HIGH LEVEL RESISTANCE TO AMINOGLYCOSIDES IN ENTEROCOCCI FROM RIYADH

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Enterococci with high level aminoglycoside resistance are being reported from different parts of the world with increasing frequency. Treatment of infections caused by such isolates is associated with a high incidence of failure or relapse. This is attributed to the loss of the synergetic effect of aminoglycosides and cell wall active agents against isolates exhibiting this type of resistance. To determine the prevalence of enterococci with high level resistance to aminoglycosides in Riyadh, Saudi Arabia, 241 distinct clinical isolates were examined by disk diffusion method using high content aminoglycoside disks. Seventy-four isolates (30%) were resistant to one or more of the aminoglycosides tested. The most common pattern of resistance was that to streptomycin and kanamycin. Of the 241 isolates tested, 29 (12%) were resistant to high levels of gentamicin, 35 (15%) to tobramycin, 65 (27%) to kanamycin and 53 (22%) to streptomycin. The highest rate of resistance to a high level of gentamicin was found among enterococcal blood isolates (30%). Eighteen of the isolates were identified as *Enterococcus faecium*, 13 (72%) of these showed high level resistance to two or more of the aminoglycosides tested. *Ann Saudi Med 1944;14(4):290-293.*

Enterococci are an important cause of mortality and morbidity around the world. The mortality of the septicemic illness ranges between 19.6 and 71.4%.[1] These organisms are also emerging as a significant cause of nosocomial infections;[2] currently they are the second leading cause of nosocomial infections in hospitals located in the United States.[3]

Management of enterococcal infections is complicated not only by natural resistance of the organism to a large number of antimicrobial agents, but also by the remarkable ability to acquire new resistance determinants.[4] These organisms are naturally resistant to low levels of aminoglycosides. The average minimum inhibitory concentration (MIC) for gentamicin and tobramycin is 8 to 64 μg/mL and that for streptomycin is around 250 μg/mL.[4] In spite of this, the combination of an aminoglycoside with a cell wall active agent like a beta-lactam or vancomycin exhibits a synergistic killing effect against most enterococcal strains. This property has been traditionally utilized for management of serious enterococcal infections. Among the resistance determinants that enterococci can acquire is the resistance to high level (MIC>2000 μg/mL) of aminoglycosides (HLA). This type of resistance eliminates the cell wall active agents' aminoglycoside synergy.[5-7] In patients infected with such isolates, the use of aminoglycosides (HLA). This type of resistance of an aminoglycoside to which high level resistance is exhibited is of no therapeutic value. The first aminoglycoside used with penicillin for the synergistic effect against enterococci was streptomycin.[8] Later, in the early 1970s, resistance to high level streptomycin (associated with resistance to synergism) was widely recognized.[5,6] This led to replacement of streptomycin by gentamicin for the synergistic effect.[9] In 1979, Horodniceanu[10] described resistance to a high level of gentamicin in three enterococcal isolates; since then, similar resistant strains have been reported from other parts of the world.[7,11-13] Due to therapeutic implication, screening for resistance to HLA is currently recommended in all serious enterococcal infections.[2,7,14]

Data on the frequency of enterococci with HLA resistance outside Europe and North America is limited. We conducted this study to determine the frequency of such resistance among enterococcal isolates from two major teaching hospitals in Riyadh, Saudi Arabia.

Material and Methods

The organisms used in this study were isolated from clinical specimens submitted between January and July 1992 to the following two hospitals - King Faisal Specialist Hospital and Research Centre (KFSH&RC) and King

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TABLE 1. *Pattern of resistance to HLA.*

Species	No. of strains tested	Strains with high level resistance to: GM, TM KM, SM	GM, TM & KM	SM & KM	SM	KM	KM & TM	SM, KM & TM	Total (%)
E. Faecalis	223	10	13	24	9	2	3	-	61(27)
E. Faecium	18	4	2	4	-	-	1	2	13(72)
Total(%)	241	14(6)	15(6)	28(12)	9(4)	2(.1)	4(.2)	2(.1)	74(30)

HLA=human lymphocyte antigen; GM=gentamicin; TM=tobramycin; KM=kanamycin; SM=streptomycin.

Khalid University Hospital (KKUH), Riyadh, Saudi Arabia. Both are teaching hospitals providing primary and tertiary care with a bed capacity of 550 and 630 respectively. Only one isolate per patient was included in the study. Isolate identification and aminoglycoside susceptibility were conducted at the microbiology laboratory of the KFSH&RC, which serves as a reference facility for Saudi Arabia and the Middle East.

