ORIGINAL ARTICLE

PREVALENCE OF VANCOMYCIN RESISTANT ENTEROCOCCUS AND ITS ANTTIBIOTIC RESISTANCE PATTERN IN PATIENTS ADMITTED IN TERTIARY CARE HOSPITAL

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Abstract

Enterococci are indigenous flora of the intestinal tract, oral cavity & genitourinary tract of human & are important opportunistic pathogens, especially in hospitalized patients. This genus is resistant to many antimicrobial agents commonly used in hospitals including β-lactam antibiotics, glycopeptides and aminoglycosides. In recent yrs the incidence of enterococcal infection has increased making the second most common nosocomial pathogen. The present study reveals the problem of mutidrug resistant enterococci and emergence of VRE. Emergence of high-level resistance to aminoglycosides (HLAR), 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.

Key words: Nosocomial infection, Vancomycin resistant enterococcus, Polymicrobial infection.

Introduction

Enterococci are indigenous flora of the intestinal tract, oral cavity & genitourinary tract of human & are important opportunistic pathogens, especially in hospitalized patients.1 E.faecalis(80-90%) & E.faecium(5-10%) are two commonly prevalent species which are human pathogens capable of causing urinary

tract infections, intra-abdominal infections, pelvic wound infections, biliary tract infections, respiratory infections, neonatal sepsis accompanied by bacteremia or meningitis or both2. Other enterococcal species are identified less often. This genus is resistant to number of antimicrobial 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 elements3. In recent yrs the incidence of enterococcal infection has increased, making it the second most common nosocomial pathogen reported to the National Nosocomial Infection surveillance system4. However emergence of high-level resistance to aminoglycosides (HLAR), 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.5

Material & Method:

This observational study was conducted after approval from institutional ethical committee in the Department of Microbiology, Era's Lucknow Medical College and Hospital, Lucknow among indoor patients from December 2007 to December 2008.Eighty six enterococcus strains were isolated from various clinical specimens including pus, urine, wound swab, catheters, blood, sputum, throat swab, cerebrospinal fluid, high vaginal swab and other body fluids, collected from patients of all age group admitted in the Departments of Surgery, Gynecology and Obstetrics, Medicine, Pediatrics and Orthopedics. Past history of the patients was recorded for diabetes mellitus, chronic renal illness and 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 Minimum Inhibitory Concentration(MIC) by Agar Diilution Method. Disc testing was performed according to the CLSI guidelines. 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. Inhibition zones were interpreted according to CLSI guidelines. Control strains used were E.faecalis ATCC-29212(susceptible) and E.faecalis ATCC-51299(resistant).

Disk Diffusion Sensitivity by Vancomycin 30 microgram disc was carried out using Kirby Bauer method. Muller-Hinton agar (MHA) plates were overlaid with the inoculums turbidity equivalent to that of a 0.5 McFarland Standard following CLSI criteria. Zone diameters were measured at 24 hrs following CLSI criteria i.e. zone of inhibition more than or equal to 17 mm reported as sensitive and less than 17mm were further confirmed by growth on BHI(Brain Heart Infusion) screen agar and MIC testing.

Determination of Vancomycin MIC was done by Agar Dilution Method. Enterococcus strains that were resistant and intermediate sensitive by Disk Diffusion method were further tested by BHI Vancomycin Screen Agar. As per CDC guidelines, in-house prepared BHI agar (Hi-Media, India) screen plates containing 6 microgram/ml Vancomycin (Lilly Pharma, Giessen, Germany) was prepared. Inoculum suspensions were prepared by selecting colonies from overnight growth on nutrient agar plates. The colonies were transferred to sterile saline to produce a suspension that

matched the turbidity of 0.5 McFarland Table andard. The final inoculum concentration of 105 to 106 CFU per spot was prepared by adding the sterile saline to the bacterial suspension. These suspensions were spot inoculated on BHI screen agar plates and plates were incubated for 24 hrs. at 35^{o}C aerobically. Any visible growth was indicated for Vancomycin resistance.

Further detection of VRE was done by MIC by vancomycin agar dilution method using MHA. The concentrations tested ranged from 2 µg/ml to 1024 µg/ml of vancomycin.

Results :

Eighty six Enterococcus strains were isolated after identification by standard biochemical tests.All were tested for Vancomycin resistance using Mueller Hinton agar by Kirby-Bauer disc diffusion method6,7. six isolates showed resistance and six were intermediate sensitive after 18-24 hours incubation at 35ºC.

Table 1- Distribution of *enterococcus* strains isolated from the various clinical specimens

Samples	**No.of samples**	**No. of Enterococci**
Urine	1900	58
Blood	290	5
Pus	380	10
Vaginal swab	180	8
Throat swab, Pleural fluid, Ascitic fluid, Peritonial fluid Bile, CSF, tissue aspirate	365	5
Total	3115	86

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

Wards	Species	Source of Specimen	Sensitivity pattern by disc method	MIC by broth dilutionmethod
ICU	*E. faecalis*	Urine	Resistant	512 µg/ml
ICU	*E. faecalis*	Urine	Resistant	64µ g/ml
GYNAE	*E. faeicum*	Urine	Resistant	64µ g/ml
GYNAE	*E. faecalis*	Pus	Resistant	32µ g/ml
POST-OP	*E. faecalis*	Urine	Resistant	16µ g/ml
ICU	*E. faecalis*	Urine	Resistant	16 µg/ml

