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Resistance to Six Aminoglycosidic Aminocyclitol Antibiotics Among Enterococci: Prevalence, Evolution, and Relationship to Synergism with Penicillin

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Two hundred and three recent clinical isolates of enterococci were tested for susceptibility to streptomycin, kanamycin, amikacin, gentamicin, sisomicin, and tobramycin. Depending upon the source of the isolate, 36 to 54% of the enterococci demonstrated high-level resistance (minimal inhibitory concentration, >2,000 μg/ml) to streptomycin, 16 to 49% to kanamycin, and 0 to 14% to amikacin. None of the strains was highly resistant to gentamicin, sisomicin, or tobramycin. A comparison with isolates of enterococci obtained in 1968 revealed that there has been a decrease in prevalence of high-level resistance among organisms isolated from wound cultures in 1976. However, no decrease in resistance to streptomycin or kanamycin was demonstrated among blood or urine isolates. Penicillin, combined with gentamicin, sisomicin, or tobramycin, was synergistic against all 10 strains of *Streptococcus faecalis* subjected to formal testing. For streptomycin and kanamycin, the presence or absence of synergism with penicillin correlated with the absence or presence of high-level aminoglycoside resistance. High-level resistance to amikacin was seen in only 1 of the 10 strains. Nonetheless, combinations of penicillin plus amikacin failed to produce synergistic killing against 6 of the 10 strains. Indeed, the combination was synergistic only against those four strains that were susceptible to high levels of kanamycin.

Enterococci are unique among streptococci because of their relative resistance to penicillin (11). As a result, penicillin alone is not always effective in the therapy of enterococcal endocarditis and other serious enterococcal infections. In 1947, Hunter demonstrated that combinations of penicillin plus streptomycin produced a synergistic bactericidal effect against enterococci in vitro and were effective in treating enterococcal endocarditis (2). However, Jawetz and Sonne (4), Tompsett and Pizette (12), and others subsequently discovered that combinations of penicillin plus streptomycin were not synergistic against all strains of enterococci. Studies in our laboratory (5) and investigations by Standiford et al. in Seattle, Washington (9), have shown that enterococcal strains that are not synergistically killed by penicillin plus streptomycin or penicillin plus kanamycin exhibit high-level resistance to streptomycin and kanamycin, respectively. Moreover, the proportion of strains exhibiting high-level resistance to streptomycin and kanamycin in these studies performed in diverse geographical locations as well as in subsequent studies by Ruhen and Darrell in London (8) and by Iannini et al. in Denver (3) has been remarkably uniform, approximating 25 to 40% for streptomycin and 11 to 27% for kanamycin. In no studies were strains highly resistant to gentamicin found. In the 8 years since our initial study was performed, the use of kanamycin and especially streptomycin has declined in our hospital, as those agents have been progressively replaced by gentamicin. In view of this, we repeated our earlier studies with a large collection of recently isolated enterococci to determine if the change in patterns of clinical utilization of aminoglycosidic aminocyclitol antibiotics has been associated with alteration in the pattern of high-level resistance to these agents. In addition, this study provided the opportunity to investigate the prevalence of high-level resistance to several of the newer aminoglycosidic

aminocyclitol antibiotics and to examine their interaction with penicillin against recent clinical isolates of enterococci.

MATERIALS AND METHODS

Included in this study were 203 strains of enterococci isolated from patients at the Massachusetts General Hospital. These included 80 strains isolated from urine specimens and 57 strains isolated from wound cultures in July and August 1976. In addition, 41 strains isolated from the blood of patients with septicemia during the period 1973 to 1976 were included as was a separate group of isolates from 25 patients with documented enterococcal endocarditis between 1969 and 1976. All of these isolates were unique; duplicates from the same patient were excluded.

