

Penicillin-Tobramycin Synergism Against Enterococci: a Comparison with Penicillin and Gentamicin

ROBERT C. MOELLERING, JR., CHRISTINE WENNERSTEN, AND ALLAN J. WEINSTEIN
Infectious Disease Unit, Massachusetts General Hospital, and Department of Medicine, Harvard Medical School, Boston, Massachusetts 02114

Received for publication 22 December 1972

Combinations of penicillin plus tobramycin have been compared with penicillin plus gentamicin against 27 strains of enterococci isolated from blood cultures. Penicillin plus gentamicin was synergistic against all strains. The combination of penicillin plus tobramycin was equally effective against strains of *Streptococcus faecalis*, but was ineffective against all four strains of *S. faecium* tested.

Tobramycin (nebramycin factor 6) is a new aminoglycosidic antibiotic which has a broad spectrum of activity against *Staphylococcus aureus* and many aerobic gram-negative organisms (2). Preliminary studies have shown it to be ineffective when used alone against enterococci (11). This is not surprising because none of the currently available aminoglycosidic antibiotics has significant activity by itself against these organisms. The use of penicillin (or other agents which inhibit bacterial cell wall synthesis) in combination with one of the aminoglycosidic antibiotics often results in a marked synergistic killing effect against enterococci (8). Among the commonly used aminoglycosides there is a spectrum of effectiveness when they are used in combination with penicillin against enterococci. Thus, although the combination of penicillin plus gentamicin has been synergistic against all strains of enterococci tested so far (9, 13), a small, but significant, number of enterococci are resistant to penicillin plus kanamycin (7, 12), and even more are resistant to the synergistic effect of penicillin plus streptomycin (7, 12). Despite this, the latter combination has, until recently, been recommended as the optimal therapy for severe enterococcal infections (6). The following studies were undertaken to determine the effectiveness of tobramycin when used in combination with penicillin against enterococci and to determine its location in the spectrum of activity of aminoglycosides against these organisms.

Twenty-seven strains of enterococci isolated

from blood cultures at the Massachusetts General Hospital were employed in these studies. These organisms were identified by the usual criteria and grouped by the method of Rantz and Randall (10). In addition, all strains were speciated on the basis of standard biochemical reactions (3).

Antibiotics were furnished as follows: gentamicin, Schering Corp.; penicillin, Pfizer Laboratories; and tobramycin, Eli Lilly & Co.

Minimal inhibitory concentrations (MIC) and minimal bactericidal concentrations (MBC) of gentamicin and tobramycin were determined by standard tube dilution methods (1).

Tests of antibiotic synergism were performed by using methods described in a previous report (8). Briefly, organisms to be tested were grown overnight in dextrose-phosphate broth. Appropriate dilutions of these organisms were added to fresh dextrose-phosphate broth to produce an initial concentration of 10^8 or 10^7 organisms per ml in 20 ml of broth. Antibiotics were then added, and the cultures were incubated without agitation at 37 C. Samples (0.5 ml) were removed at 0, 4, and 24 h for the determination of colony counts. Antibiotic synergism was defined as a decrease of 100-fold or more in number of organisms after 24 h of incubation caused by the combination as compared with the most effective of the antibiotics used alone (penicillin in all cases). The concentrations of gentamicin and tobramycin were less than their respective MIC in all experiments.

In Fig. 1 are shown the frequency distributions of MIC and MBC values for gentamicin and tobramycin against 27 strains of enterococci isolated from blood cultures. In general, gentamicin was slightly more effective against all strains than was tobramycin when used alone. Nonetheless, all of the MIC and MBC for both drugs fell outside of the clinically achievable range.

Table 1 demonstrates the effectiveness of penicillin when combined with gentamicin and

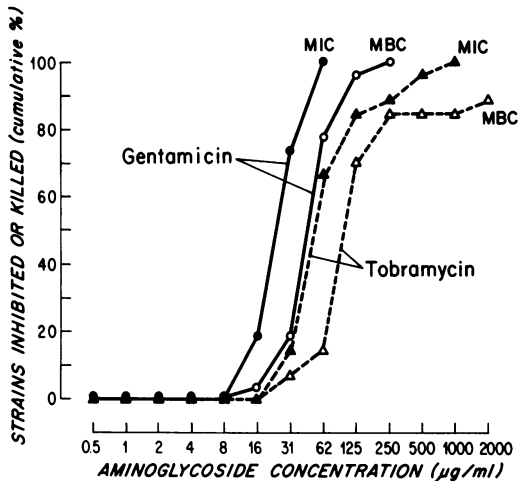


FIG. 1. Frequency distribution of MIC and MBC values for gentamicin and tobramycin against 27 strains of enterococci from blood cultures.