All organisms were serologically grouped using the streptex kit (Wellcome Diagnostics, Dartford, England) and presumptively identified as enterococci by the ability to hydrolyze esculine in the presence of bile and to grow in 6.5% NaCl.[15] To differentiate species of the isolates, the API 20S system (Analytab Products Inc., Plain View, New York, USA) was used.

Gentamicin was obtained from Schering Corp., Bloomfield, New Jersey, USA, tobramycin from Eli Lilly & Co., Indianapolis, Indiana, USA, streptomycin from Specia, Paris, France and kanamycin from Bristol Laboratories, Syracuse, New York, USA. The susceptibility to a high level of aminoglycosides was tested using high content aminoglycoside disks. This method was found superior to others in detecting high level resistance to aminoglycosides in enterococci.[16-19] The disks were prepared in-house by applying appropriate aminoglycoside stock solution to 6 mm sterile blank disks (BBL Microbiology Systems, Cockeysville, Maryland, USA) and allowing them to air dry. Final disk concentrations were 300 µg of streptomycin (SM), 120 µg of gentamicin (GM), 120 µg of tobramycin (TM) or 120 µg of kanamycin (KM) per disk.

The susceptibility testing was performed on Mueller-Hinton agar (Oxoid, Unipath LTD., Hampshire, England) supplemented with 5% sheep blood (BBL Microbiology Systems, Cockeysville, Maryland, USA). The method used was disk-agar diffusion as recommended by the National Committee for Clinical Laboratory Standards.[20] Test plates were incubated at 35°C for 20 hours and examined for zone size. Isolates showing zone size <9 mm around the aminoglycoside disks were considered resistant, while isolates with zone size >10 mm were considered susceptible to a high level of the aminoglycoside tested.[16] All isolates showing a zone size between 8 and 13 around any of the aminoglycoside disks were also tested by agar screening method[21] using an inoculum-replicating device.

Quality control was performed using *Enterococcus faecalis* ATCC#29212 (susceptible strain) and *E. faecalis* ATCC#49383 (resistant to a high level of the aminoglycosides tested). The results were found to be within expected range throughout the period of the study.

Results

A total of 241 distinct patient isolates of group D enterococci recovered from 152 (63%) females and 89 (37%) males were studied. Most of these isolates were obtained from inpatients (67%).

Enterococcus faecalis was the predominant species (92%) with *Enterococcus faecium* a distant second (7.5%). There was only one *Enterococcus avium* identified. Seventy-four isolates were found to be resistant to one or more aminoglycosides tested. The number of isolates resistant to HLA and the pattern of resistance is shown in Table 1. Out of the 18 *E. faecium* isolates identified, 13 (72%) exhibited a pattern of resistance to HLA compared to only 61 (27%) of the 223 *E. faecalis* isolates. Overall, the most common pattern of enterococcal resistance to HLA was that to streptomycin and kanamycin. This was detected in 28 isolates. Resistance to all aminoglycosides tested or to all aminoglycosides except streptomycin was exhibited by 14 and 15 isolates respectively. Resistance to streptomycin, kanamycin and tobramycin or to kanamycin alone were the least common patterns identified, detected in two isolates each.

Enterococci with HLA resistance according to the site of isolation and the overall resistance to each of the aminoglycosides tested are shown in Table 2. Of the 241

TABLE 2. *Resistance to HLA among isolates from various sites.*

Source of isolates	No. of strains tested	No. of strains with high level resistance to: GM	TM	KM	SM
Blood	10	3	4	4	3
Vascular catheters	17	4	4	5	1
Wounds	33	4	4	9	9
Urine	128	12	15	28	23
Stool	13	2	4	6	4
Miscellaneous	40	4	4	13	13
Total (%)	241	29(12)	35(15)	65(27)	53(22)

HLA=human lymphocyte antigen; GM=gentamicin; TM=tobramycin; KM=kanamycin; SM=streptomycin.

isolates tested, 29 (12%) were resistant to high levels of gentamicin, 35 (15%) to tobramycin, 65 (27%) to kanamycin and 53 (22%) to streptomycin. Blood isolates showed the highest rate of resistance to gentamicin (30%) and tobramycin (40%). Of the three blood isolates resistant to high-level gentamicin, one was susceptible to streptomycin. Resistance to HLA was more common among isolates obtained from inpatients (33%) than outpatients (21%).