All strains were identified as enterococci on the basis of colonial morphology and Gram stain; all were capable of growth in 40% bile, were able to hydrolyze esculin, and could grow in 6.5% salt; and all were serologically grouped by the method of Watson et al. (13). Those strains selected for testing in synergism experiments were identified as to species with physiological tests suggested by Facklam (1). All were *Streptococcus faecalis*.

Susceptibility and resistance to high concentrations (2,000 μg/ml) of the various aminoglycosidic aminocyclitols in agar-containing medium were determined by methods described previously (5). Minimal inhibitory concentrations of the aminoglycosidic aminocyclitols were determined by the agar dilution technique with a Steers replicator (10). The inoculum consisted of an undiluted overnight culture of each organism grown in dextrose-phosphate broth (Pfizer-Albimi). Dextrose-phosphate broth with 0.8% agar was utilized as the growth medium for agar dilution studies. Appropriate control organisms were included with each set of determinations.

Tests of antibiotic synergism were performed in dextrose-phosphate broth by determining time-kill curves for organisms exposed to various agents alone and in combination. These methods have been described in detail in an earlier publication (6). A starting concentration of 10^7 colony-forming units/ml was used. Synergism was defined as an increase in killing of 100-fold or more by the combination of antibiotics after 24 h of incubation as compared with the most effective agent alone. In all instances, the concentration of the aminoglycosidic aminocyclitol was chosen to be less than the minimal inhibitory concentration. Concentrations of antibiotics used in the synergism studies were as follows: penicillin, 10 U/ml; streptomycin, kanamycin, amikacin, 20 μg/ml; gentamicin, tobramycin, sisomicin, 5 μg/ml.

Penicillin, streptomycin, and kanamycin were commercially obtained. Gentamicin and sisomicin standard powders were gifts from the Schering Corp.; amikacin standard was furnished by Bristol Laboratories, and tobramycin standard was provided by Eli Lilly & Co.

RESULTS

Figure 1 details the susceptibility of all 203 strains of enterococci isolated from blood, urine, and wound cultures. Gentamicin, sisomicin, and tobramycin were clearly more active on a weight basis than amikacin, kanamycin, or streptomycin. Indeed, no strains demonstrated high-level resistance (minimal inhibitory concentration >2,000 μg/ml) to gentamicin, sisomicin, or tobramycin. However, 45% of the strains demonstrated high-level resistance to streptomycin, 38% to kanamycin, and 7% to amikacin.

Data concerning the prevalence of high-level resistance by anatomical site of origin are given in Table 1. There was little difference in the

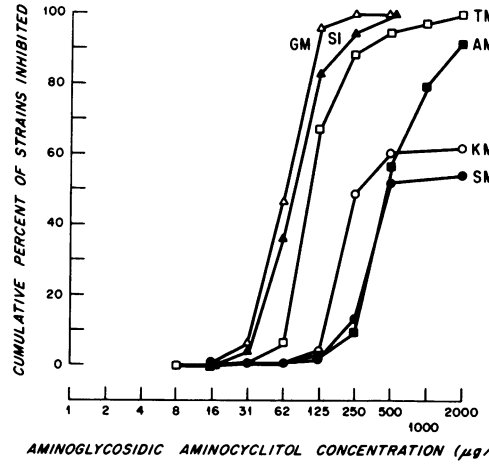

FIG. 1. *Susceptibility of 203 strains of enterococci to six aminoglycosidic aminocyclitol antibiotics. Abbreviations: AM, amikacin; GM, gentamicin; KM, kanamycin; SI, sisomicin; SM, streptomycin; TM, tobramycin.*

TABLE 1. *Prevalence of high-level resistance to various aminoglycosidic aminocyclitol antibiotics among clinical isolates of enterococci*

Source of isolate	No. of strains	% Resistant to ≥ 2,000 μg/ml of:					
		Streptomycin	Kanamycin	Amikacin	Gentamicin	Tobramycin	Sisomicin
Blood (septicemia)	41	53.7	48.8	4.9	0	0	0
Blood (endocarditis)	25	36.0	16.0	0	0	0	0
Blood (septicemia and endocarditis)	66	47.0	36.4	3.0	0	0	0
Urine	80	41.3	37.5	5.0	0	0	0
Wound	57	49.1	42.1	14.0	0	0	0

susceptibility to aminoglycosides of organisms isolated from different sites, although the isolates from patients with endocarditis demonstrated slightly less overall high-level resistance to streptomycin and kanamycin.