tobramycin against the same 27 strains of enterococci. A starting inoculum of 10^7 organisms per ml and clinically achievable levels of penicillin, gentamicin, and tobramycin were employed in all of these studies. Penicillin plus tobramycin was synergistic against all 23 strains of *Streptococcus faecalis*. Penicillin plus gentamicin produced a synergistic killing of 22 of the 23 strains tested. In the remaining strain, the increase in killing by the combination was just less than 2 log units. When the study was repeated for the one "nonsynergistic" strain by using a starting inoculum of 10^6 organisms per ml, it also was killed synergistically by the combination of penicillin and gentamicin. We could demonstrate no significant qualitative or quantitative difference between tobramycin and gentamicin when used in combination with penicillin against *S. faecalis*. Figure 2 shows the results of a representative experiment. The situation was somewhat different for the four strains of *S. faecium*. The penicillin-tobramycin combination exhibited no evidence of synergism against these strains when a starting inoculum of 10^7 organisms was employed. When a starting inoculum of 10^6 organisms per ml was used, *S. faecium* remained resistant to the synergistic effect of penicillin plus tobramycin. Even the use of much higher concentrations of both penicillin and tobramycin failed to produce any significantly increased killing of these strains of *S. faecium*, in clear contradistinction

TABLE 1. Effectiveness of penicillin combined with tobramycin or gentamicin against 27 strains of enterococci^a

Organism	Number of strains tested	Antibiotic combination	Magnitude of increased killing (differential reduction in colony counts) by antibiotic combination at 24 h (no. of strains)					
			Nonsynergistic ^b		Synergistic ^b			
			0	10^1	10^2	10^3	10^4	10^5
<i>Streptococcus faecalis</i> ^c	23	PCN, 10 U/ml + TM, 5 µg/ml	0	0	4	10	6	3
	23	PCN, 10 U/ml + GM, 5 µg/ml	0	1 ^d	4	8	3	7
<i>S. faecium</i>	4	PCN, 10 U/ml + TM, 5 µg/ml	4	0	0	0	0	0
	4	PCN, 10 U/ml + GM, 5 µg/ml	0	3 ^e	0	0	1	0

^a Abbreviations: PCN, penicillin; GM, gentamicin; and TM, tobramycin. Starting inoculum, 10^7 organisms per ml.

^b See text for definitions.

^c Includes four strains identical to *S. faecalis* in all respects except that they were able to ferment arabinose and raffinose.

^d The combination of penicillin plus gentamicin produced increased killing of $>10^3$ against this strain when a starting inoculum of 10^6 organisms per ml was employed.

^e Combined penicillin and gentamicin produced increased killing of $>10^2$ in all three strains when the starting inoculum was 10^6 organisms per ml.

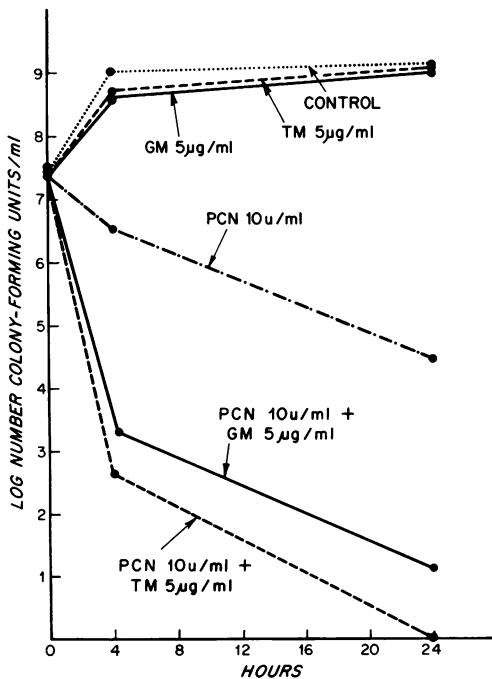


FIG. 2. Effect of penicillin plus gentamicin and penicillin plus tobramycin on *Streptococcus faecalis*, strain 7778.

to the results when penicillin plus gentamicin was used. The latter combination produced increased killing against all four strains, although in three it was less than 100-fold with a starting inoculum of 10^7 organisms per ml. When either higher concentrations of gentamicin or a lower starting inoculum (10^5 organisms per ml) was employed, penicillin plus gentamicin was synergistic against all four strains of *S. faecium*.

