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Resistance pattern of enterococcus for high level aminoglycosides and vancomycin in blood culture of admitted patients

Shadma Yaqoob*, Fareya Haider, Priyanka Shukla
Department of Microbiology, ELMC & H, Lucknow, Uttar Pradesh, India.

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Abstract: Enterococci have emerged as important nosocomial pathogens from a variety of clinical conditions and the major reason for this is the trend of increasing antimicrobial resistance and enterococcal bacteraemia results in a high mortality. The present study was undertaken to determine the occurrence, species prevalence, antibacterial resistance, with a special reference to vancomycin and high level aminoglycoside resistance. Material and methods: The study was conducted on blood culture isolates. The antibiotic susceptibility of isolates by the Kirby Bauer disc diffusion method was done according to the CLSI guidelines. Screening tests for high level aminoglycosides and vancomycin and minimum inhibitory concentration (MIC) tests for vancomycin was done. The blood specimens were cultured and suspected growths were identified to species level and found to consist mostly of *E. fecalis* (70%). VRE accounted for 2 (91%) isolates and high level aminoglycoside resistance was seen in 47.82 and 60.86 isolates. The *E. faecium* isolates were more drug resistant than the *E. faecalis* isolates. Linezolid and Teicoplanin showed good anti-enterococcal activity. This study shows an emergence of Vancomycin resistant enterococcus (VRE) along with increased rate of multidrug-resistant enterococci. Regular surveillance of antimicrobial susceptibilities and treatment of enterococcal infections should be done effectively to limit the spread of multidrug resistance.

Key words: Nosocomial infection; VRE, High level aminoglycoside resistance; Enterococcal bacteremia.

Introduction

Enterococci are indigenous flora of the intestinal tract, oral cavity & genitourinary tract of human & have emerged as important nosocomial pathogens in the last few decades and the major reason for this is the trend of increasing antimicrobial resistance seen in these organisms[1] *E. faecalis* (80-90%) & *E. faecalis* (5-10%) are two commonly prevalent species which are human pathogens capable of causing bacteremia[2]. Other enterococcal species are identified less often.

This genus is resistant to a numbers of anti-microbial agents commonly used in hospitals including β-lactam antibiotics, glycopeptides and aminoglycosides. They can also rapidly express resistance to many antibiotics by acquisition of plasmids & transposable elements[3]. The incidence of enterococcal infection has increased making the second most common nosocomial pathogen reported to the National Nosocomial Infection surveillance system[4]. However, emergence of high-level resistance to aminoglycosides (H.L.A.R), β-lactam antibiotics and to vancomycin by some strains, with multidrug resistance has led to the failure of synergistic effects of combination therapy, more often in hospitalized patients and previously treated with antibiotics. According to the National Nosocomial Infections Surveillance (N. N. I. S) data, more than 28 per cent of all nosocomial enterococcal strains are vancomycin resistant[5].

Material and Methods

The present study comprised of the blood culture specimens referred for bacteriological cultures from patients of all age groups and both sexes who were admitted in Era's Lucknow medical college and hospital The present study was conducted from December 2013 to December 2014. Twenty-three enterococcus strains were isolated from blood culture specimens. Past history of the patients was recorded for diabetes mellitus, chronic renal illness and any other chronic illness leading to prolonged hospitalization.

Specimen Processing was Done in Two Parts

Part I- Isolation and identification of Enterococcus by culture and biochemical tests.

Culture of Specimens: All the specimens received in the bacteriology laboratory were inoculated on Blood agar and McConkey agar plates & incubated at 37°C for 24-48 hours.

Identification and speciation of Enterococcus: Presumptive identification was done on the basis of colony characteristics, Gram's staining, catalase test. Confirmation was done by growth in 6.5% NaCl, bile aesculin hydrolysis, Production of acetoin, Pyruvate utilization, Arginine decarboxylation, Haemolysin production, Tellurite reduction.

Part II- In Vitro Antibiotic Susceptibility Testing by Disc Diffusion Method of Kirby Bauer and MIC by Agar Diilution Method.

