HIGH LEVEL GENTAMICIN RESISTANCE AND SUSCEPTIBILITY TO VANCOMYCIN IN ENTEROCOCCI IN A TERTIARY CARE HOSPITAL OF DHAKA CITY

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Abstract

Vancomycin and high level gentamicin resistant enterococci detection is important for effective treatment and control of nosocomial infection. The present study was undertaken to determine the species distribution of *Enterococcus* and the rate of vancomycin and high level gentamicin resistant enterococci (HLGRE) in clinical samples in a tertiary care hospital of Dhaka city. Enterococci were identified to species level by standard biochemical and serological methods. Their susceptibilities to antibiotics were determined by disc diffusion method according to CLSI guideline. Minimum inhibitory concentration (MIC) of vancomycin and gentamicin were determined by agar dilution method. The study was conducted from July 2009 to February 2010.

Among 80 isolates, 95% and 5% were identified as *Enterococcus faecalis* and *Enterococcus faecium* respectively. Out of 80 isolates 72 (90%) were sensitive and 8 (10%) were intermediate resistant to vancomycin (30μg) by disc diffusion method, but all isolates were susceptible by agar dilution MIC method. Out of 80 enterococci, 37 (46.25%) showed high level resistance to gentamicin (MIC: > 500 μg/ml) by MIC method but, initially six of which showed sensitive result to gentamicin by disc diffusion method using 120 μg disc.

The study indicated high prevalence of HLGRE in our hospital population. MIC method was more accurate in detecting high level gentamycin resistant enterococci compared to disc diffusion method with 120 μg gentamicin disc. However, none of the enterococcal strains showed resistance to vancomycin. HLGRE should be monitored regularly in clinical samples as it is difficult to treat.

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Introduction

Enterococcus is a leading cause of nosocomial infections and important for its ability to acquire antibiotic resistance determinant from other organisms. *Enterococcus* includes more than 17 species. *Enterococcus faecalis* (90-95%) and *Enterococcus faecium* (5-10%) are the two species commonly present in human intestine as commensal. Other species account for less than 5% of clinical isolates. Enterococci are estimated to cause 5-15% of all cases of bacterial endocarditis.[1] Recently, the treatment of enterococcal infections is increasingly become difficult due to emegernce of antibiotic resistant strains. *E. faecium* represents most vancomycin resistant enterococci, but vancomycin resistant strains of *E. faecalis* also occur. Vancomycin resistant enterococci (VRE) are now a common cause of hospital-acquired infection and are difficult to treat pathogen with currently available antibiotics.[2,3]

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Combination of a cell wall active antibiotic such as penicillin and an aminoglycoside such as gentamicin is essential for severe enterococcal infection. Although enterococci have intrinsic low-level resistance to aminoglycoside, they have synergistic susceptibility when treated with a cell wall acting antibiotic and an aminoglycoside. However, some aminoglycosides are not susceptible to synergism.[4,5] Emergence of high level resistance to gentamicin (MIC of >500 μg/ml) by some enterococci has caused the failure of synergistic effects of combination therapy.[6]

Along with the antibiotic pattern, rapid and accurate identification of enterococci in species level is important for appropriate drug therapy. Although studies on the rate of enterococcal infection, detection of VRE and HLGRE has been done in many countries, a few studies are done in Bangladesh. So, the present study was undertaken to determine the distribution of enterococcal species and rate of vancomycin and high level gentamicin resistant enterococci in clinical samples in a tertiary care hospital of Dhaka city.

Material and Method

Study place, samples and organisms

The study was carried out in the Department of Microbiology, Ibrahim Medical College and BIRDEM hospital, Dhaka. It was conducted from July 2009 to February 2010. All the enterococci, isolated during the period, from different clinical samples of patients attending BIRDEM hospital were included in the study for species identification and antibiotic sensitivity tests.

Microbiological methods

All samples collected during the above period were routinely cultured on blood agar media. All suspected colonies of enterococci were identified by Gram staining, cultural characteristics, motility, growth in bile esculin media and in media containing 6.5% NaCl, catalase, litmus milk reduction and L-arabinose hydrolysis tests using the standard microbiological techniques.[7] Specific antiser (Streptex, Ramel Europe Ltd. UK) was used to determine the serogroups.

Antibiotic susceptibility testing

Antibiotic susceptibility to different antibiotics was done by Kirby-Bauer disc diffusion method and as per the recommendations of the CLSI.[8,9] Antibiotic potency of the disks was standardized against the reference strain *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923. The control organisms were included in each batch of test. All disks were obtained from Oxoid Ltd., Basingstoke, Hampshire, UK except the gentamicin 120 μg disc which was obtained from Hi-Media Laboratories Pvt. Ltd, India. Minimal inhibitory concentration (MIC) of vancomycin and gentamicin was determined by agar dilution method.[10] Muller-Hinton agar plates were prepared with vacncomycin concentration ranging from 0.125 μg/ml to 128 μg/ml and for gentamicin 0.125 μg/ml to 4096 μg/ml. A fixed inoculums of bacteria standardized with 0.5 McFarland standards was prepared and inoculated on to the respective plates. The reading was taken after 24 hours of incubation. The highest dilution that inhibited the growth of the organism was taken as MIC of the test organism. Any *Enterococcus* showing a MIC of >500 μg/ml to gentamicin was considered as HLGRE. Any strain showing a MIC of ≥ 32 μg/ml to vancomycin was considered as VRE.

