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ENTEROCOCCUS CAUSING HUMAN INFECTIONS WITH SPECIAL REFERENCE TO VANCOMYCIN RESISTANCE: A MATTER OF CONCERN

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Abstract- *Background*- Vancomycin resistant *enterococci* (VRE) pose an emerging problem in hospitals worldwide. This study was to determine the prevalence of Vancomycin Resistant *enterococci* (VRE) isolated from various clinical specimens. *Materials and Methods*- Between time periods of June 2014 to May 2015, total 208 *Enterococcal* strains were isolated from various clinical samples and confirmed by standard biochemical test methods. Antibiotic susceptibility assay was performed by Kirby-Bauer disc diffusion method and results were interpreted according to the Clinical Laboratory Standard Institute (CLSI) guidelines 2014. For all the isolates initial Vancomycin Resistant *enterococci* screening by disc diffusion method was done and later confirmed by determination of Minimum Inhibitory Concentration (MIC) by Epsilometer test. *Results*- 208 isolates of *enterococcal spp.* were obtained from various clinical specimens .76 (36.53%) accounted for *E.faecalis* and 128 (61.53%) for *E.faecium* and 4(1.92%) isolates were other *enterococcal spp*. 84.14% of isolated *Enterococcus* were resistant to ampicillin, followed by amikacin(73.08%), ciprofloxacin (66.83%), Levofloxacin(30.77%), HLG (High Level Gentamicin) (12.01%), vancomycin (4.81%). All Vancomycin Resistant *enterococci* showed high Minimum Inhibitory Concentration value for vancomycin by Epsilometer test. *Conclusion*- Our study reveals the emerging problem of Vancomycin Resistant *enterococci* thus all laboratories should have prompt and appropriate detection methods for Vancomycin resistance to restrict the spread of infection, which is a real threat to the community, Treatment of infection by VRE with drugs such as Quinupristin-Dalfopristin, Linezolid & Tigecycline.

Keywords- Antibiotic susceptibility assay, Epsilometer test, Minimum Inhibitory Concentration(MIC), Vancomycin Resistant *Enterococci* (VRE)

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Introduction

Emergence of gram-positive organisms as leading causes of hospital acquired infection in the 1990s [1]. One of the organisms that caused real threat was Vancomycin resistant *enterococcus* (VRE) that was first reported in 1988[2]. In past century enterococcus was only an intestinal commensal organism of little clinical significance but now it is emerging as the second most common pathogen causing Nosocomial urinary tract infection and third most common pathogen causing bacteremia[3] . They belong to Lancefield group D streptococci ,whose taxonomy has changed in the last few years.

In recent years, *Enterococcus* emerged as real threat to community because of they are causing fatal infections like bacteremia, surgical site infection(SSI) and urinary tract infection (UTI) especially from hospitalized patients on indwelling catheter, endocarditis, cause increasing resistance to many antimicrobial agents[4-6]. Serious Nosocomial *enterococcal* infections are more resistant to treatment and mortality is high, especially in elderly patients with underlying diseases like malignancy and diabetes [7]. During 2004, VRE caused about one of every three infections in hospital intensive-care units, based on data from Centers for Disease Control and Prevention. *Escherchia coli* as a sole agent of nosocomial urinary tract infection followed by *enterococci* according to CDC surveillance of nosocomial infection [8].

Emergence of HLAR(high level aminoglycoside resistance) with resistance to beta-lactam antibiotics like ampicillin and vancomycin together has led to failure of synergistic effects of combination therapy[4,5,6]. Therefore, VRE with HLAR pose challenge to clinicians for treatment of such infections [4]. Reason behind rapid emergence of *E. faecium* as causative agent of nosocomial infections due to it is more difficult to be treated by aminoglycosides and glycopeptides in the last two decades [9-10].

Thus, the present study was conducted in tertiary care teaching hospital,Western India to determine the susceptibility pattern of isolated *enterococcal* strains and by this it is possible to control the spread of *enterococcal* infections. Proper hand hygiene by washing with soap is the best way to prevent spread of *enterococci* in hospitalized patients.The CDC Hospital Infection Control Program encourages hospitals to develop their own plans to prevent spread of VRE in hospitalised patients [8].These control measures can be:

- Prudent vancomycin use by clinicians.
- Education regarding vancomycin resistance to all hospital staff members.

