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# Emergence of *vanA* gene among vancomycin-resistant enterococci in a tertiary care hospital of North - East India

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***Background & objectives*: Vancomycin-resistant enterococci (VRE) have become one of the most challenging nosocomial pathogens with the rapid spread of the multi-drug resistant strain with limited therapeutic options. It is a matter of concern due to its ability to transfer vancomycin resistant gene to other organisms. The present study was undertaken to determine the emergence of vancomycin-resistant enterococci and the *vanA* gene among the isolates in a tertiary care hospital of North-East India.**

***Methods*: A total of 67 consecutive enterococcal isolates from different clinical samples were collected and identified by using the standard methods. Antibiogram was done by disk diffusion method and VRE was screened by the disk diffusion and vancomycin supplement agar dilution method. The minimum inhibitory concentration (MIC) value for vancomycin was determined by E-test. The VRE isolates were analyzed by PCR for *vanA* gene.**

***Results*: A total of 54 (81%) *Enterococcus faecalis* and 13 (19%) *E. faecium* were detected among the clinical isolates and 16 (24%) were VRE. The VRE isolates were multidrug resistant and linezolid resistance was also found to be in three. MIC range to vancomycin was 16-32 µg/ml among the VRE. The *vanA* gene was found in nine of 16 VRE isolates.**

***Interpretation & conclusions*: Emergence of VRE and presence of *vanA* in a tertiary care hospital setting in North-East India indicate toward a need for implementing infection control policies and active surveillance.**

**Key words** *Enterococcus faecalis* - *Enterococcus faecium* - nosocomial infection - vancomycin-resistant enterococci (VRE) - *VanA* gene

Enterococci have emerged as the leading causes of multiple drug resistant hospital-acquired pathogens especially with the emergence of glycopeptide-resistant *enterococcus* (GRE) species. It is the second most common pathogen causing mortality and morbidity[1] and the third leading cause of hospital acquired bloodstream infection[2]. These not only pose challenge to the clinicians but also result in treatment failure, selection pressure and spread of resistant strains in the health care institutes.

GRE strains were observed after almost 25 years of vancomycin use since 1980s. In the United States, vancomycin-resistance *Enterococcus faecium* accounted for 4 per cent of healthcare-associated infections[3]. In Europe, prevalences of VRE is diverse ranging from <1 to >40 per cent[4]. Resistance to glycopeptides is mediated by the *van* gene clusters, which produce resistance by altering the drug target from D-alanine-D-alanine to D-alanine-D-lactate[5]. So far, eight genotypes of glycopeptide resistance, which differ in the level and range of resistance and in transferability to glycopeptides, have been described for enterococci. Five of the *van* genes are acquired (*vanA, B, D, E, G*) and three (*vanC1, C2, C3*) are intrinsic. Of these, *vanA* is the most prevalent and is predominantly found in *E. faecium* and *E. faecalis*, the enterococcal species responsible for most infections in human[6]. This study was undertaken to detect the presence of vancomycin resistant enterococci (VRE) and that of *vanA* among the enterococcal isolates in a tertiary care hospital in North-East (NE) India.

## Material & Methods

A total of 67 consecutive, non-repetitive clinical isolates of *Enterococcus* species from different clinical samples (urine, blood, sputum, pus and throat swabs) were obtained in the department of Microbiology and this study was carried out in the Genetic Laboratory, under department of Microbiology, Gauhati Medical College and Hospital and National Institute of Pharmaceutical and Education and Research, Guwahati. The approval from the institutional ethical committee was obtained prior to the study.

*Enterococcus* species were identified in accordance with the standard procedures[5]. The isolates were stored for further processing in Luria Bertani (LB) broth with 20 per cent glycerol at -80°C.

*Antibiotic susceptibility testing*: Antibiotic susceptibility testing was done using Kirby-Bauer disk diffusion method as per the CLSI (Clinical Laboratory Standards Institute) guideline[7] against vancomycin (30 µg), teicoplanin (30 µg), linezolid (30 µg), penicillin G (10 U), tetracycline (30 µg), ciprofloxacin (30 µg), chloramphenicol (30 µg) and doxycycline (30 µg). The quality control organisms were *E. faecalis* ATCC 51299 (VRE) and *E. faecalis* ATCC 29212. (Hi-media, Mumbai).

*Phenotypic detection of vancomycin resistant Enterococcus*: Vancomycin resistance in all enterococcal isolates was detected by disk diffusion method and VRE agar dilution screening test[7]. Vancomycin supplement (6 µg, Hi-media, Mumbai) was added to 1 ml of sterile distilled water and then mixed to 1000 ml of the autoclaved brain heart infusion (BHI) agar at 45°C and the medium was pour in a petridish and the bacteria was inoculated and incubated at 37°C for 48 h. Growth of even single colony was considered as vancomycin-resistant *Enterococcus* (VRE).