In Table 2 the prevalence of high-level aminoglycoside resistance among current isolates of enterococci is compared with data obtained from similar clinical isolates tested 8 years earlier. There was no decrease in the prevalence of high-level aminoglycoside resistance among blood and urine isolates. Indeed, there was a slight increase in resistance, especially to kanamycin, among these strains. The pattern was different, however, for the wound isolates, where a decreased prevalence of high-level resistance to both streptomycin and kanamycin was seen in 1976, as compared with 1968.

Ten strains of enterococci (all *S. faecalis*) were selected for more detailed testing. These were not randomly selected, but an attempt was made to include a large proportion of organisms with high-level resistance. Time-kill curves were performed for all strains with combinations of penicillin with streptomycin, kanamycin, amikacin, gentamicin, sisomicin, or tobramycin. There was an excellent correlation between the presence or absence of high-level resistance to a given aminoglycoside and absence or presence of synergism for each of the aminoglycosides, except amikacin, when combined with penicillin (Table 3). There were no strains with high-level resistance to gentamicin, sisomicin, or tobramycin, and all 10 strains were synergistically killed by combinations of penicillin with each of these three agents. For one strain, studies using an initial concentration of 10^7 colony-forming units/ml resulted in enhanced killing of only 1 to 2 logs with both penicillin-gentamicin and penicillin-tobramycin combinations. However, repeat experiments, using an inoculum of 10^5 colony-forming units/ml, demonstrated clear-cut synergism in both instances.

Strains with high-level resistance to streptomycin or kanamycin were not killed synergistically when these antibiotics were combined with penicillin. However, combinations of penicillin plus streptomycin or penicillin plus kanamycin demonstrated synergism against all strains inhibited by 2,000 μg of streptomycin or kanamycin per ml, respectively. For amikacin, the relationship between the absence of high-level resistance and the presence of synergism

TABLE 2. *Prevalence of high-level resistance among clinical isolates of enterococci to streptomycin and kanamycin—1968 versus 1976*

Source of isolate (year)	No. of strains	% Resistant to 2,000 μg/ml of:	
		Streptomycin	Kanamycin
Blood (1968)	27	40.7	18.5
Blood (1973–1976)	41	53.7	48.8
Urine (1968)	103	39.8	27.2
Urine (1976)	80	41.3	37.5
Wound (1968)	26	80.8	76.9
Wound (1976)	57	49.1	42.1

TABLE 3. *Penicillin aminoglycoside synergism: relationship to high-level resistance to various aminoglycosides*

Susceptibility to aminoglycosides	No. of strains	Increase in killing by antibiotic combination (log_{10}):		
		>2 (synergism)	1–2	<1 (no synergism)
Resistant to high levels (2,000 μg/ml) of:				
Streptomycin	7	0	0	7
Kanamycin	6	0	0	6
Amikacin	1	0	0	1
Gentamicin	0	–[a]	–	–
Tobramycin	0	–	–	–
Sisomicin	0	–	–	–
Susceptible to high levels (2,000 μg/ml) of:				
Streptomycin	3	3	0	0
Kanamycin	4	4	0	0
Amikacin	9	4	0	5
Gentamicin	10	9	1[b]	0
Tobramycin	10	9	1[b]	0
Sisomicin	10	10	0	0

[a] –, Not determined.