Anti-microbial sensitivity testing was performed according to the C. L. S. I guidelines[6]. Muller-Hinton agar was used as media. It was inoculated with a suspension of each organism equivalent to 0.5 McFarland turbidity standard and discs were applied. Control strains used were *E. faecalis* A.T.C.C 29212 (susceptible) and *E. faecalis* A.T.C.C-51299(resistant) The various antibiotics tested were Ampicillin (10µg), Tetracycline (30µg), Erythromycin (15µg), Ciprofloxacin (5µg), Gentamicin (10µg), Vancomycin (30µg) and Linezolid (30µg). For high level aminoglycoside resistance detection, Gentamicin (120µg) and Streptomycin (300µg) discs were used. The source of the anti-microbials

***Corresponding Author:**
Dr. Shadma Yaqoob,
Assistant Professor,
Department of Microbiology, ELMC & H,
Lucknow, Uttar Pradesh, India.

was Hi-Media Ltd (Mumbai) India. The standard strains, *E. faecalis* A.T.C.C 29212 and *E. faecalis* A.T.C.C 51299 were used as the susceptible and resistant quality control strains.

Enterococcus strains that were resistant and intermediate sensitive to vancomycin and High Level Amynoglycosides by Disk Diffusion method were further tested by B. H. I Screen Agar. As per C. D. C guidelines, in-house prepared B.H.I agar (Hi-Media, India) screen plates containing 6 microgram/ml Vancomycin (Lilly Pharma, Giessen, Germany) was prepared. In same way B. H. I agar screen plates with gentamicin concentration of 500µg/ml and streptomycin concentration of 2000µg/ml also prepared. Further detection of VRE was done by M. I.C by vancomycin agar dilution method using M. H. A. The concentrations tested ranged from 2 µg/ml to 1024 µg/ml of vancomycin.

Results

The blood specimens were cultured and screened for growth of enterococci and Suspected growths were isolated and identified. A total of 23 strains of enterococci were isolated from blood samples. Two species of *Enterococcus* were isolated *E. fecalis* 19(86.22%) and *E. fecium* 4 (17.39%).

Out of 23 isolates 20 were isolated in pure culture while the rest 3 i.e: 13.04 % were in combination with other bacteria i.e.: polymicrobial infections. By disc diffusion method the high-level resistance to gentamicin in this study was present in 10 (43.47%) isolates and 1 was intermediate sensitive. The high-level resistance to streptomycin was 13 (56.5%) and 1 was intermediate sensitive. Both intermediate strains become resistant after screening tests and the number of H. S. G resistant isolates changed to 11 and H. S. S resistant isolates to 14.

In disk-diffusion method, of the 19 *E. fecalis*, 8 (42%) and 11(58.9%); of the 4 *E. fecium*, 2 (50%) and 2(50%); showed high-level resistance to gentamicin and streptomycin. However, by agar-screen method, 9 (47.3%) and 12(63%) *E. fecalis*; 2 (50%) and 2 (50%) *E. fecium* showed high-level resistance to gentamicin and streptomycin. The highest resistance was observed among *E. fecium* followed by *E. fecalis* both by disk-diffusion method and agar-screen method. Isolates which were resistant to one aminoglycoside, not necessarily be resistant to another aminoglycoside. 2 strains were resistant to vancomycin by both disc and agar dilution methods. Both were *E. faecalis* species. Amongst 2 VRE strains, one was teicoplanin and linezolid resistant by disc diffusion method. MIC of two isolates were 64µg/ml.

Table 1: Pathogen isolated from various clinical specimens

Sample	Total No. of samples	*E. coli*	Klebsiella	CONS	Pseudomonas	*S. aureus*	Candida	Enterococcus	Other org.	Sterile/ NPO/ Contaminants
Blood	897	87	47	59	9	51	44	**23**	107	470

CONS, Coagulase negative staphylococci
Other organisms include Proteus, Acinetobacter, Enterobacter, Citrobacter, Gonococcus and Streptococcus.
NPO- Non-pathogenic organisms:which present normally in healthy individuals not producing disease in normal conditions (Diphtheroids and Micrococcus).

Table 2: Phenotypic identification of enterococci

No. Of Isolates (n=23)	MAN	SOR	ARG	ARA	SBL	RAF	TEL	MOT	PIG	SUC	PYU	Species
19	+	-	+	-	+	-	+	-	-	+	+	*E. faecalis*
4	+	-	+	+	V	V	-	-	-	+	-	*E. faecium*

Abbreviations and symbols: Man, Mannitol; SOR, Sorbose; ARG, Arginine; ARA, Arabinose; SBL, Sorbitol; RAF, Raffinose; TEL, 0.04% Tellurite; MOT, Motility; PIG, Pigment; SUC, Sucrose; PYU, Pyruvate; +,>90% Positive; -, <10% Positive.