- Screening of health care workers.
- Surveillance cultures in operation theatres, intensive care units and neonatal intensive care units.
- Possible early detection with reporting of vancomycin resistance by respective microbiology laboratory.
- Infection control measures to prevent VRE transmission.

Materials and Methods

The clinical samples were received from hospitalised patients in tertiary care hospital, western India. Total 208 *enterococcal* strains were isolated from urine of urinary tract infection patients; blood and body fluids from septicemic patients; cerebrospinal fluid (CSF) from meningitis cases; endotracheal tube secretions from intensive care unit patients and wound swabs from surgical ward patients, between time periods of June'14 to May'15. The specimens were inoculated on bile-esculin medium which is selective medium for isolation of *Enterococcus.* The specimens were also inoculated on blood agar and MacConkey's agar for the isolation of concomitant organisms mixed with *enterococci.*Presumptive diagnosis of *enterococci* was based on their growth characteristics on sheep blood agar, nutrient agar MacConkey agar, gram staining i.e. gram positive cocci arranged in pairs at angles to each other, catalase negative biochemical reaction, ability to grow in 6.5% Nacl and bile esculin hydrolysis test [Fig-1]. Identification upto *enterococcal spp.* level by carbohydrate fermentation tests using following sugars-glucose, arabinose, mannitol, raffinose, lactose, sucrose, sorbitol and trehalose (standard biochemical tests) [Fig-2]. Haemolysin production was detected in the strains of *E. faecalis* and *E.faecium* on sheep blood agar.

Fig. 1- Bile esculin hydrolysis test positive

Antibiotic susceptibility testing- The susceptibility of the isolates against most commonly used antibiotics such as ampicillin, gentamicin(HLG), levofloxacin, ciprofloxacin, amikacin, Vancomycin, teicoplanin , linezolid, tetracycline, doxycycline were evaluated by using Kirby-bauer disc diffusion method and results were interpreted according to Clinical and Laboratory Standards Institute (CLSI 2014) guidelines. All the isolates first undergone to initial Vancomycin Resistant *enterococci* screening by disc diffusion method using disc of vancomycin(30µg) and later confirmed by determination of Minimal inhibitory concentration (MIC) using Epsilometer test(E-strip) [Fig-3]. *Enterococcus faecalis* ATCC 51299 and *Enterococcus faecalis* ATCC 29212 were used as quality control strains.

