ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF ENTEROCOCCUS SPECIES ISOLATED FROM VARIOUS CLINICAL SPECIMENS IN A TERTIARY CARE HOSPITAL, KATHMANDU, NEPAL

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ABSTRACT

Resistance to vancomycin and high level aminoglycosides are common among *Enterococcus* spp. and are being increasingly reported from different parts of the world. These resistance phenomena in enterococci have limited the therapeutic options to treat the infections caused by them. The objective of our study was to determine the antimicrobial resistance patterns of *Enterococcus* spp. (n=60) isolated over a year from clinical specimens received from patients visiting Nepal Medical College Teaching Hospital, Kathmandu, Nepal. All enterococci were subjected to antimicrobial susceptibility testing, high level gentamicin resistance testing by disc diffusion method and minimum inhibitory concentration of vancomycin by agar dilution method. Prevalence of high level gentamicin resistance among enterococci was 55%. None of the isolates were resistant to vancomycin by both disc diffusion and agar dilution method. However 8.3% of them were intermediate to vancomycin. All of these vancomycin intermediate isolates were from samples from hospital admitted patients and resistant to ampicillin, ciprofloxacin, erythromycin and high level gentamicin. Present findings were suggestive of possible emergence of vancomycin resistant enterococci in the hospital if immediate and adequate control measures are not implemented.

KEYWORDS

Enterococci, high level gentamicin resistance, Nepal, vancomycin resistant enterococci

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INTRODUCTION

Enterococcus spp., the natural inhabitants of the intestinal tract of humans and animals have received attention in recent times due to their increasing role in nosocomial infections.[1] Inherent resistance among *Enterococcus* spp. to the most commonly used antimicrobial agents like cephalosporins, sulphonamides, low level aminoglycosides, polymyxins has left limited therapeutic options to treat the infections caused by them.[2]

Combination of beta-lactams and aminoglycosides that show synergistic bactericidal effect is being traditionally used to treat serious enterococcal infections like endocarditis, bacteraemia, intra-abdominal infections.[3,4] However, this synergy is not achieved when there is high level resistance to either class of drugs.[5] Increasing trend of resistance to these antimicrobials has raised the question of using combination therapy which may lead to therapeutic failure.[6] Glycopeptides like vancomycin, teicoplanin have been in use to treat infections by such resistant bacteria.[7] But unfortunately vancomycin resistance among *Enterococcus* spp. has been reported all over the world and is in rising trend.[8-10]

Resistance to vancomycin and high level gentamicin (HLG) among *Enterococcus* spp. has been reported also from Nepal.[11-13] It does raise the question of whether the antimicrobial resistance pattern among these bacteria in Nepal has changed. Improved knowledge of local and regional epidemiology and susceptibility patterns of bacteria is crucial in order to optimize empiric antibiotic treatment strategies. This study on antimicrobial resistance patterns of *Enterococcus* spp. along with their resistance to vancomycin and HLG seems to be essential, report of which could be highly beneficial for infection control and formulation of antibiotic policies in hospital set-up in this region.

MATERIALS AND METHODS

A descriptive cross sectional study was conducted over a year (July, 2017- June 2018) in the microbiology laboratory of Nepal Medical College Teaching Hospital (NMCTH), Kathmandu, Nepal. All the enterococci isolated from the clinical specimens submitted for bacterial culture were included in the study. *Enterococcus* spp were identified by studying colony characters, gram staining, catalase test and biochemical tests according to the standard microbiological techniques.[14]

The antimicrobial susceptibility testing was done by the Kirby Bauer disc diffusion method[15] in Mueller Hinton agar (MHA) as per the Clinical and Laboratory Standards Institute (CLSI) guidelines by using the following commercially available antimicrobial discs from Hi-media, Laboratories, Mumbai, India: [Ampicillin (10µg), erythromycin (15µg), doxycycline (30µg), ciprofloxacin (5µg), vancomycin (30µg) and teicoplanin (30µg)]. For urinary isolates, sensitivity against nitrofurantoin (300µg) was also tested.