The results of the agar screening and the disk diffusion methods correlated well except for two *E. faecium* isolates showing zone size of 10 and 11 mm around the tobramycin disks and growing on the agar containing 2000 µg of tobramycin. The first was found to be resistant to a high level of tobramycin while the second was susceptible using the macrotube dilution method.

Discussion

Serious enterococcal infections caused by strains resistant to HLA pose a significant therapeutic challenge. In spite of limited experience, there are good indications that treatment of these infections with an aminoglycoside and a beta-lactam is associated with a high incidence of failure or relapse. Moellering[3] summarized eight case reports of endocarditis caused by enterococci resistant to a high level of gentamicin. One of the isolates was susceptible to streptomycin and the patient was successfully treated with a combination of streptomycin, penicillin and vancomycin. The diagnosis of another patient was made at postmortem and no antibiotics were used. Out of the remaining six cases, four (67%) either relapsed or had a primary failure with initial management using antibiotics alone. These poor results compare unfavorably with a relapse rate of only 12% or less in previous studies of penicillin-aminoglycoside therapy of enterococcal endocarditis.[22-24] Although the number of cases in that series was small, the findings clearly illustrate the clinical implications of resistance to HLA and the need for routine screening.

Our finding of 12% prevalence of enterococci resistant to high-level gentamicin in Riyadh is comparable to that reported from other parts of the world: 14% in Thailand,[12] 15% in Chile[12] and 13% in one center in the United Kingdom.[25] The prevalence of such isolates in the United States is variable and ranges between 4.5% and 55% at different centers.[7,26-28] Ann Arbor, Michigan, USA and Philadelphia, Pennsylvania, USA, with prevalence rates of 13% and 15.1% respectively,[26,27] are similar to the findings in Riyadh.

Of the 29 isolates identified with high-level resistance to gentamicin, 23 were recovered from clinically significant sites including three from blood and 20 from vascular catheters, urine and wounds. These three sites are known to be among the most common sources of enterococcal bacteremia with the latter two being the source in 77% of the cases.[29] Blood isolates in this study showed the highest rate of resistance to high-level gentamicin compared to isolates from other sites. Three of the 10 blood isolates exhibited such resistance compared to only 29 of the total 241 isolates tested. Although the number of blood isolates in this study is small, this observation is consistent with previous reports from Thailand and the United Kingdom.[12,30] The higher rate of resistance among isolates causing septicemia is alarming, since it is during such an infection that the use of aminoglycosides is needed most.

Among the 29 isolates resistant to high levels of gentamicin, tobramycin and kanamycin, 15 (52%) were susceptible to a high level of streptomycin. The high rate of this resistance profile is similar to that of 46% reported from Chicago, Illinois, USA,[31] but is much higher than the 0% to 33% prevalence reported from other centers in the United States and elsewhere.[4,13,26,32] This high prevalence of gentamicin-resistant, streptomycin-susceptible isolates may, in part, be due to the limited use of streptomycin in this country. In treating patients with serious enterococcal infections caused by isolates resistant to high-level gentamicin, the use of gentamicin has no therapeutic benefit. Streptomycin may be used instead for the synergistic effect if the isolate is susceptible.

We found a high prevalence of *E. faecium* resistant to HLA. Among the 18 isolates identified, 13 (72%) were highly resistant to two or more of the aminoglycosides tested. Resistance to high-level gentamicin and streptomycin was present in 33% and 55% of the isolates respectively. The 33% prevalence of highly gentamicin-resistant isolates in this study is in sharp contrast to the lower prevalence of 3.9% to 6% recently reported by other investigators.[33,34] It causes concern to see this high prevalence among *E. faecium* isolates less than five years since such resistance was first described in this species.[35] To our knowledge, the prevalence of highly gentamicin resistant *E. faecium* isolates in this study is the highest reported. Treatment of patients with serious *E. faecium* infections is further complicated by the inherent resistance of the organism to the synergistic effect of cell wall active agents combined with either tobramycin or kanamycin, regardless of the sensitivity results.[36] This fact negates their use in such infections.

Enterococcal resistance to HLA, vancomycin[37] and beta-lactams[32,34] has been described with increasing frequency. Management of serious infections caused by strains resistant to HLA is becoming more difficult and is almost beyond the boundaries of medical management alone if associated with resistance to beta-lactams and vancomycin. The findings in this study again stress the importance of routine screening for resistance to HLA in serious enterococcal infections and the need for further

research to develop and test new antibiotics and antibiotic combinations against these organisms.

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