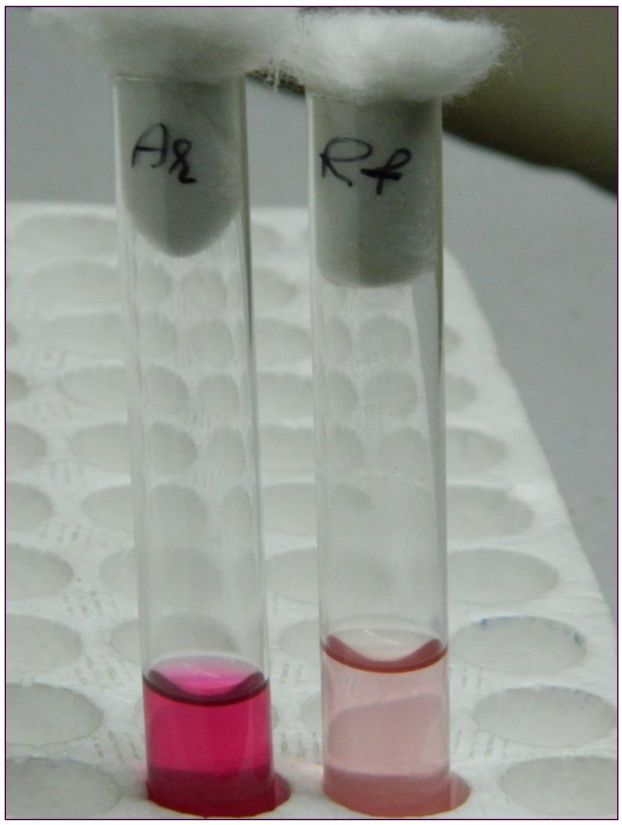

Fig. 2- Arabinose, Raffinose fermentation test

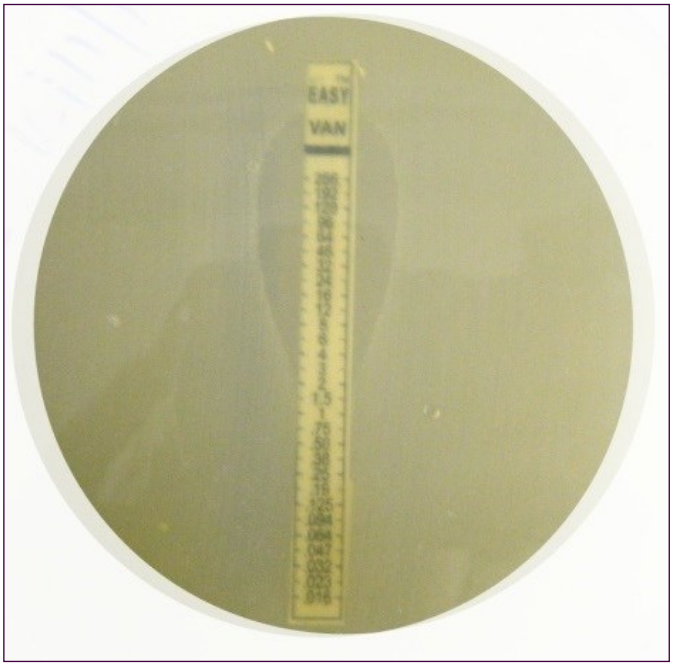

Fig. 3- Confirmation of VRE by E- strip

Results

Between time periods of June 2014 to May 2015, total 36823 samples were received from patients with infection in different wards, among them; various micro-organisms were isolated in 14139 samples. Among these 208 *enterococcal* strains isolated, 76 were *E.faecalis*, 128 were *E.faecium* and 4 were other *Enterococcal spp*.

Total 208 *enterococcal* strains were isolated from various clinical samples [Table-1].

Table 1- Prevalence of *enterococci* and VRE from various clinical samples

Clinical specimens	Isolated Enterococcal strains No. (%)	Total VRE isolation (No.)
Urine	106 (50.96%)	2
Blood	79 (37.98%)	8
Pus	07 (3.36%)	0
Swab	07 (3.36%)	0
CSF	04 (1.92%)	0
Body fluids	04 (1.92%)	0
ET secretions	01 (0.48%)	0
Total	208	10

Highest isolates of *Enterococci* were from urine (50.96%), followed by Blood (37.98%) [Table-1].

Among 208 *enterococcal* isolates, 78 were from surgical wards, 59 from medicine wards, 41 from pediatric wards and 30 from ICU.

Antimicrobial susceptibility pattern of isolated *enterococci* is summarised in [Table-2].

Table 2- Antimicrobial susceptibility pattern of *Enterococci* by Modified Kirby-bauer disc diffusion method

Antibiotic tested	% Sensitive	% Resistant
Amikacin (30µg)	26.92	73.08
Ampicillin (10µg)	15.86	84.14
Tetracycline (30µg)	51.92	48.08
Doxycycline (30µg)	59.61	40.39
Ciprofloxacin(5µg)	33.17	66.83
Levofloxacin (5µg)	69.23	30.77
Moxifloxacin (5µg)	92.3	7.7
Gentamicin [HLR](120µg)	76.45	23.55
Vancomycin (30µg)	95.19	4.81
Teicoplanin (30µg)	98.07	1.93
Linezolid (30µg)	100	0

Antibiotic susceptibility tests showed higher resistance to various antibiotics tested [Table-2]. Out of 208 isolates, 198 were sensitive to vancomycin and 10 were resistant to vancomycin. The vancomycin MIC for 10 isolates was more than 8 µg/ml. Among 10 isolates of VRE, 6 were *Enterococcus faecium* and 4 were *Enterococcus faecalis*. In this study, two VRE isolates were resistant to teicoplanin, and they all were sensitive to Linezolid. Phenotypes with Minimum inhibitory concentration value greater than 8µg/ml were VanA and VanB. The detection of high-level gentamicin resistance in 23.55% of *Enterococcal* isolates is also a real threat due to beginning of a major resistance problem.

Discussion

Widespread use of vancomycin and extended-spectrum cephalosporins in hospitals likely contributed to the emergence and dramatic increase of VRE over the past 20 years [6].