[b] Use of lower inoculum (10^5 colony-forming units/ml) resulted in demonstrable synergism ($\geq 10^3$ increase in killing).

did not hold true. Synergism with penicillin plus amikacin could not be demonstrated against six of the strains, despite the fact that only one was highly amikacin resistant. However, all six strains with high-level kanamycin resistance were resistant to synergism not only with penicillin and kanamycin, but also with penicillin and amikacin. Conversely, the four strains sensitive to high levels of kanamycin were killed synergistically by combinations of both penicillin plus kanamycin and penicillin plus amikacin. Figures 2 and 3 provide representative time-kill curves for two strains of enterococci.

DISCUSSION

In general, the prevalence of bacterial strains resistant to a given antimicrobial agent is related to the selective pressure resulting from the clinical or other use of the antibiotic. We have previously demonstrated that high-level resistance to streptomycin and kanamycin (with concomitant lack of synergism when exposed to penicillin plus streptomycin or kanamycin) was found among a significant number of enterococci isolated from clinical specimens in 1968 at the Massachusetts General Hospital (5). Similar observations have been made in Seattle, Washington (9), Denver, Colorado (3), and London, England (8). Since our original study in 1968, the use of both streptomycin and kanamycin has declined at the Massachusetts General Hospital. Despite this fact, there has been no major decline in the prevalence of blood and urine isolates of enterococci that are highly resistant to streptomycin and kanamycin, although there was a significant decrease in resistance to both agents among wound isolates. Despite extensive use of gentamicin during this period, there were no isolates of enterococci that had developed high-level resistance to that agent. The observation that 7% of the 203 strains of enterococci studied here had high-level resistance to amikacin is of interest since

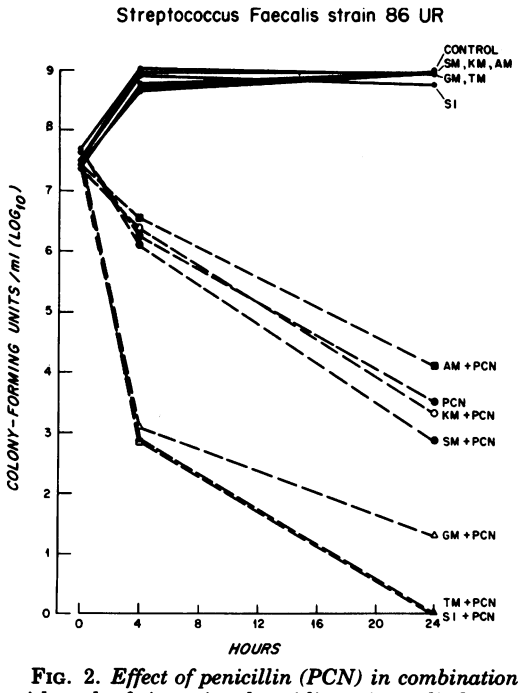

FIG. 2. *Effect of penicillin (PCN) in combination with each of six aminoglycosidic aminocyclitol antibiotics against S. faecalis strain 86 UR, which is highly resistant to kanamycin and streptomycin but not to amikacin, gentamicin, sisomicin, or tobramycin. Minimal inhibitory concentrations: amikacin (AM), 500 μg/ml; gentamicin (GM), 62 μg/ml; kanamycin (KM), >2,000 μg/ml; sisomicin (SI), 62 μg/ml; streptomycin (SM), >2,000 μg/ml; tobramycin (TM), 125 μg/ml. See text for concentrations of antibiotics used in this experiment.*