Enterococcus strains that were resistant and intermediate sensitive by Disk Diffusion method were further tested by BHI Vancomycin Screen Agar. Vancomycin screen agar showed only 6 resistant strains and the other 6 intermediate strains became susceptible after screening. MIC also showed concordant results with Vancomycin screen agar ie. the percentage of sensitive strains had increased from 86.04% to 93.02%. The MIC of VRE ranged from 16 - 512µgm/ml. All 6 VRE isolates were further tested for different antibiotics susceptibility and resistance pattern.8

Table 3- Resistance pattern of VRE strains among the patients

VAN MIC µgm/ml	Amp	HSG	Nx	Cp	E	Te	Lz	Pm
16 µg	R	R	S	R	R	S	S	R
16 µg	R	S	R	R	R	S	S	S
32 µg	R	R	R	R	R	R	S	R
64 µg	R	R	R	R	R	S	S	S
64 µg	R	S	R	R	R	S	S	S
512 µg	R	R	R	R	R	S	S	R

*(*Van-Vancomycin, Amp-Ampicillin, HSG-High strength Gentamycin, Nx- Norfloxacin, Cp-Ciprofloxacin,E-Erythromycin, Te-Teicoplanin, Lz-Linezolid, Pm-Pristinomycin)

One of the VRE strain was found to be resistant to teicoplanin and remaining 5 were sensitive. This drug is rapidly and completely absorbed after oral administration with a mean bioavailability of approximately 100%9. We observed that this drug is effective against multiple drug resistant strains of Enterococcus .

Discussion

Out of 86 isolates, 75 were isolated in pure culture while the rest 11 were in combination with other bacteria ie: 12.7% of enterococcal infections were polymicrobial10. It suggests that enterococci can act synergistically with other intestinal bacteria to enhance the rate of infection.

The MIC of VRE ranged from 16 - 512µgm/ml. Chaudhary U et al. 200711, had also reported approximate result. Out of 6 VRE , five resistant isolates were from urine specimen and one from pus that was approximately similar to Taneja et al. (2004) from PGI Chandigarh. All were E.faecalis species except one that was E.faecium. All 6 VRE were isolated from admitted patients. Of 6 VRE isolates, 4 were female patients, 2 in post operative ward , 2 in Gynae ward and 2 were males - 1 in I.C.U. and 1 in male medicine ward and were associated with urinary tract infection and catheterization with prolonged hospital stay.

One of the VRE strain was found to be resistant to teicoplanin and remaining 5 were sensitive so this study had given the conclusion that isolate that was

VRE and teicoplanin resistant was phenotype VanA and the remaining 5 isolates which were teicoplanin sensitive, probably VanB or any other phenotypes12. An indoor female patient who was suffering from UTI with septicaemia and the isolate was E.faecalis which was resistant to all drugs except Linezolid but she did not respond to the treatment& died during hospital stay only.

Conclusion: An important feature in the emergence of the enterococci as a cause of nosocomial infection is their increasing resistance to a wide range of antibiotics and their ability to acquire resistance to all currently available antibiotics either by mutation or by receipt of foreign genetic material through the transfer of plasmids and transposons13. High Level Aminoglycoside Resistant strains with multidrug resistance has led to the failure of synergistic effects of combination therapy14,15.The present study reveals the problem of mutidrug resistant enterococci and emergence of VRE. Thus we suggest to promote more the rational use of antibiotics in health care settings, more surveillance studies in order to monitor changes in enterococcal resistance patterns.

So a good antibiotic policy should be laid down between the clinician and microbiologist in all tertiary care hospitals and a strict antibiotic regimen should be applied by clinicians. Those patients identified with history of chronic illness like diabetes mellitus, renal failure, peritoneal dialysis should be dealt with utmost care.

Competing Interest: None declared

Funding: Nil

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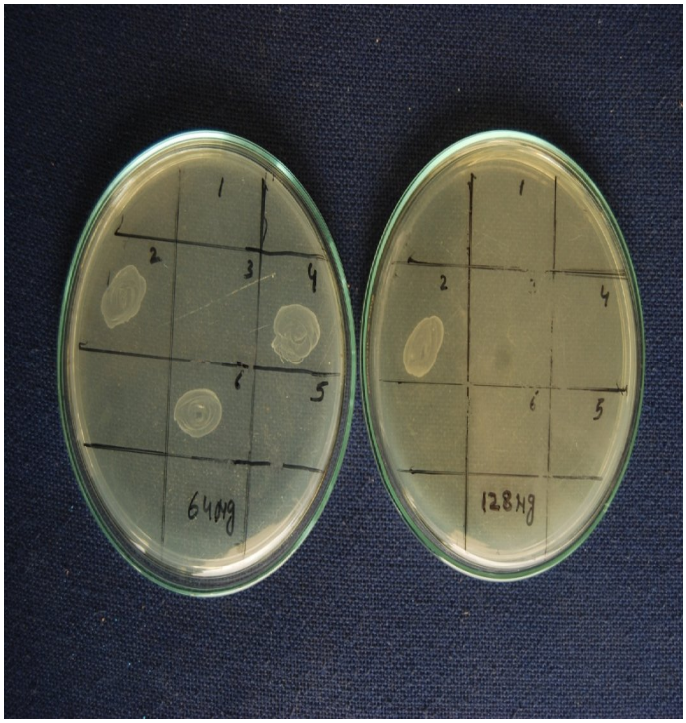

Figures showing results of MIC of Vancomycin