The screening test for high level aminoglycosides resistance (HLAR) was done by disk diffusion method using CLSI guidelines on MHA using gentamicin (120µg) disk. Zone size of ≤ 6mm was regarded as resistant.[16]

Minimum inhibitory concentration (MIC) of vancomycin was determined by agar dilution method as per the CLSI guidelines. Ten microlitre of 0.5 McFarland standard turbidity matched colony suspension was spot inoculated in MHA with different concentrations of the drug. The plates were incubated at 35°C for 24 hours. The minimum concentration of vancomycin that inhibited the bacterial growth was considered the minimum inhibitory concentration (MIC) for that isolate. MIC of ≤4 µg/ml was considered as susceptible, 8-16 µg/ml as intermediate and ≥32 µg/ml as resistant. *E. faecalis* ATCC 29212 was taken as control.[17]

RESULTS

From both the in-patients and out-patients, a total of 18480 clinical specimens (Urine 8880, blood 5002, sputum 1880, pus 1685 and body fluids 1033) were processed. Of the 60 isolates of *Enterococcus* spp. 27 were from male patients and 33 from female patients. The isolates obtained were 27 (45.0%), 23 (38.3%), and 10 (16.7%) from pus, urine and blood respectively. The highest positivity rate among the processed sample was found in pus sample (1.6%) followed by urine (0.3%) and blood (0.2%). Majority of the enterococci isolates were from the inpatient's samples and from the patients of age group 21 to 40 years (Fig. 1 & Table 1).

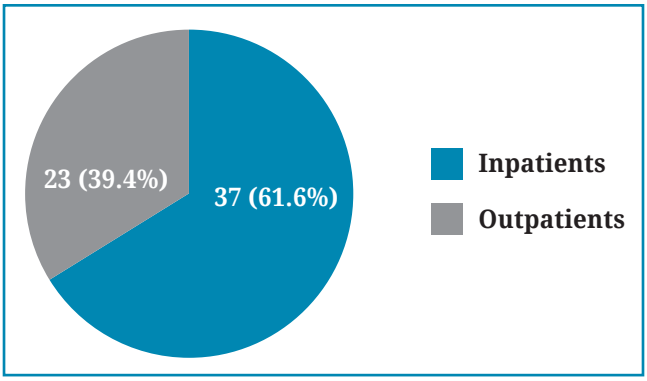

Fig. 1 *Enterococcus* spp. in specimens from inpatients and outpatients

Table 1: Distribution of clinical isolates of *Enterococcus* spp. according to the age of patients (n=60)

Age of patients in years	n (%)
< 10	9 (15.0)
11-20	7 (11.7)
21-30	13 (21.7)
31-40	13 (21.7)
41-50	6 (10.0)
51-60	7 (11.6)
61-70	3 (5.0)
>70	2 (3.3)

Table 2: Antimicrobial resistance pattern of *Enterococcus* spp. including HLGR

Antibiotics	Resistance n (%)
Ampicillin	27 (45.0)
Ciprofloxacin	30 (50.0)
Erythromycin	31 (51.6)
Gentamicin (120 microgram)	33 (55.0)
Doxycycline	4 (6.7)
Vancomycin	00 (00)
Teicoplanin	00 (00)
Nitrofurantoin	4 (17.4) of 23 urine isolates

Prevalence of high level gentamicin resistance (HLGR) among the enterococci isolates was 55% (n=33). Half of the isolates were resistant to ampicillin, ciprofloxacin and erythromycin. None of the isolates were resistant to vancomycin and teicoplanin (Table 2). As in disc diffusion method none of the isolates were found to be resistant to vancomycin by agar dilution method. However 5 (8.3%) of them showed intermediate susceptibility to vancomycin (Table 3).

Table 3: MIC of vancomycin and susceptibility pattern of *Enterococcus* spp. (n=60)

MIC (µg/ml)	Standard interpretation[17]	n (%)
≤4	Susceptible	55 (91.7)
8-16	Intermediate	5 (8.3)
≥32	Resistant	00 (00)

All five enterococci isolates (3 from pus and one each from urine and blood) showing intermediate susceptibility to vancomycin were from inpatient's samples and resistant to ampicillin, ciprofloxacin, erythromycin and HLG. Three of the isolates were susceptible to doxycycline.