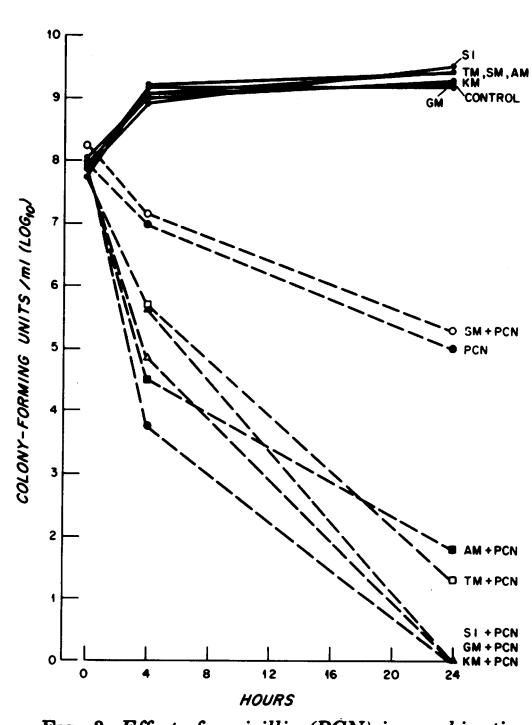

FIG. 3. *Effect of penicillin (PCN) in combination with each of six aminoglycosidic aminocyclitol antibiotics against S. faecalis strain 6 END, which is highly resistant to streptomycin but not to amikacin, kanamycin, gentamicin, sisomicin, or tobramycin. Minimal inhibitory concentrations: amikacin (AM), 1,000 μg/ml; gentamicin (GM), 62 μg/ml; kanamycin (KM), 250 μg/ml; sisomicin (SI), 125 μg/ml; streptomycin (SM), >2,000 μg/ml; tobramycin (TM), 125 μg/ml. See text for concentrations of antibiotics used in this experiment.*

this drug had had only minimal usage (in 40 patients) at the Massachusetts General Hospital prior to this survey.

In none of the previous studies of high-level resistance to aminoglycosides among blood isolates of enterococci were strains from patients with bacterial endocarditis specifically differentiated from those representing bacteremia without endocarditis. Hence we examined a subgroup of isolates of enterococci from 25 patients with documented endocarditis occurring between 1969 and 1976. Although the prevalence of high-level resistance to streptomycin and kanamycin was slightly lower among these strains as compared with those having simple bacteremia, it was still great enough to be of clinical significance.

This study also provided the opportunity to look for high-level resistance among enterococci to certain of the newer aminoglycosidic aminocyclitol antibiotics, including tobramycin, sisomicin, and amikacin. Such studies have not been previously reported. We found no example of high-level resistance to gentamicin, tobramycin, or sisomicin among the 203 isolates screened, and all isolates tested demonstrated synergism when exposed in vitro to penicillin plus sisomicin, gentamicin, or tobramycin. The latter findings are consistent with previous studies from our laboratory concerning penicillin-tobramycin synergism against enterococci (7). However, it should be noted that combinations of clinically achievable concentrations of tobramycin did not produce synergism against any of four strains of *Streptococcus faecium*, even though the latter were not highly resistant to tobramycin (7). Penicillin-gentamicin combinations were synergistic against all strains of both *S. faecalis* and *S. faecium* (7).

The results with amikacin are of considerable interest and may have important clinical implications. Previous studies (3) have demonstrated that combinations of penicillins (i.e., ampicillin) plus amikacin can be synergistic against enterococci. Our findings suggest that this is not always the case, however. Though it is true that the strains of enterococci we used for the in vitro tests of synergism were weighted in favor of resistant organisms, it is nonetheless obvious that there are a number of strains of enterococci against which the combination of penicillin plus amikacin fails to produce synergism. Unfortunately, this cannot be predicted from tests of high-level resistance to amikacin, as can be done for streptomycin and kanamycin. However, our data strongly suggest that the results of testing for high-level kanamycin resistance also may be applied to amikacin. None of the six organisms resistant to greater than 2,000 μg of kanamycin per ml was killed synergistically by combinations of either penicillin plus kanamycin or penicillin plus amikacin. In addition, all four strains susceptible to 2,000 μg of kanamycin per ml demonstrated synergism with penicillin and amikacin. The mechanism for the apparent cross-resistance between amikacin and kanamycin is currently under active investigation in our laboratory.

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