High-level gentamicin resistance and vancomycin resistance in clinical isolates of enterococci in a tertiary care hospital in eastern Nepal

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ABSTRACT

High-level gentamicin resistance and vancomycin resistance in enterococci, a family of important opportunistic pathogens, have emerged as a significant clinical problem over recent years. The present study was conducted to determine the high-level gentamicin and vancomycin resistance among the clinical isolates of enterococci. A total of 110 phenotypically identified enterococcal isolates were subjected to determination of high-level gentamicin resistance (by disk diffusion and agar dilution methods) and vancomycin resistance (by agar screening and agar dilution methods). About 36% of the isolates were found to have high-level gentamicin resistance, which indicates that gentamicin no longer remains an appropriate choice for inclusion in combination therapy with cell wall-active agents. Ten percent isolates exhibited resisance to vancomycin during screening. However, agar dilution confirmed that the isolates did not have resistance to vancomycin but had reduced susceptibility to it, which indicates their impending emergence of resistance to vancomycin.

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

INTRODUCTION

Enterococci, members of the normal flora in the gut of humans and animals, have emerged as important agent of nosocomial and community-acquired infections in the recent years.[1] They have been associated with various infections such as infection of the urinary tract, sepsis, endocarditis, intra-abdominal infection, wound infection and meningitis.[2]

A common regime for treatment of serious enterococcal infections such as sepsis is the combination of cell wall inhibitors such as penicillin, ampicillin or vancomycin with aminoglycosides such as streptomycin or gentamicin.[3] The combination is synergistic in action.[4] However, when an enterococcal strain is resistant to the cell-wall active agent or has high-level aminoglycoside resistance, there is no synergism and the combination therapy is likely to be unsuccessful.[5] Therefore, it is very important to detect resistance to the aminoglycosides as well as the cell-wall active agents in order to predict likelihood of their synergism. Thus, we aimed to determine the prevalence of high-level gentamicin and vancomycin resistance among the clinical isolates of enterococci in BP Koirala Institute of Health Sciences (BPKIHS), a tertiary care hospital in eastern Nepal.

MATERIALS AND METHODS

A total of 110 phenotypically characterized isolates of enterococci, recovered during a period of one year from January 2008 to December 2008 from various clinical specimens comprising of urine, blood, high vaginal swab, pus, sputum, wound swab, peritoneal fluid, ascitic fluid and cerebrospinal fluid obtained from patients attending BPKIHS were studied.

High-level gentamicin resistance screening: Screening for high-level gentamicin resistance was carried out by the disk diffusion method for all the 110 enterococcal isolates using 120 µg disk of gentamicin on Mueller Hinton blood agar medium as recommended by the Clinical Laboratory Standards Institute (CLSI) guidelines.[6,7] *Enterococcus faecalis* ATCC 29212 was used as control.

Agar screening for vancomycin resistance: Screening of vancomycin resistance was performed for all the isolates according to the guidelines of Clinical Laboratory Standards Institute (CLSI).[8] After preparation of colony suspension of 0.5 McFarland turbidity, approximately 5 µL of the suspension was spotted onto the BHI agar incorporated with 6 µg/mL of vancomycin. After incubation at 35^0C at ambient air for 24 hours, growth was observed. *Enterococcus faecalis* ATCC 29212 was used as control. The interpretation was made as: >1 colony: presumptive resistance.

Determination of minimum inhibitory concentration (MIC) of gentamicin and vancomycin: MIC of gentamicin and vancomycin for the isolates was determined using agar dilution technique. [8] Mueller Hinton blood agar was supplemented with different concentrations of gentamicin and vancomycin. After the test organism was grown in broth, the turbidity was matched with McFarland 0.5 standard (approximately 1.5×10^8 cfu/mL). Spot inoculation on the agar medium was done using 10 µL of bacterial culture. *Staphylococcus*

Table-1: Results of high-level gentamicin resistance screening by disk diffusion method

Species	Number of isolates tested	Susceptibility interpretation[6]		
		Resistant (≤6 mm)	Intermediate (7-9 mm)	Susceptible (≥10 mm)
E. avium	1	-	-	1
E. cecorum	2	-	-	2
E. dispar	1	-	-	1
E. faecalis	73	24	-	49
E. faecium	25	13	-	12
E. gallinarum	1	-	-	1
E. hirae	1	-	-	1
E. mundtii	1	-	-	1
E. saccharolyticus	5	3	-	2
Total	110	40	-	70

