Prevalence of high level gentamicin and vancomycin resistance among clinical isolates of enterococci from a tertiary care hospital in central Nepal

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ABSTRACT
High level aminoglycoside resistance is an established phenomenon in enterococci. With the increasing use of broad spectrum cephalosporins and glycopeptides, vancomycin resistant enterococci are being increasingly reported from different parts of the world. The objective of our study was to determine the prevalence of high level gentamicin resistant (HLAR) enterococci among the clinical isolates in our hospital and to find the minimum inhibitory concentration (MIC) of vancomycin against these isolates. The enterococci isolated over a year (n=41) were subjected to HLAR screening by disc diffusion and MIC of vancomycin by agar dilution method. HLAR prevalence was 63%. MIC of vancomycin showed none in the resistant range. However, 4 (9.7%) isolates were in the intermediate range heralding the eminent emergence of VRE if adequate control measures are not implemented in time.

Keywords: High-level gentamicin resistance, vancomycin resistance, enterococci, Nepal

INTRODUCTION
Enterococci have been recognized as an important cause of endocarditis for almost a century.1 Currently they have emerged as a common cause of hospital acquired infection.2 Bactericidal synergy between beta-lactam and glycopeptide antibiotics are required for treatment of serious enterococcal infections. However, this synergy is not achieved when there is high level resistance to either class of drugs.1 Vancomycin has been the drug most used against these multidrug resistant strains. Unfortunately, after the first report in 1988 from United Kingdom, vancomycin resistant enterococci (VRE) have been reported from world over.3,4 To the best of our knowledge, till date minimum inhibitory concentration (MIC) of vancomycin falling in the resistant range has been reported in only one isolate of enterococci from Nepal.5 We have therefore attempted to find the prevalence of high level aminoglycoside resistant (HLAR) entercocci in our set-up and the MIC of vancomycin for these clinical isolates.

MATERIALS AND METHODS
This study was carried out for a period of one year (April 2013-March 2014). All the enterococci isolated from the clinical specimens submitted for bacterial culture in the Microbiology unit of Central Laboratory Services at Nepal Medical College Teaching Hospital, were studied. Besides the antibiotics susceptibility test for the routinely used drugs, HLAR screening was done followed by MIC determination of vancomycin. Screening for HLAR was done by disc diffusion method as recommended in Clinical Laboratory Standards Institute (CLSI) guidelines.6 McFarland 0.5 standard turbidity matched broth culture of the isolate was lawn cultured on Mueller Hinton Agar medium and a 120μg gentamicin disc (HiMedia) was placed on it. After incubating at 37º C at 24 hours in an aerobic environment, zone of inhibition (ZOI) was recorded, ZOI of less than 6mm was considered as HLAR.

MIC of vancomycin was determined for all isolates of enterococci using the agar dilution technique. Different concentrations of vancomycin were incorporated into Mueller Hinton Agar plates. McFarland 0.5 standard turbidity matched broth culture of the isolates were spot inoculated (10μl) on the drug incorporated plates. The plates were incubated at 37º C for 24 hours and examined. The minimum concentration of vancomycin that inhibited the bacterial growth was considered the MIC for that isolate. MIC of ≤4μg/ml was considered sensitive; 8-16μg/ml as intermediate and ≥32μg/ml as VRE. Enterococcus faecalis ATCC 29212 was used as the control strain.

RESULTS
There were 41 enterococci isolates of which 16 (39%) were from the out-patient departments (OPD) and 25 (60.9%) were from admitted patients. Most of the isolates 35 (85.3%) were from urine; 5 (12.1%) were from exudates and 1 (2.4%) was from blood sample. Of the 41 isolates, 12 (29%) were resistant to amoxicillin. A high percentage 26 (63%) were resistant to ciprofloxacin. HLAR was seen in 26 (63%) of the isolates tested. Vancomycin resistance was noted by disc diffusion in 3 (7%) of the enterococci.
However, the MIC of vancomycin performed against these isolates showed none to be vancomycin resistant (Table 1).