*MIC for vancomycin-resistant Enterococcus*: Minimum inhibitory concentration (MIC) value for vancomycin was determined using E-test strip (Hi-media, Mumbai). Any *Enterococcus* was considered VRE if the MIC was ≥ 16 µg/ml[7].

*Polymerase chain reaction (PCR) for vanA gene*: The isolates resistant to vancomycin were taken for plasmid DNA isolation and amplification. A single colony was picked from a freshly streaked blood agar plate and inoculated in 3 ml L-B broth where it was grown at 37°C for 12-16 h with constant shaking. The culture was incubated for 1 h at 37°C with lysozyme (5 mg/ml). The bacterial cells were harvested by centrifugation at 6000 x *g* for 15 min at 4°C. The pellet was taken and resuspendend in tris-acetate-EDTA (TAE) buffer and heated in a heat block at 95°C for 5 min. It was further centrifuged at in 'g' 15000×*g* for 5 min at 4°C and the supernatant containing DNA was used as a source of template for amplification.

The PCR amplification for *vanA* was performed[8] with some modifications. The reaction mixture with a final volume of 50 µl contained 3 µl of purified plasmid DNA, 1 × PCR buffer (20 mM Tris-HCl/50 mM KCl, *p*H 8.4), 7 mM $MgCl_2$, 0.2 mM each deoxynucleoside triphosphate, 0.5 nM each primer, and 2.5 units of Taq polymerase (Qiagen, Mumbai). The PCR conditions were 95° C for 5 min for the first cycle; 95°C for 1 min, 50°C for 1 min and 72$^0$C for 1 min for the next 30 cycles and final extension at 72°C for 10 min.

The *vanA* gene primer sequence (5'-3') used was: forward primer - A1 GGGAAAACGACAA TTGC and reverse primer -A2 GTACAATGCGGCCGTTA[9]. Amplification of gene was carried out by DNA thermal cycler with the help of specific primers for *vanA* gene. *E. faecalis* ATCC 51299 was used as positive control for *vanA* and the negative control consisted of all reagents but no DNA template. PCR product was analyzed by electrophoresis with 1.5 per cent agarose gel and 0.5 mg ethidium bromide added separately. Gel was photographed on an ultraviolet light transilluminator by gel documentation system (Perkin

**Table I.** Distribution of enterococcal isolates in various clinical specimens

| Clinical samples | No. of enterococci recovered | Isolation rate (%) | No. of VRE screened | Isolation rate (%) |
|---|---|---|---|---|
| Urine | 56 | 84 | 12 | 75 |
| Blood | 4 | 6 | 3 | 18.75 |
| Sputum | 3 | 4 | 1 | 6.25 |
| Pus | 2 | 3 | - | - |
| Throat swab | 2 | 3 | - | - |
| Total | 67 | | 16 | |

VRE, vancomycin resistant enterococci

Elmer, USA). 100 bp molecular marker was used and a final product of 732 bp was considered as *vanA* gene.

## Results & Discussion

A total of 67 enterococcal isolates were collected from 896 culture-positive samples screened over the one year period. The majority of isolates were recovered from urine specimens followed by isolates from blood, sputum, pus and throat swab; 49 (73%) of the enterococcal isolates were obtained from hospitalized patients and 18 (27%) were from outpatient department. Among these 67 enterococcal isolates, 16 (24%) VRE and 51 (76%) vancomycin sensitive enterococcus (VSE) were detected (Table I); 54 (81%) were *E. faecalis* and 13 (19%) *E. faecium* and among the VRE, nine (56.3%) *E. faecalis* and seven (43.7%) *E. faecium* were detected.

Of the 67 isolates, 46 (68.6%) were multiple drug resistant to more than one class of antibiotics, three (4.5%) were resistant to linezolid (Table II) and were found to be VRE. The linezolid resistance was found in two *E. faecalis* and one *E. faecium* VRE isolates. The VRE isolates were resistant to ciprofloxacin (69%), tetracycline (56%), penicillin (58%) and erythromycin (100%). Most of the VRE were found to be sensitive to tecoplanin and linezolid (Fig. 1). Among the VRE, MIC to vancomycin ranged between 16-32 µg/ml.

Nine of 16 VRE isolates were found to possess *vanA* gene with a 732 bp PCR product (Fig. 2) of which six were *E. faecium* and three *E. faecalis.*

In the present study, 81 per cent *E. faecalis* and 19 per cent *E. faecium* were detected. Earlier studies from various parts of India had shown 55-87 per cent of *E. faecalis* as the predominant species followed by 10-20 per cent of *E. faecium*[10-13]. In some studies a relatively high proportion of *E. faecium* has been reported[14,15]. In our study maximum isolation was from the urine followed by blood. This finding was consistent with other studies[12,16,17].