DISCUSSION

Enterococci, traditionally regarded as low- grade pathogens now have emerged as an important cause of infections.[1-4] Increased use of broad spectrum antibiotics, rising number of severely ill patients, lack of proper infection control measures etc. have contributed to their involvement in infections especially in hospital set up.[18] This study showed higher number of the *Enterococcus* spp. from clinical samples of inpatients (61.6%; 37 of 60) compared to outpatients. This is similar to findings of other studies from Nepal[11-12] and the rest of the world.[8,19,20] This could be due to the acquisition of infection from the hospital environment. Natural ability of enterococci to readily acquire, accumulate and share extrachromosomal genetic elements encoding virulence traits or antibiotic resistance genes give advantages to their survival under unusual environmental stresses and in part this explains their presence in hospital environment. Moreover, hospitalized patients are usually immunologically weak and are prone to acquire infections from hospital environment.[21] Age wise, most of the *Enterococcus* spp. were isolated from 21-40 years age group of patients. Females were predominant among enterococcal isolates compared to males. This finding is also similar to that of other studies.[22, 23] *Enterococcus* spp. are the normal flora in gastrointestinal tract which could be the source of infections like urinary tract infections, surgical site infections[21] and young females are more prone to having urinary tract infections. This might explain the reason why most of the enterococcal isolates were from reproductive age group females in our study. *Enterococcus* spp. are more commonly isolated from urine and pus samples.[8, 11, 12, 19] This study also showed similar findings. Since these bacteria colonize most commonly the soft tissues wounds, ulcers and gastrointestinal tract in hospitalized patient they are more frequently isolated from urine and pus samples.[21]

HLGR in enterococci is of great concern these days because this results in failure of synergistic bactericidal effect of beta-lactam and aminoglycosides therapy against enterococcal infections. [6,12] Resistance to high concentration of aminoglycosides in enterococci is due to the production of aminoglycosides modifying enzymes and gene mutation of antibiotic target.[2]

As per the CLSI recommendation, screening for HLAR in enterococci should include testing for both HLG and high level streptomycin, we could test only HLG due to unavailability of streptomycin (300 µg) disc.

The overall prevalence of HLGR enterococci in this study was 55% which is almost similar to other studies conducted in Nepal[11,12] and other countries.[22,24,25] However in contrast to this finding, higher rate of resistance was reported in different studies from India.[19,26] Less prevalence of HLGR enterococci in our set up could be due to geographical variations, differences in antibiotic prescribing policies and infection control practices. Since only few studies were conducted in our region on limited number of samples this may not reflect the entire scenario of Nepal. This highlights the need of further study to be conducted on larger sample size in other parts of our country.

Our study showed various resistance patterns of enterococcal isolates against different antibiotics that were tested. Resistance rate of enterococcal isolates to erythromycin was 51.6%, to ciprofloxacin was 50% and to ampicillin was 45%. Nitrofurantoin, one of the important effective drugs for urinary isolates of enterococci showed better result in vitro

(resistance rate of 6.7%) as compared with other antibiotics tested which is similar to the study conducted in India.[22]

After the first report of vancomycin resistant enterococci (VRE) in mid 1980s, these are being constantly reported from different parts of the world. Their prevalence has varied according to place and time.[1,2,5,7] To the best of our knowledge, there is no report of VRE in Nepal till date except one report of vancomycin resistant *Enterococcus faecium* from a case of peritonitis in a continuous ambulatory peritoneal dialysis patient in eastern part of Nepal.[27] This isolate had a MIC of vancomycin of 32 ug/ml. Investigators from western Nepal have reported VRE by disc diffusion method however they did not confirm their findings by MIC determination.[13] Amatya *et al*[12] from this same institute reported three isolates of VRE by disc diffusion methods in 2014 but none of them were found to have MIC in the resistant range by agar dilution method. However they reported 9.7% vancomycin intermediate enterococci (VIE) by agar dilution method. Our study showed none of the isolates was resistant to vancomycin by both disc diffusion and agar dilution method. But showed similar rate (8.3%) of VIE by agar dilution method. Similar rate of VIE was reported by Nepal *et al.*[11] previously in hospital from eastern Nepal. In this study all these VIE isolates were from inpatients samples and were resistant to ampicillin, ciprofloxacin, erythromycin and HLG. Resistance to multiple antibiotics and reports of intermediate susceptibility to vancomycin in our study indicates initiation of development of vancomycin resistance by enterococci in our set up.

To conclude, multidrug resistant *Enterococcus* spp. have already made their presence in our hospital set up. High rate of HLAR and reduced susceptibility to vancomycin especially from hospital isolates has raised question of their treatment efficacy and signifies the need of control measures to be implemented immediately to prevent the upcoming treatment challenges posed by VRE. Vigilant use of antimicrobials, strict infection control practices and continuous laboratory monitoring for HLAR and vancomycin resistance using proper technique can help to prevent these issues.

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