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.
Materials and Methods: Enterococcal isolates were isolated from various clinical samples according to standard protocol and sample size of the study was 52. Vancomycin resistance genes *VanA* and *VanB* were detected using conventional PCR.
Results: The six isolates resistant to Vancomycin confirmed phenotypically were confirmed using Polymerase Chain Reaction.
Conclusion: in the present study 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.

Keywords: Vancomycin resistance, Enterococci and polymerase chain reaction.

Introduction
Enterococci are the most common aerobic and anaerobic, gram positive cocci found as normal flora in bowel of humans and other animals.[1] For many years Enterococcus *spp* were believed to be harmless to humans and was considered not important medically.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 toother parts of the body during abdominal surgeries.In the beginning enterococci have been considered as relatively of low virulence.[2] Recentlyaccording to Centre for disease control (CDC) survey enterococci has become the second most common cause of hospital aquired urinary tract infections next to E.coli, surgical wound infections and the third most common cause of nosocomial bacteraemia.[12]

The infections caused by enterococci are urinary tract infection, intra abdominal and pelvic infections, bacteremia wound and soft tissue infections, endocarditis, respiratory tract infections, neonatal sepsis and meningitis.[3] Although enterococci can cause human infection in the community & in hospital, these microorganisms began to be recognized with increasing cause of nosocomial infection in late 1970s, paralleling to the increasing resistance to currently used antimicrobials.[8] Hence enterococci emerged as leading therapeutic challenges with life threatening infections.

Monitoring the antibiotic resistance of enterococci isolated from various clinical specimens gives us information about the prevalence of VRE and will be essential in keeping a check on the spread of bacterial resistance. Despite the increasing reports of VRE in different countries, there is a distinct lack of data regarding the molecular characterization of VRE isolates, originating from the Middle East.[11]

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.[4,5] 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. [3,5] 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[6]:
Detection of vancomycin resistance genes (*VanA* and *VanB*) were performed as described by Kariyama*et al*., 2000[6]. 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^{0}C.Extracted bacterial DNA was stored at -20ºC[7].

PCR Master Mix

Buffer	-	3µl
dNTP's	-	1 µl
$MgCl_2$	-	1 µl
Forward primer	-	3 µl
Reverse primer	-	3 µl
Template	-	2 µl
MilliQ	-	6 µl

PCR CYCLING TEMPERATURE

Initial Denaturation at	-	94^o C for 5 mins
Denaturation at	-	94^o C
Annealing at	-	54^o C
Extension at	-	72^o C
Final Extension at	-	72^{o}C for 10 mins
Holding at	-	4^{o}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.*

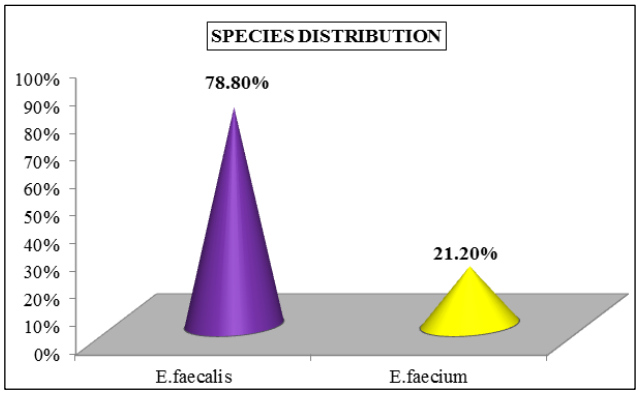

Fig. 1: Showing Species Distribution

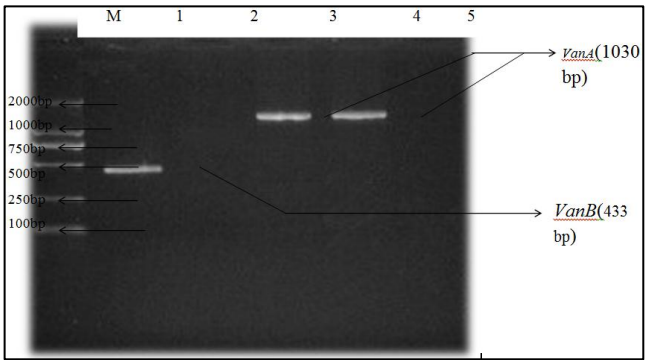

Fig. 2: Gel picture shows Lane-1 containing 250bp ladder. Lane1 shows positive for Gene *VanB*(433BP). Lane 3&4 shows Positive for Gene *VanA* (1030BP). Lane 2&5 shows negative controls.

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

Primer	Sequence	Product size (bp)
*VanA*forword	5′-CATGAATAGAATAAAAGTTGCAATA-3′	1030
VanA reverse	5′-CCCCTTTAACGCTAATACGATCAA-3′	
*VanB*forword	5′-GTGACAAACCGGAGGCGAGGA-3′	433
VanB reverse	5′-CCGCCATCCTCCTGCAAAAAA-3′	

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 that 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[9] who have isolated 81.03% of *E.faecalis* and 18.7% of *E.faecium*. Our isolation percentage rate is slightly lower than compared with Agarwal*et al*.,1999[10] who have isolated 86% of *E.faecalis* and 14% of *E. faecium*.

Several researchers investigated that in India and abroad reports that 80% to 90% of Enterococci are *E.fecalis* 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[(14)] study who showed 87.5% of *VanA* phenotype. Similarly Nelson *et al* 2000[15] 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.

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