Table 3: Distribution of enterococcal isolates according to IPD/OPD

Specimen	Species	No. of Isolates	IPD	OPD
Blood	*E. faecalis*	19	19(86.6%)	0
	E. faecium	4	4(17.39%)	0
	Total	**23**	**23**	

IPD= Indoor patient department, i.e. admitted patients.
OPD= Outdoor patient's department, not admitted before culture of specimens.
Table 3 shows that all 23 isolates were isolated from the hospitalized patients and none from the outdoor patient

Table 4: AST pattern of various enterococcal species

Sample	n=	Species	A			HSG			HSSm			Cx			E			Va			Te			Lz			Pm		
			S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Blood	23	*E. faecalis* (n=19)	6	-	3	0	1	8	7	1	1	1	-	8	4	2	3	6	1	2	8	-	1	8	-	1	0	-	9
		E. faecium (n=4)	-	-	4	2	-	2	2	-	2	3	-	1	1	-	3	4	-	-	4	-	-	4	-	-	2	-	2
Total	23		6		7	2	1	10	9	1	4	8	-	9	5	2	6	1		2	2		1	2		1	2		11

A - Ampicillin; HSG- high strength gentamicin; HSSm- High strength streptomycin; Cx- ciprofloxacin; E- erythromycin; Va- vancomycin; Te- teicoplanin; Lz- Linezolid; Pm- Pristinomycin; S- sensitive, I-Interm

Table 5: High Level Aminoglycosides Resistance (HLAR) Amongst Enterococci Isolated

Species	Total No. of Isolates	Resistant to Gentamicin		Resistant to Streptomycin	
		Disk Diffusion	Screen Agar	Disk Diffusion	Screen Agar
E. faecalis	19	8(42%)	9(47.3%)	11(58.9%)	12(63%)
E. faecium	4	2(50%)	2(50%)	2(50%)	2(50%)
Total	23	43.47%	47.82%	56.52%	60 .86%

Table 6: MIC of the VRE isolated (n=2)

S. No.	Species	Source of Specimen	Sensitivity pattern by disc method	MIC by agar dilution method
1	*E. faecalis*	Blood	Resistant	64 µgµml
2	*E. faecalis*	Blood	Resistant	64 µgµml

Table 7: Presence of HLAR amongst VRE

S.No	Vancomycin	Gentamycin	Steptomycin
1	R	R	R
2	R	R	R

ediate,

Discussion

Enterococci have been the third most common cause of nosocomial bacteraemia[1]. The enterococci are commensal micro-organisms and are opportunistic pathogens. They cause infections in immunocompromised persons, particularly in elderly patients with serious underlying disease, patients who have been hospitalized for prolonged periods, use invasive devices, and/or have received broad spectrum antimicrobial therapy.

In our study isolation rate of enterococci was 23 in 897 blood samples ie: 2.5%. in similar study by Mendiratta *et al.,* M. G. I. M. S, Maharashtra 2008 isolation rate was 1.16%[7]. In study by Jyotsana *et al.,* 2007 it was reported 21(1.26%) enterococci from 1666 cases of blood culture samples[8]. The above results were similar to our results although the species wise difference was noted from their studies.

The vast majority of the isolates in this study were *E. faecalis* which caused 86.6% infection followed by *E. faecium* which was responsible for about 17.4% of infection which was comparable to the distribution of enterococcal species in other studies.[9,10,11,12]. Out of 23 isolates 20 were isolated in pure culture while the rest 3 ie: 13.04 % were in combination with other bacteria ie: polymicrobial infections. In a study from South India by Prakash, *et al.,* 13% of enterococcal infections were polymicrobial and study by Chaudhary U *et al.,* in 17.7% patients the infection was polymicrobial[13]. So both the above studies were similar to our study. These studies suggest that enterococci can act synergistically with other intestinal bacteria to enhance the rate of infection.

