Molecular characterization of vancomycin resistant enterococci from various clinical samples in a tertiary care hospital

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Abstract
Aim: The aim of this present study was to investigate the molecular characterization of vancomycin resistant enterococci isolated from various clinical samples. Enterococci are aerobic and anaerobic gram positive cocci found in gastrointestinal tract of humans and other animals. Enterococci are bacteria that normally live in the human intestine and female genital tract. It normally does not cause disease, but it can cause infections in someone whose immune system is weakened, or it can infect from the intestinal tract to other parts of the body during abdominal surgeries. In the beginning enterococci have been considered as relatively of low virulence. Recently according to Centre for disease control (CDC) survey enterococci has become the second most common cause of hospital acquired urinary tract infections next to E.coli, surgical wound infections and the third most common cause of nosocomial bacteraemia. Enterococci are one of the common isolates from various samples in our hospital. Several reports of VRE all over the world ranges from 0.3 in 1989 to 11% in 1996. In 2012 the incidence of VRE in hospital was 37% to 46.5%. A study done in Lady Hardinge Medical college, New Delhi, Chandigarh and Mumbai indicates 8%, 5.5% and 23% respectively all being of VanB phenotype. Hence this study was done to know the prevalence of VRE and its phenotypes hence this study was done to know the antibacterial resistance, prevalence percentage rate of VRE & its phenotypic, genotypic characterization in and around Kancheepuram.

Materials and Methods
Experimental Design
Various clinical samples such as Pus, Urine, Sputum, Body fluids & Blood of Inpatient and Out Patient department of Meenakshi medical college, Enathur, Kancheepuram, were processed according to standard protocol and Enterococcal isolates were collected. The study was conducted during the period from March 2012 to February 2013. An informed consent was obtained from all the subjects participating in the present study.

Inclusion Criteria
All the Enterococcal isolates from clinical samples such as blood, urine, pus, sputum, wound swab, catheter tip and other body fluids are included.

Exclusion Criteria
All commensally Enterococcal isolates from anatomical sites like gastrointestinal tract, female genital tract, stool and oral throat swab were not included in the study.
Collection of Samples
All clinical samples were collected according to standard guidelines and processed according to standard protocol and speciated. All Enterococcal isolates were screened for vancomycin resistance using Vancomycin screen agar and MIC was done using microbrothdilution method. VRE isolates were screened for genes VanA and VanB using conventional PCR.

Genotypic characterization of Vancomycin Resistance Genes By Polymerase Chain Reaction
Detection of vancomycin resistance genes (VanA and VanB) were performed as described by Kariyama et al., 2000. Genomic DNA was extracted was done.

DNA Extraction
DNA was extracted by boiling of all enterococcal isolates. In brief a loop full of the colonies were picked and suspended in 200µl of sterile distilled water and boiled for 10 mins at 95°C. Extracted bacterial DNA was stored at -20°C.

PCR Master Mix
<table>
<thead>
<tr>
<th>Buffer</th>
<th>3µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>dNTP’s</td>
<td>1 µl</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>1 µl</td>
</tr>
<tr>
<td>Forward primer</td>
<td>3 µl</td>
</tr>
<tr>
<td>Reverse primer</td>
<td>3 µl</td>
</tr>
<tr>
<td>Template</td>
<td>2 µl</td>
</tr>
<tr>
<td>MilliQ</td>
<td>6 µl</td>
</tr>
</tbody>
</table>

PCR CYCLING TEMPERATURE
Initial Denaturation at 94°C for 5 mins
Denaturation at 94°C
Annealing at 54°C
Extension at 72°C
Final Extension at 72°C for 10 mins
Holding at 4°C for 10 mins

Analysis of PCR products (Amplicons):
After amplification, the amplicons were visualized on 1.5% agarose gel with 0.5x Tris-borate-EDTA buffer. A100-bp DNA ladder was used as a molecular size marker. The gels were stained with ethidium bromide and photographed under UV light for presence of band with the Gel Documentation system.