A study from Lucknow reported VRE in 55.17 per cent of the isolates[18]. Other Indian studies reported VRE isolation between 0-5 per cent[10,11,13]. Studies from Indore and Nagpur reported 14.29 and 11.38 per cent VRE, respectively[19,20]. In our study the VRE isolation was 24 per cent.

Studies from New Delhi reported MIC range of vancomycin >32 µg/ml[10], from Chandigarh 8-16 µg/ml[11] and from Mumbai 8-128 µg/ml[13]. Studies from Italy[21], Japan[22] and Turkey[23] showed MIC range of vancomycin 16-32 µg/ml similar to our study.

The *vanA* gene was found in 56.25 per cent of the VRE isolates in our study. Mathur *et al*[10] reported 80 per cent of *vanA* phenotype which was higher than our findings. Studies from Taiwan[24] and Brazil[25] reported

**Table II.** Antibiogram of enterococcal isolates from the clinical samples (n=67)

| Antibiotics | Sensitive (%) | Resistant (%) |
|---|---|---|
| Vancomycin | 51 (76) | 16 (24) |
| Teicoplanin | 62 (92.5) | 5 (7.5) |
| Linezolid | 64 (95.5) | 3 (4.5) |
| Ciprofloxacin | 22 (33) | 45 (67) |
| Doxycycline | 41 (61) | 26 (39) |
| Chloramphenicol | 42 (63) | 25 (37) |
| Tetracycline | 37 (55) | 30 (45) |
| Penicillin | 28 (42) | 39 (58) |

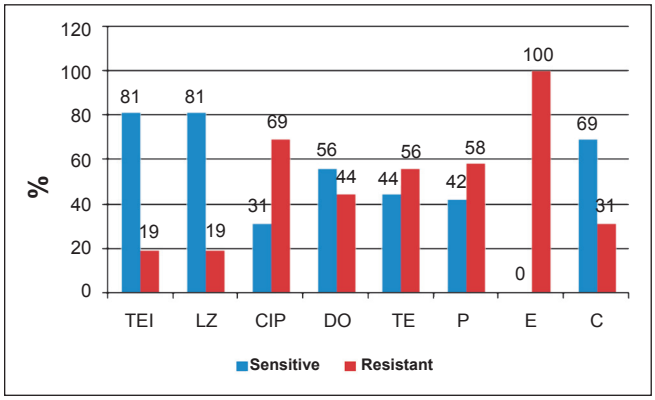


**Fig. 1.** Antibiogram sensitivity of VRE isolates. TEI, teicoplanin; LZ, linezolid; CIP, ciprofloxacin; DO, doxycycline; TE, tetracycline; P, penicillin; E, erythromycin; C, chloramphenicol.

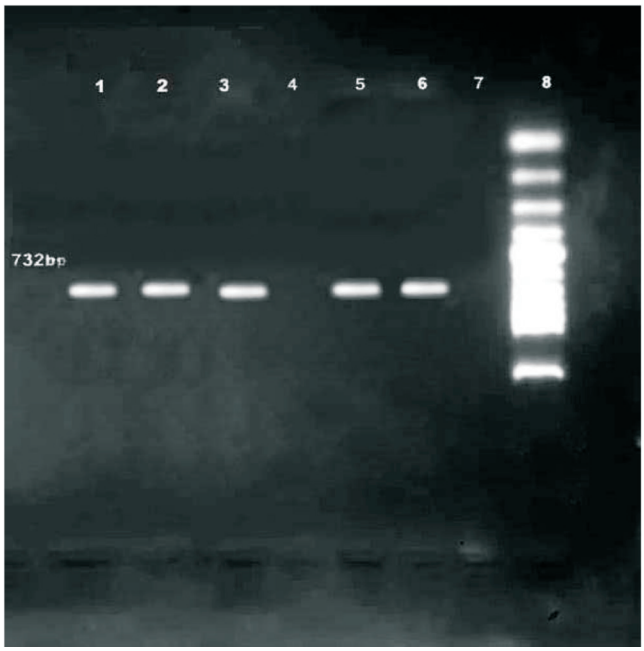


**Fig. 2.** Agarose gel electrophoresis showing amplification of 723 bp fragment for vanA of VRE from the clinical samples. Lanes 1-3, 5-6: VRE strains represents the vanA amplified 732 bp fragment, Lane 7 - *E. faecium* ATCC 29212 (negative control), Lane 8-100 bp ladder marker.

*vanA* gene in all the VRE isolates. The steady rise in the proportion of vancomycin-resistant *E. faecalis* isolates in the United States from 2.7 per cent in 1999 to 3.9 per cent in 2010 has been reported[26].

In conclusion, emergence of VRE and *vanA* gene among the isolates in a tertiary care hospital setting in NE India points towards implementing prevention and control policies.

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