Results

A total of eighty enterococci were isolated during the study period. Of the 80 *Enterococcus* isolates, 71 were from urine, 8 from wounds/pus and 1 from tracheal aspirate. Among 80 isolates, 76 (95%) were *Enterococcus faecalis* and 4 (5%) were *E. faecium*. The detail antimicrobial susceptibility pattern of the 80 isolates to different antibiotics is shown in Table-1. Most of the isolates were sensitive to the tested antibiotics except ciprofloxacin and cotrimoxazole. About 82-95% enterococci was sensitive to penicillin and ampicillin. Out of 80 isolates, 72 (90%) were sensitive while 8 (10%) were intermediate resistant to vancomycin (30 μg) by disc diffusion method. But all the intermediate resistant isolates were found susceptible by agar dilution method. The MIC range of those 8 intermediate resistant enterococci was 2-4 μg/ml. Of the 80 isolates, 49 (61.25%) were sensitive while 31 (38.75%) were resistant to gentamicin by disc diffusion method. All 31 gentamicin resistant enterococci by disc diffusion method showed high level resistance (MIC $>$ 500 μg/ml) by agar dilution method (Table-2). But, out of 49 gentamicin sensitive isolates by disc diffusion method, six isolates showed high level resistance to gentamicin. Both MIC_{50} and MIC_{90} of

Table-1: *Antimicrobial susceptibility patterns of Enterococcus by disk diffusion method (n=80)*

Antimicrobial agents	Susceptibility pattern		
	Sensitive	Intermediate Resistant	Resistant
	n/%	n/%	n/%
Vancomycin (30µg)	72/90.0	8/10.0*	0
Gentamicin (120µg)	49/61.25	0	31/38.75
Amikacin (30µg)	46/57.5	0	34/42.5
Netilmicin (30µg)	58/72.5	0	22/27.5
Penicilling (10µg)	65/81.25	0	15/18.75
Ampicillin (10µg)	76/95.0	0	4/5.0
Ciprofloxacin (5µg)	23/28.75	0	57/71.25
Cotrimoxazole (30µg)	21/26.25	0	59/73.75

Note: *All the 8 intermediate resistant enterococci were sensitive to vancomycin by MIC method.

vancomycin were 2 μg/ml while for gentamicin it was 64 μg/ml and 4096 μg/ml respectively (Table 2).

Discussion

In our series, 88.7% enterococci were isolated from urine samples and the predominant enterococcal species identified was *E. faecalis* (95%). The reported isolation rate of *E. faecalis* from clinical samples ranged from 57% to as high as 90% in different places.[11-14] This high rate of *E. faecalis* infection could be due to its virulence or its presence in the hospital environment as most of our samples were from hospitalized patients. No vancomycin resistant enterococci or VRE was detected among our eighty isolates. However, 8 (10%) isolate showed intermediate resistance to vancomycin by disc diffusion method. But all of them were found sensitive by agar dilution MIC method (MIC range 2-4 μg/ml) which indicated that disc diffusion method sometimes could be misleading in detecting vancomycin resistance. Though no VRE was detected, about 46.25% (37/80) enterococci showed high level resistance (MIC > 500 μg/ml) to gentamicin. Similar isolation rate of HLGRE has been reported by others.[14,15] It was to be noted that about 12% (6/49) of the gentamicin sensitive isolates as detected by 120μg gentamicin disc diffusion test showed high level resistance (MIC: > 500 μg/ml) to gentamicin by MIC method. It therefore, indicated that disc diffusion test with 120μg gentamicin disc was not accurate and sensitive enough to detect HLGRE. Agar dilution MIC method was superior in identifying HLGRE.

Table-2: *Comparative susceptibility pattern of isolated enterococci to vancomycin and gentamicin by disc diffusion and agar dilution MIC method*

Antimicrobial agents	Disc Diffusion		MIC by agar dilution		MIC_{50} (µg/ml)	MIC_{90} (µg/ml)
	Zone diameter break-point (mm)	No of isolates	MIC break-point (µg/ml)	No of isolates		
Vancomycin (30 µg)	S (≥17) I (15-16) R (≤14)	72 8 0	S (≤4) I (8-16) R (≥32)	80 0 0	2	2
Gentamicin (120 µg)	S(≥10) Incon(7-9) R (≤6)	49 0 31	S (≤500) -- R (>500)	43 -- 37 (31+6)	64	4096

Note: S= Susceptible I = Intermediate resistant and R= Resistant, Incon= Inconclusive. MIC of gentamicin > 2000 µg/ml was considered high level resistance.

The results of the study indicated the absence of VRE and high prevalence of HLGRE in tertiary care hospital of Dhaka city. Enterococci have both an intrinsic and acquired resistance to antibiotics, making them important nosocomial pathogens. As VRE and HLGRE are difficult to treat, resistance should be monitored regularly in a wide range of clinical samples.

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