Ethical Concern
Ethical clearance was obtained from the Ethical committee meeting conducted at Meenakshi Medical College Hospital and Research Institute Kanchipuram, Tamil Nadu, India.

Results
A total of 52 isolates of enterococci were isolated from various clinical samples.

Species Distribution of Enterococcal isolates
Fig 1 Shows that the species distribution among various enterococcal isolates. Among the 52 isolates of enterococci 41 isolates (78.8%) were E. faecalis and 11 isolates (21.2%) were E. faecium.

Fig. 1: Showing Species Distribution

Fig. 2: Gel picture shows Lane-1 containing 250bp ladder. Lane1 shows positive for Gene VanB(433BP). Lane 3&4 shows Positive for Gene VanA (1030BP). Lane 2&5 shows negative controls.

Fig. 2 shows that the six isolates resistant to Vancomycin confirmed phenotypically (by vancomycin screen agar) were taken for PCR. The presence of VanA &VanB genes were detected. Out of 6 isolates 2(33.3%) showed presence of VanA (1030bp) gene out of which 1(50%) was from E. faecalis and 1(50%) was from E. faecium. The presence of VanB (433bp) gene was positive in only 1(16.7%) in E. faecium. E. faecalis isolates were negative for presence of VanB gene.

Primer Sequences

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
<th>Product size (bp)</th>
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<tbody>
<tr>
<td>VanA forword</td>
<td>5’-CATGAATAGAATAAAAGTTGCAATA-3’</td>
<td>1030</td>
</tr>
<tr>
<td>VanA reverse</td>
<td>5’-CCCCCTTTAAGCTAATACGATCAA-3’</td>
<td></td>
</tr>
<tr>
<td>VanB forword</td>
<td>5’-GTGACAAACCGAGGCAGGA-3’</td>
<td>433</td>
</tr>
<tr>
<td>VanB reverse</td>
<td>5’-CCGCCATCCTCTTGCAAAAAA-3’</td>
<td></td>
</tr>
</tbody>
</table>
Discussion
Enterococci in the recent years have gained increased importance because of their ability to cause serious infections and also their increasing resistance to antibiotics.

In the present study isolated 78.80% of *E. faecalis* and 21.20% of *E. faecium*. In our isolation percentage rate is very similar to Vinod Kumar et al.2011 who have isolated 81.03% of *E. faecalis* and 18.7% of *E. faecium*. Our isolation percentage rate is slightly lower than compared with Agarwalet al.,1999 who have isolated 86% of *E. faecalis* and 14% of *E. faecium*.

Several researchers investigated in that India and abroad reports that 80% to 90% of Enterococci are *E. faecalis* and 10 to 20% of enterococci are *E. faecium*. This finding is of potential concern *E. faecium* is more commonly associated with Vancomycin resistance than other enterococci.13

In our study, VanA was detected in 2(33.3%) of the isolates which are resistant to vancomycin at a MIC range of >16μg/ml. Among the 2 isolates positive for the presence of VanA gene 1 was found in *E. faecalis* & 1 was *E. faecium*. VanB gene was found in only 1(16.7%) isolate which was found to be *E. faecium*. 

Our study showed comparatively higher percentage of resistance in *E. faecium* than in *E. faecalis* which is less compared to Sanal et al.2013 study who showed 87.5% of VanA phenotype. Similarly Nelson et al.2000 also showed higher rate of detection for VanA compared to VanB genotype which is coherent with our study. In our study there was 33.3% of VanA and only 16.7% of VanB were detected by PCR.

Conclusion
We conclude that, Vancomycin resistant enterococci is a major health problem in the coming years and hence it is necessary to take all adequate measures to identify the resistant strains. Routine testing of all enterococcal isolates for vancomycin resistance & judicial use of vancomycin and effective surveillance of VRE suspected patients will limit the spread of VRE infections.

Conflict of Interest: None.

References

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