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Original Research Article

Study on prevalence of Vancomycin Resistant *Enterococcus* and high level Gentamicin resistance among *Enterococcus* isolates in a Pediatric tertiary care hospital

Kaarthiga S[1], J Bhavana[1,*], Mahantesh[1], Yadukul S[2]

[1]*Dept. of Microbiology, Indira Gandhi Institute of Child Health, Bengaluru, Karnataka, India*
[2]*Dept. of Forensic Medicine, Chamarajanagar Institute of Medical Sciences, Yadapura, Karnataka, India*

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ABSTRACT

Vancomycin resistance and high level aminoglycoside resistance in Enterococci has limited its treatment, as Enterococci are susceptible to only narrow spectrum of antibiotics. VRE is known to cause hospital acquired infections. This study was aimed to determine prevalence of vancomycin resistance and HLG resistance among Enterococci in a Pediatric hospital. 310 *Enterococcus* spp was isolated between Jan 2018- May 2020 from pediatric patients from various samples. Among them 206 (66.5%) isolates were *Enterococcus faecalis*, 104 (33.5%) isolates were *Enterococcus* spp including *Enterococcus faecium*. Most of the isolates were from urine (68%). High Level Gentamicin (HLG) resistance was seen in 69% of the isolates. Vancomycin resistance (VRE) was seen in 29 (9.4%) isolates. HLG resistance among VRE was 79% (23/29), all of them were sensitive to Linezolid. 17 of the VRE isolates were from urine sample and 15 (88%) of these isolates were sensitive to Nitrofurantoin. 90% (26/29) of the VRE isolates were isolated from nosocomial infections. 58% of VRE isolates were *Enterococcus faecalis.*

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1. Introduction

Enterococcus spp are present as commensals mainly in the gastrointestinal tract in humans. They can also survive in environment, also in adverse environmental conditions like drying and high temperature to a certain extent. They are known to cause infections like urinary tract infections, wound infection and also blood stream infections primarily in debilitated patients. Enterococci are intrinsically resistant to many of the commonly used antibiotics, hence there are only a limited groups of antibiotics effective against Enterococci.[1] Resistance to fluoroquinolones and high level Gentamicin(HLG) has become common among *Enterococcus*. So, Vancomycin is the main drug of choice for treating systemic infections. Emergence of Vancomycin Resistant *Enterococcus* (VRE) has possessed a greater threat in its treatment.[2] VRE is also a potent nosocomial pathogen.[1,2] Surveillance for VRE becomes very important to prevent its cross infection.[3]

Objective of the study is to look for the prevalence of Vancomycin resistance and HLG resistance among *Enterococcus* isolates in our institute .

2. Materials and Methods

The study was conducted from Jan 2018- May 2020 in pediatric patients aged between 1 day- 18 years, in Department of Microbiology, in a Pediatric tertiary care hospital.

Non repetitive isolates of *Enterococcus* species from various samples were included in the study identified by Gram's stain of the colonies which were Gram positive diplococci, grown on 5 % Sheep blood agar which were about 1 mm and non hemolytic or alpha hemolytic, and

* *Corresponding author.*
E-mail address: bhavana_druthi@yahoo.co.in (J. Bhavana).

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minute Lactose fermenting colonies from Mac Conkey agar, after 24- 48 hrs of incubation at 37^0C. Further identification was done by catalase test, where *Enterococcus* are catalase negative; Bile aesculin hydrolysis and heat tolerance test.[4,5] Further Antibiotic Susceptibility (AST) was tested for Penicillin (10 units), Ciprofloxacin (5μg), Levofloxacin (5μg), High level Gentamicin (120μg), Vancomycin (30μg) and Linezolid (30μg); Nitrofurantoin (300μg) was included for urine isolates. AST was done by Kirby Bauer Disk diffusion method on cation adjusted Muller Hinton Agar. CLSI guidelines were followed for interpretation of the zones.[6] Vancomycin resistance was confirmed by Vitek 2 compact.

3. Results

A total of 310 *Enterococcus* spp was isolated between Jan 2018- May 2020 from pediatric patients aged between 1 day- 18 years and from various samples in Department of Microbiology, in a Pediatric tertiary care hospital. Among 310 isolates, 206 (66.5%) isolates were *Enterococcus faecalis*, 104(33.5%) isolates were *Enterococcus* spp including *Enterococcus faecium*. Most of the isolates were from urine (68%) followed by pus (16%) and blood (14%), 6(2%) isolates were from body fluids. 61.6% of isolates were from male and 39.4% were from female patients. 25% of the isolates were from Pediatric ICU, from Pediatric Surgery department 20%, Neonatal ICU (14%) and Pediatric Medicine department(13%), 9% of isolates were from Out patient Department. Antibiotic susceptibility, most of the isolates were resistant to Penicillin (90%), Ciprofloxacin (81%). High Level Gentamycin (HLG) resistance was seen in 69% of the isolates. 34% of the isolates were sensitive to Levofloxacin, 90.6 % of isolates were sensitive to Vancomycin, all the isolates were sensitive to Linezolid. 90.5% of the urine isolates were sensitive to Nitrofurantoin.

