**Glycopeptide Resistance in Enterococci: Plasmid-mediated Resistance to Vancomycin and Teicoplanin in *Enterococcus***

Mohammed A.M. Marie¹, Krishnappa Lakshmana Gowda¹*, Yazeed A Al-Sheikh¹, James John², Sangeetha Gopalkrishnan³, Cs. Pradeep¹ and Khaled Homoud M. Dabwan¹

¹Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, King Saud University, Riyadh, Kingdom of Saudi Arabia.
²Department of Clinical Microbiology, Christian Medical College and Hospital, Vellore, India.
³Department of Microbiology, Central Leprosy Training and Research Institute, Chennai, India.

(Received: 19 November 2013; accepted: 29 December 2013)

*Enterococcus faecalis* is the most frequent cause of nosocomial infections, especially bacteremia, sepsis in children, endocarditis, urinary tract infection (UTI), and wound infections. There are five resistance genes whose products are responsible for resistance to glycopeptides antibiotics in vancomycin-resistant enterococci strains (VRE) (Honarm *et al.* 2012). Two of such genes (Van A and Van B) are most common than others, especially in *E. faecalis* and *E. faecium*. We selected 70 well-characterized *E. faecalis* from blood (89%) and feces (11%) and antimicrobial susceptibility testing, these isolates were investigated for the presence of vanA and vanB gene by polymerase chain reaction. This study showed an overall prevalence of vanA gene and none of them had vanB gene. Since all our isolates were positive for van A gene, there may be an increased probability of the dissemination of VRE gene in other strain especially *s.aureus*. This is an important issue that needs to be monitored and additional studies are in progress to help determine the impact of VRE and its patterns of transmission in our hospital.

**Key words:** Enterococci, antibiotics, Vancomycin.

---

*Enterococcus faecalis* is the most frequent cause of nosocomial infections, especially bacteremia, sepsis in children, endocarditis, urinary tract infection (UTI), and wound infections. Bacteremia due to *E. faecalis* is usually caused by strains resistant to most antibiotics¹. Successful administration of the disease is dependent on rapid detection and characterization of the bacteria, and determination its sensitivity pattern to antimicrobial drugs.

The glycopeptide vancomycin is the first choice alternative to a penicillin-aminoglycoside combination for the treatment of systemic enterococcal infections. Acquired glycopeptide resistance in *Enterococcus* species is due to the production of peptidoglycan precursors with reduced affinity for glycopeptide antibiotics². There are eight resistance genes whose products are responsible for resistance to glycopeptides antibiotics in vancomycin-resistant enterococci strains (VRE)³. Two of such genes (Van A and Van B) are most common than others, especially in *E. faecalis* and *E. faecium*⁴. Strains with Van A gene are resistant to vancomycin and teicoplanin, and strains with VanB are resistant to vancomycin but sensitive to tycoplanin⁴⁵. The first report of enterococci resistant to high
concentrations of glycopeptide antibiotics (vancomycin and teicoplanin) was reported the occurrence in an outbreak of vancomycin-resistant *E. faecium* infecting patients in a hospital renal unit because of the increasing use of intravascular devices combined with the high prevalence of methicillin resistance among staphylococci. The increased prevalence of vancomycin resistance possesses the possibility that the resistance genes present in VRE can be transferred to other gram positive bacteria especially *Staphylococcus aureus*. Several recent nosocomial VRE outbreaks attest to the importance of early detection of VRE so that preventive measures including the isolation of infected patient can be instituted. We describe here a convenient multiplex PCR assay which can be performed to investigate for the presence of *van A* and *vanB* gene conferring glycopeptide resistance to enterococci.

Table 1. PCR primer for *van A* and *Van B*

<table>
<thead>
<tr>
<th>Primer</th>
<th>Nucleotide sequence (5’→3’)</th>
<th>Size(bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>VanB</td>
<td>CATCGCCGTCCCGAATTTCAA GATGCGGAAGATACCGTG</td>
<td>667</td>
<td>Lee et al. 2001.</td>
</tr>
</tbody>
</table>

Fig. 1. Minimal inhibitory concentration of *Enterococcus* sp

Fig. 2. Agarose gel electrophoresis of *van A* and *Van B* PCR
MATERIALS AND METHODS

In this study, we selected 70 well-characterized *E. faecalis* from blood (89%) and feces (11%) and antimicrobial susceptibility testing (AST) was done as per standard procedure.\(^9\) The minimum inhibitory concentration (MIC) of vancomycin, teicoplanin, daptomycin, and linezolid for these non-duplicate isolates were determined by the E-test (AB Biodisk) as per manufacturer instruction. Further, these isolates were investigated for the presence of *vanA* and *vanB* gene by polymerase chain reaction (Table 1).

RESULTS

Out of 70 isolates studied, percentage of *E. faecalis* and *E. faecium* were 87% and 13% respectively. The susceptibility pattern of the isolates showed a 100% resistance to vancomycin, 1% susceptible to teicoplanin, 92% susceptible to daptomycin and 93% to linezolid. 94% of the isolates studied had vancomycin MIC >256 and teicoplanin MIC >16 μg/ml (Fig. 1).

DISCUSSION

This study showed an overall prevalence of *vanA* gene and none of them had *vanB* gene by polymerase chain reaction (Fig. 2). Molecular detection of VRE may help to provide timely treatment and implementation of infection control interventions. With the increasing incidence of *vanA* type VRE, linezolid and daptomycin are the alternatives. Interestingly, although daptomycin is used frequently, 92% of VRE were found to be susceptible to daptomycin.

Rapid diagnosis is very critical in the treatment of bacteremia. Routine assay is time-consuming and expensive, and commercial automatic screening tests and disc diffusion agar are not efficient for highly resistant bacteria that make most of the hospital isolates. The sensitivity of PCR was the same as that of routine phenotypic test. Generally Enterococci are not regarded as extremely virulent bacterial pathogens, though; resistance to many antimicrobial drugs such as acquired resistance to high concentrations of glycopeptide antibiotics, specifically vancomycin, may complicate treatment of enterococcal infections. In the near term, VRE will become established, endemic, nosocomial pathogens with a certain percentage of enterococcal isolates typically being vancomycin-resistant (e.g. 25–50%) and episodic outbreaks producing upward spikes in the prevalence of resistant isolates.\(^9\) The mechanism for relative resistance to vancomycin that has been seen in staphylococci differs from that of acquired glycopeptide resistance in enterococci\(^\text{4,9}\), but the potential spread of enterococcal vancomycin resistance determinants to other species will remain a concern. Since all our isolates were positive for van A gene, there may be an increased probability of the dissemination of VRE gene in other strain especially *S. aureus*. Additionally, fact that enterococci are enteric bacteria and the intestinal lumen offers an encouraging environment for bacterial conjugation, thus *vanA* gene could be disseminated to other enterococci species and to other bacteria genera. This is an important issue that needs to be monitored and additional studies are in progress to help determine the impact of VRE and its patterns of transmission in our hospital.

ACKNOWLEDGMENTS

The authors would like to extend their sincere appreciation to the Deanship of Scientific Research at King Saud University for its funding of this research through the Research Group Project no. RGP-VPP-314.

Disclosure

The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

REFERENCES