Enterococci show intrinsic low-level cross resistance to all aminoglycosides due to decreased uptake of antibiotics. Therefore, there is no meaning in testing susceptibility of clinical isolates of enterococci to low-level aminoglycosides. Enterococci can also exhibit acquired resistance to high level of aminoglycosides. It is very important to know whether the clinical isolate of *Enterococcus* is susceptible to high level of aminoglycosides or not. We used disk-diffusion (using high-potency gentamicin and streptomycin) and agar-screening methods to detect H. L. A. R. Agar-screen method was found superior in identifying H. L. A. R. It is possible that disk-diffusion method may not detect borderline resistance. Antimicrobial resistance has been consistently reported to be more common in *E. faecium* as compared to *E. faecalis.*

H.L.A.R was significantly higher among *E. fecium* isolates The high level resistance to gentamicin and streptomycin were observed among *E. fecium* 2 (50%) and 2(50%); followed by *E. fecalis* 8 (42%) and 11(58.9%); *both* by disk-diffusion method and agar-screen method. Our results were comparable to the results of other studies[14,15] Since the first report of vancomycin resistant enterococci (V.R.E) was given by in 1988 by Uttley *et al.,* Mathur *et al.,* [16,17] from New Delhi were the first to report V. R. E from India in 1999. There are various other reports on isolation of V.R.E from India Chaudhary U *et al.,* 2007; Mendiratta *et al.,* 2008; Ghoshal *et al.,* etc. [13,7,18]Out of 23 isolates, 2 were resistant, 1 intermediate sensitive and 20 sensitive to vancomycin. Vancomycin screen agar showed only 2 resistant strains and the other 1 intermediate strains became susceptible after screening. M.I.C also showed concordant results with Vancomycin screen agar ie. 1 intermediate sensitive strain and 2 resistant strains were further studied by using M.I.C (minimum inhibitory concentration). Out of these 3 isolates, 1 intermediate strain became sensitive and remaining 2 strains were resistant, the percentage of sensitive strains had increased from 87% to 91%. According to Chaudhary U *et al.,* 2007[13], who had reported 98% vancomycin sensitive strains and 88.5% sensitive to teicoplanin.

The MIC of VRE isolates were 64µgm/ml.

We found that both V. R. E strains were *E. faecalis* . Of 2 V. R. E isolates 1 was of female patient in female medicine ward and 1 male in I.C.U. and were associated with urinary tract infection, septicaemia, lung infection and catheterization with prolonged hospital stay. The male patient did not respond to the treatment as this strain was resistant to all the drugs and died after prolonged stay Sensitivity to vancomycin was 94% that was much higher compared to the sensitivity of other antibiotics so this drug should be kept as reserve drug and should not be used indiscriminately, otherwise resistance to this drug may also occur posing a threat to treatment in future.

V. R. E isolates were further tested for teicoplanin susceptibility and resistance pattern, 1 was found resistant to teicoplanin and remaining 22 were sensitive (95.6%) so this study had given the conclusion that isolates that was V. R. E and teicoplanin resistant were phenotype VanA and the remaining isolates which were teicoplanin sensitive, probably VanB or any other phenotypes.

In our study with Linezolid only 1 strain (4.34% was resistant and all are sensitive to linezolid). Jones *et al.,* identified only 5% resistance to linezolid.

Conclusion

The present study reveals the problem of multidrug resistant enterococci and emergence of V. R. E. Studies on the risk factors associated with the acquisition of enterococci expressing resistance to vancomycin had identified multiple antibiotics and prolonged hospital stays as independent factors. However, emergence of multidrug resistant has led to the failure of synergistic effects of combination therapy.

Thus we suggest intensified actions to promote more the rational use of antibiotics in health care settings, more surveillance studies in order to monitor changes in enterococcal resistance patterns, better isolation procedure and better susceptibility test needs to measure the vancomycin resistance accurately.

It appears that this may just be the beginning of the problem, screening of symptomatic patients with significant isolates of enterococci obtained in pure culture is recommended.

Recommendations on the basis of this study

The study recommends routine testing of enterococcal isolates for H.L.A.R and vancomycin susceptibility. Agar-screen method should be preferred for detection of H.L.A.R in enterococci. M.I.C for vancomycin should be performed in all laboratories to keep record of increasing resistance of enterococci to vancomycin and for early detection of vancomycin resistance by strain of enterococci.

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