Vancomycin resistance was seen in 29(9.4%) isolates(VRE), most of the VRE isolates were resistant to Penicillin (100%), Ciprofloxacin (90%), Levofloxacin (86%) and HLG (79%), all of them were sensitive to Linezolid. 17 of the isolates were from urine sample (59%), and 15(88%) of the urine VRE isolates were sensitive to Nitrofurantoin. From Pus sample 7(25%) and from blood 5 (17%) VRE were isolated. 90% (26/29) of the VRE isolates were isolated from nosocomial infections. 14% (4/29) of VRE isolates were from Central line associated blood stream infection (CLABSI), all the urine VRE isolates were from Catheter associated urinary tract infections (CAUTI), 17% (5/29) of the VRE isolates were from Surgical site infections (SSI). Most of the VRE isolates were *Enterococcus faecalis* (58%), the rest were *Enterococcus faecium* and other *Enterococcous* spp.

Table 1: Sample wise distribution of Enterococcal isolates.

Sample	E. faecalis	E.faecium+ other Enterococcus spp	Total
Urine	150	61	211 (68%)
Pus	27	24	51(16%)
Blood	26	16	42 (14%)
Body fluids	3	3	6 (2%)
Total	206(66.5%)	104 (33.5%)	310

Table 2: Antibiotic sensitivity of the Enterococcal isolates.

Antibiotic	S	R	I
Penicillin	32 (10%)	278 (90%)	0
Ciprofloxacin	54 (17%)	252 (81%)	4(2%)
Levofloxacin	106 (34%)	203 (65.5%)	1(0.5%)
HLG	95(31%)	215 (69%)	0
Linezolid	310 (100%)	0	0
Vancomycin	281(90.6%)	29(9.4%)	0
Nitrofurantoin (urine isolates)	191(90.5%)	16 (7.5%)	4(2%)

Table 3: Percentage of VRE isolated out of 310 *Enterococcus* isolates from various samples

Sample	VRE (E. faecalis)	VRE (E.faecium+ other Enterococcus spp)	Total VRE
Urine	11	6	17(5.4 %)
Pus	3	4	7 (3%)
Blood	3	2	5 (2%)
Total	17 (5.4%)	12 (4%)	29 (9.4%)

Table 4: Antibiotic sensitivity of VRE isolates

Antibiotic	S	R	I
Penicillin	0	29 (100%)	0
Ciprofloxacin	2 (7%)	26 (90%)	1(3%)
Levofloxacin	4 (14%)	25 (86%)	0
HLG	6 (21%)	23 (79%)	0
Linezolid	29 (100%)	0	0
Nitrofurantoin (urine isolates)	15(88%)	2 (12%)	0

Table 5: VRE isolated from Nosocomical infections

VRE (n=29)	NICU	PICU	Total
CLABSI	2(7%)	2(7%)	4(14%)
CAUTI	1(3%)	16(56%)	17(59%)
SSI	5(17%)		5(17%)
Total	7(24%)	18(62%)	26(90%)

4. Discussion

The prevalance of VRE in the present study was 9.4%. High Level Gentamycin (HLG) resistance among *Enterococcus* was 69%. Similar results were seen in many of the studies in India, Banerjee T et al in their study conducted from September 2010 to March 2014 in Varanasi, prevalance of VRE was 7.09%, HLG resistance was seen in 47.41% of the *Enterococcus* isolates.[7] Sachan.S et.al, in their study in Uttarakhand during November 2013 to October 2015 observed High-level gentamicin (HLG) and vancomycin resistance among 55.57% and 6.01% of enterococcal isolates, respectively.[2] Khanal LK et.al, in their study in Khanpur during November 2017 to May 2018 had 12% prevalence of VRE , and HLG resistance was seen in 60% of the Enterococcal isolates.[3] Hathiwala R et.al, in their study in Chhattisgarh HLAR were seen in 58% and VRE in their study was only 1.6% and all were identified as E. faecium.[1] Manimala E et al, in their study in Tirunelveli, Tamil Nadu from April 2017 to May 2018 had a prevalance of 4% of VRE in their center, HLG resistance was seen in 50% of their samples.[8]

The present result is comparable with the other similar studies taken up in India with few studies showing low prevalence, probable reasons being their location or shorter study duration.

VRE is challenging as a nosocomial pathogen because of its rapid spread, better survival in environment and limited option for treatment. It is also capable of transferring the resistance genes to other organisms. It has also been observed that there is high mortality associated with VRE infections. Hence surveillance and immediate hospital infection control measures on detecting VRE becomes more important for preventing its nosocomial infection.[1,2]

5. Conclusion

Nosocomial infection with VRE has been increasing and treatment options for VRE is also limited. So, prompt detection, treatment and hospital infection control measures for VRE is utmost important to prevent it from causing HAI.

Further study of interest will be taken up; detection of Vancomycin resistance genes (Van A and Van B) in the VRE isolates.

6. Source of Funding

None.

7. Conflict of Interest

None.

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Author biography

Kaarthiga S Senior Resident

J Bhavana Assistant Professor

Mahantesh Professor and HOD

Yadukul S Assistant Professor

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