Abbreviation: (-), no isolates obtained

aureus ATCC 25923 and *Enterococcus faecalis* ATCC 29212 were used as control strains in each series of the test. The plates were incubated at 37°C for 24 hours and examined. The minimum concentration of the antibiotic which inhibited bacterial growth was considered MIC. The isolates which exhibited MIC of gentamicin ≥500 µg/mL were regarded as high-level gentamicin resistant (HLGR) enterococci and those which had MIC of vancomycin ≥32 µg/mL as vancomycin resistant enterococci (VRE).

RESULTS

Results of high-level gentamicin resistance screening by disk diffusion method: Of the isolates tested, 40 isolates (36.4%) had zone of inhibition ≤6 mm and were considered as HLGR enterococci (Table-1).

High-level gentamicin resistance among enterococcal isolates determined by agar dilution method

Forty of the 110 isolates (36.4%) exhibited MIC of gentamicin above the breakpoint of 500 µg/mL and were regarded as HLGR enterococci (Table-2). The results corresponded with the values obtained after screening by disk diffusion method.

Among the nine different species of *Enterococcus* in our study, only three species- *E. faecalis* (32.9%), *E. faecium* (52%) and *E. sachharolyticus* (60%) exhibited high-level gentamicin resistance.

Agar screening for vancomycin resistance: After screening for vancomycin resistance, 11 isolates (10%) demonstrated resistance to it (Table-3).

MIC of vancomycin: The following table (Table-4) depicts the relation of MIC with the susceptibility pattern of the enterococcal isolates. Ninety percent of the isolates had MIC level below the breakpoint of 4 mg/mL and were considered to be susceptible to vancomycin. Vancomycin resistant isolate (MIC level of e"32 mg/mL) was not observed in this study. However, 10% of the isolates exhibited intermediate susceptibility.

Among the 11 isolates which had MIC level 8-16 µg/mL (intermediate susceptibility), majority of them (8 isolates) belonged to *E. faecalis.* The other species were*: E. faecium* (1 isolate), *E. gallinarum* (1 isolate) and *E. mundtii* (1 isolate).

Vancomycin intermediate isolates with high-level gentamicin resistance: Among the 11 (10%) vancomycin intermediate isolates, four (36.4%) had high-level gentamicin resistance: three isolates of *E. faecalis* and single isolate of *E. faecium.*

DISCUSSION

High-level aminoglycoside resistant enterococci were first reported in France in 1979 and since then, they have been isolated from all the continents. [10] Studies have shown that they comprise significant fraction of the clinical enterococcal isolates.[11] Enterococci with high-level resistance to streptomycin and kanamycin have been relatively common, but high-level resistance to gentamicin has become a clinical problem only in the recent years.[2]

The prevalence of HLGR enterococci differed from one study to other. A study by Zervos and associates[11] documented a prevalence of 55 per cent of high-level gentamicin resistance in enterococci in an US centre. Another study[12] reported a prevalence rate varying from 1 to 49% in the 27 European countries studied.

Of the 110 isolates tested by both the disk diffusion and agar dilution technique in this study, 40 isolates (36.4%) demonstrated high-level resistance to gentamicin. The

Table-2: MIC of gentamicin and susceptibility pattern of isolates

Total no. of isolates tested	MIC(µg/mL)	Standard interpretation[6,9]	Number of isolates obtained (%)
110	≤4	Susceptible	35 (31.8)
	8	Intermediate	16 (14.5)
	≥16	Resistant	59 (53.7)
	≥500	High-level gentamicin resistant (HLGR)	40 (36.4)