Table 1: MIC of vancomycin and susceptibility pattern of enterococcus

<table>
<thead>
<tr>
<th>Total number of isolates</th>
<th>MIC (μg/ml)</th>
<th>Standard interpretation (CLSI)</th>
<th>Number of isolates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤4</td>
<td>Susceptible</td>
<td>37 (90.3)</td>
</tr>
<tr>
<td></td>
<td>8-16</td>
<td>Intermediate</td>
<td>4 (9.7)</td>
</tr>
<tr>
<td></td>
<td>≥32</td>
<td>Resistant</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

One isolate from urine of a male inpatient had a MIC of 16μg/ml. This isolate was HLAR as well as resistant to amoxicillin, ciprofloxacin and teicoplanin by disc diffusion. It was sensitive to nitrofurantoin and chloramphenicol. Of the 3 isolates with MIC 8μg/ml, two were also HLAR while one was sensitive to Gentamicin 120μg. Two of these were from urine of outpatients.

DISCUSSION

During the study period, 41 Enterococci were recovered. Inpatient source outnumbered the outpatients. This could be due to the acquisition of the infection from the hospital environment. Enterococcus is the second most common cause of nosocomial UTI and wound infection in the United States of America. Such a distribution is reported by other investigators as well. Resistance, both inherent and acquired (by mutations, plasmid and transposons) give enterococci a survival advantage and establish them as a successful nosocomial pathogen. Clustering among inpatients was also seen with HLAR enterococci (69% from inpatients vs 31% from outpatients). The usual mechanism of resistance to high concentrations of aminoglycoside is by the production of aminoglycoside modifying-enzymes. Penicillin-aminoglycoside synergy does not occur in HLAR enterococci (streptomycin MIC,>2000μg/ml); Gentamicin MIC,>500μg/ml.

The overall HLAR enterococci prevalence in this study was 63.4% (n=26). A study from eastern Nepal showed HLAR enterococci as 36.4% in 2008. They arrived at the same result by disc diffusion and agar screening techniques. Prevalence studies for HLAR enterococci from other parts of Nepal are lacking. In India, 37% prevalence was reported from a hospital in South India and 46% from another in Maharashtra. Parameswarappa J et al found a prevalence of 68% in their setup. The difference in the prevalence seen in different hospitals may be due to the difference in the type of patients, antibiotics prescribing policies, infection control practices and the size of the samples studied. The HLAR enterococci prevalence is reported as low as low as 1% to as high as 49% from 27 European countries.

In this study, although 3 enterococci showed vancomycin resistance by disc diffusion method, MIC by agar dilution showed none in the resistant range. Of the 3 VRE by disc diffusion, one had an intermediate MIC (16μg/ml) while two others were in the sensitive range. A total of 4 (9.7%) vancomycin intermediate enterococci (VIE) were recorded by agar dilution method in our study. Another study from Nepal encountered 10% VIE. These are reported from hospitals elsewhere where VRE are not yet isolated. Of the 4 isolates of VIE in our study, 2 were from urine of outpatients. These could be community acquired. (However, history of recent or previous hospitalization could not be ruled out). VRE colonization of the intestines of healthy human volunteers and farm animals have been demonstrated. VRE was isolated from frozen pork and poultry. Thus, humans can acquire VRE or the gene for VRE through food or contact with domesticated animals; colonized patient after leaving hospital may also be a source of VRE to the community.

Nepal et al reported a single isolate of vancomycin resistant Enterococcus faecium from a case of peritonitis in a continuous ambulatory peritoneal dialysis patient. This isolate had a MIC of vancomycin of 32μg/ml. This is probably the first and only reported case of VRE from Nepal detected by agar dilution method till date. Investigators from western Nepal reported VRE by disc diffusion but they did not confirm their findings by MIC determination. VRE are constantly being reported from elsewhere in the world; the prevalence varying according to place and time. In India, since the first report in 1999 by Mathur et al, prevalence of VRE in a North Indian hospital was 1% in 2003 (Mathur) while Parija et al reported 8.7% prevalence from Puducherry in 2008-2009. VRE is an established pathogen in the Western world with as many as 17% in all enterococci strains from the USA and in up to 28% of all nosocomial strains. The spread of glycopeptides resistance in enterococci is promoted by the use of oral glycopeptides as well as by the non glycopeptides antibiotics like extended spectrum cephalosporins and anti-anaerobic agents.

To conclude, HLAR enterococci have already made their niche in our hospital setup with a high prevalence of 63%. Resistance against vancomycin, the drug commonly prescribed for treating resistant enterococci, is seen to be emerging. Although none of our enterococci isolates had a MIC in the resistant range, 3 had MIC in the intermediate range. If immediate and adequate
control measures are not put in place, we will soon be facing the treatment challenges posed by VRE. Continuous laboratory monitoring for HLAB and vancomycin resistance using valid techniques, judicious use of antibiotics and strict infection control practices can help prevent this situation.

REFERENCES