identification of the Vancomycin-resistant phenotype of Enterococci. This highlights the necessity for regulating frequent surveillance programs for prompt recognition of VRE in hospitals and communities. This also focuses attention on the necessity for the fulfillment of severe infection control measures such as rational use of antibiotics especially controlling the use of Vancomycin to the lowest and effectual treatment of VRE infections, proper handwashing practices, education of the healthcare employees, and additional personnel associated in the patient management.

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Conflict of Interest Statement

The authors proclaim that they have no conflicts of interest.

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