Table-3: Result of agar screening for vancomycin resistance

Species	Number of isolates tested	Presumptively	
		Resistant	Susceptible
E. avium	1	-	1
E. cecorum	2	-	2
E. dispar	1	-	1
E. faecalis	73	8	65
E. faecium	25	1	24
E. gallinarum	1	1	-
E. hirae	1	-	1
E. mundtii	1	1	-
E. saccharolyticus	5	-	1
Total	110	11	99

rate of isolation of HLGR enterococci in our study is in accordance with the findings from India [13] (36.9%) and USA [14] (38%). The prevalence rate of HLGR enterococci in our study is much higher than the rate observed in Belgium[15] (10%) and Kuwait[16] (13.9%). The higher rate seen in our study may be ascribed to the source of the isolates being from a tertiary care set up where wider usage of broad spectrum antibiotics is common. Thus, high occurrence of high-level gentamicin resistance highlights that gentamicin remains a poor choice for inclusion in combination therapy with cell wall-active agents. Moreover, enterococci exhibiting high level gentamicin resistance are also resistant to virtually all of the clinically available aminoglycosides, except for streptomycin.[17] Therefore, there is need to develop more potent aminoglycosides that will be resistant to modification by a broad spectrum of aminoglycoside-modifying enzymes present in *Enterococci*.[17]

In the present study, the degree of high-level gentamicin resistance in *E. faecium* isolates (52%) was higher than that in *E. faecalis* (32.9%), similar to findings from other regions, including Maharashtra, India[18] which revealed the resistance among *E. faecium* isolates (95.5%) being significantly higher than *E. faecalis* (37.5%) and Iran[19] which reported the resistance in 79% of *E. faecium* and 61.5% of *E. faecalis* strains. However, predominance of the resistance in *E. faecalis* over *E. faecium* has been reported from Greece[20] and Japan.[21]

The emergence of VRE is a cause for concern because of the limited therapeutic options for treating serious infections and because of their potential to transfer vancomycin-resistance genes to other organisms, such as methicillin-resistant *Staphylococcus aureus*.[1] The rate of isolation of VRE differed from study to study: 1% by Mathur et al[22] from northern India, 2.6% by Udo et al from kuwait[16], 17.4% by Prakash et al[23] from Pondicherry, India. In some hospitals in United States, they have been associated with more than 20% of enterococcal infections.[24]

In the current study, vancomycin resistance was determined by agar screening and agar dilution methods. During screening, 11 isolates (10%) exhibited resistance. However, agar dilution confirmed that those isolates did not have resistance to vancomycin but had reduced susceptibility to it, indicating their impending emergence of resistance to vancomycin.

Of the 11 vancomycin intermediate isolates in the present study, 8 belonged to *E. faecalis*, 1 to *E. faecium* and 1 each to *E. gallinarum* and *E. mundtii*. Isolation rate of vancomycin intermediate isolates in our study is similar to observation of Azevedo et al [25] and Moaddab et al.[26] Azevedo et al [25] reported that among 455 isolates tested, no isolate with resistance to vancomycin was detected but 20 isolates (4.4%) were found to be vancomycin intermediate, including those belonging to species traditionally associated with intrinsic resistance to vancomycin (seven of *E. gallinarum* and two of *E. casseliflavus*) and the remaining belonging to *E. faecalis*. Moaddab et al [25] also noted that 11 of 198 strains (5.5%) were vancomycin intermediate and there was no vancomycin resistant strain in their study. One of the 11 strains in their study was *E. faecalis* and the remaining 10 strains were *E. faecium*. Though the rate of isolation of vancomycin intermediates isolates in our study is similar to that reported by these studies, the species distribution among the intermediate isolates remains differed.

Vancomycin resistance in enterococci has coincided with the increasing incidence of high-level enterococcal resistance to penicillin and aminoglycosides, thus presenting a challenge for physicians who treat patients who have infections caused by these microorganisms.[27,28] In a study conducted by Hayden et al [29], 53.8% VRE isolates exhibited high-level gentamicin resistance. Though we did not encounter any VRE isolates in the present study, 4/11 (36.4%) vancomycin intermediate isolates had high-level gentamicin resistance. Three isolates of *E. faecalis* and single isolate of *E. faecium* were found to have such existence.

Table-4: MIC of vancomycin and susceptibility pattern of the isolates

Total number of isolates tested	MIC (μg/mL)	Standard interpretation (CLSI)[6,8]	Number of isolates (%)
110	≤4	Susceptible	99 (90)
	8-16	Intermediate	11 (10)
	≥32	Resistant	0 (0)

Finally, it is emphasized that continuous surveillance, prudent use of antimicrobials and strict adherence to the infection control practices are crucial in the effective management of enterococcal infection as well as for the prevention of development and spread of resistance among the local isolates.

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