The glycopeptide- vancomycin is the first choice alternative to penicillin-aminoglycoside combination for treatment of serious *enterococcal* infections. Many different types of vancomycin resistance genes have been reported in enterococci. *Enterococci* have two types of resistance- acquired and intrinsic (natural) to vancomycin. Some types of *enterococci* acquire the resistance when other bacteria come in contact with *enterococci* and share genetic information that resists vancomycin. Acquired resistance has been noted with both clinically important forms of *E. faecium* and *E. faecalis*.

Glycopeptide-vancomycin resistant genotypes in *enterococci* include VanA (high-level resistance), which is detected in a most *enterococcal species*; VanB, VanD with moderate to high-level resistance and VanC causing intrinsic low-level resistance [11]. Vancomycin resistance is most commonly seen in *E. faecium* which is encoded by *VanA* gene cluster on the mobile genetic element Transposon 1546[12].As *Enterococci* are reservoirs of antibiotic resistance genes, they can transfer their resistance genes to other bacteria like methicillin-resistant *Staphylococcus aureus* [13]. Monitoring the antibiotic resistance of *enterococci* isolated from clinical specimens is a useful tool to get information about the prevalence of VRE and will be essential for controlling the spread of bacterial resistance.

Risks factors for acquiring Vancomycin resistant *enterocoous* infection [8] are:

- Persons who have taken previous treatment with vancomycin and combinations of other antibiotics like penicillin and high level gentamicin.
- Hospitalized patients who are on long term antibiotic therapy.
- Persons with weak immunity, such as patients in intensive-care units, in transplant wards , patients with malignancy ,elderly patients particularly in long term care facility.
- Surgical ward patients who have undergone abdominal or any other surgical procedure.
- Persons with central intravenous catheters or urinary catheters for long duration.

VRE is transmitted among hospitalized patients most commonly by healthcare workers whose hands have inadvertently become contaminated, either from feces, urine, body fluids or blood of a patient carrying the organism.

Table 3- Comparison of VRE isolation with other studies

	Marthur, et al [14]	De, et al [15]	Shah L [16]	Present study
Isolated enterococcal spp.	444	200	92	208
VRE (%)	5(1%)	03(1.5%)	08(8%)	10 (4.80%)
Phenotypes	VanA, VanB	VanA	VanA, VanB	VanA, VanB

According to CLSI guidelines 2014, MIC of vancomycin for *enterococci* between 8-16 µg/ml is considered as intermediate resistant and MIC greater than or equal to 32 µg/ml as resistant [17]. In present study, 4.80% of isolated *enterococcal* strains showed resistance by MIC [Table-3]. Among reported VRE isolates, *E. faeci-*

um was the commonest.

Ampicillin along with High level gentamycin considered as treatment of choice. Therefore resistance of *Enterococci* against these antibiotics has important clinical implications. Present study showed 84.14% resistance to ampicillin due to resistance mechanism involving low affinity penicillin binding proteins or production of β lactamases. Resistance to amino glycosides in *Enterococci* is with multidrug resistance.In present study, HLAR was seen in 23.55% of the strains for gentamicin (HLAR).HLAR was more in *E.faecium* than *E.faecalis*. These findings also reported in some other study [2,16]. Vancomycin resistance was noted in 4.81% of isolated *enterococcus* strains. All isolates were susceptible to linezolid and 98.07% sensitive to teicoplanin. So, choice of treatment for VRE isolates is linezolid and teicoplanin.

All laboratories should have prompt and appropriate detection methods for vancomycin resistance, which will be helpful in reducing the spread and ultimately morbidity and mortality due to VRE in hospitalized patients. VRE with community acquired sources and health care workers should be detected early as this will limit the spread of VRE to the hospital environment. Surveillance of family members of VRE infected patients and community to detect reservoirs of VRE should done from time to time to limit the spread of infection [12].

Conclusion

This study signifies the emergence of VRE in our hospital and so there is importance of screening for vancomycin resistance among isolated *enterococcus* from various clinical samples. Thus there is a need to study the antibiogram of *enterococcal* isolates to reduce the spread of such strains. As MIC detection is expensive, all *enterococcal* isolates can be screened by Vancomycin disc diffusion method and only those isolates resistant by this method tested further for vancomycin MIC, as by boith above mentioned methods correlation was seen in this study. Quick diagnosis and appropriate measures on infection control can reduce its spread.

Conflicts of Interest: None declared.

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