Until very recently the combination of penicillin plus streptomycin has been considered by many to represent optimal therapy for severe enterococci infections (6). However, it has become increasingly clear that penicillin plus streptomycin does not produce a synergistic effect against all strains of enterococci. Recent studies, in fact, show that as many as 30 to 80% of strains of enterococci are resistant to the synergistic effects of penicillin plus streptomycin (7, 12). Failure of synergism in these strains has been shown to be related to ribosomal resistance to streptomycin (14). Fewer, but significant, numbers of enterococci also are resistant to synergism of penicillin plus kanamycin (7). The exact clinical relevance of these observations remains to be defined. However, because we have thus far encountered no strains of enterococci resistant to the synergis-

tic action of penicillin plus gentamicin (9), a finding which has been confirmed by others (13), we have suggested that penicillin plus gentamicin may constitute the optimal therapy for severe enterococcal infections, at least in a situation in which formal testing for in vitro synergism cannot be done. Our recent observations have confirmed the apparent clinical efficacy of penicillin-gentamicin therapy of severe enterococcal infections (A. J. Weinstein and R. C. Moellering, Jr., in press). The present studies suggest that penicillin plus tobramycin may be equally as effective as penicillin plus gentamicin against infections due to *S. faecalis*, which comprises the majority of strains isolated from clinical enterococcal infections (4). This observation may have clinical relevance in several situations. If tobramycin (which is currently an investigational drug) is released for general clinical use, it is quite likely that combinations of penicillin plus tobramycin will, on occasion, be employed as initial "broad spectrum" coverage for serious infections before culture results are known. In such instances, the physician could assume that this regimen includes adequate coverage for the majority of group D streptococci. In addition, penicillin plus tobramycin could be considered as a substitute for penicillin plus gentamicin in the therapy of serious infections due to *S. faecalis*.

In situations where biochemical speciation of enterococci or facilities for in vitro testing for synergism are not available, however, penicillin plus gentamicin must remain the treatment of first choice for severe enterococcal infections, for this combination is also effective against *S. faecium* which are resistant to penicillin plus tobramycin. The reason for the resistance of *S. faecium* to penicillin-tobramycin combinations is not entirely clear, but we have also noted similar resistance of *S. faecium* to synergism of penicillin plus kanamycin (7). This is not surprising in view of the chemical similarity of tobramycin and the kanamycins (5). In fact, tobramycin appears to be identical to kanamycin B, except for the absence of a 3-hydroxyl group on the amino sugar. Additional studies concerning the mechanism of resistance of *S. faecium* to antibiotic synergism are currently in progress.

The organisms used in this study were grouped and biochemically speciated by Barbara Watson.

This work was supported by Public Health Service grant GRSG RR-05486-08 and a grant from Eli Lilly & Co.

LITERATURE CITED

1. Anderson, T. G. 1970. Testing of susceptibility to antimicrobial agents in body fluids, p. 299-310. In J. E.

- Blair, E. H. Lennette, and J. P. Truant (ed.), Manual of clinical microbiology. American Society for Microbiology, Bethesda, Md.
2. Black, H. R., and R. S. Griffith. 1971. Preliminary studies with nebramycin factor 6. *Antimicrob. Ag. Chemother.* 1970, p. 314-321.
 3. Deibel, R. H. 1964. The group D streptococci. *Bacteriol. Rev.* 28:330-366.
 4. Facklam, R. R. 1972. Recognition of group D streptococcal species of human origin by biochemical and physiological tests. *Appl. Microbiol.* 23:1131-1139.
 5. Koch, K. F., and J. A. Rhoades. 1971. Structure of nebramycin factor 6, a new aminoglycosidic antibiotic. *Antimicrob. Ag. Chemother.* 1970, p. 309-313.
 6. Mandell, G. L., D. Kaye, M. E. Levison, and E. W. Hook. 1970. Enterococcal endocarditis. *Arch. Intern. Med.* 125:258-264.
 7. Moellering, R. C., Jr., C. Wennersten, T. Medrek, and A. N. Weinberg. 1971. Prevalence of high-level resistance to aminoglycosides in clinical isolates of enterococci. *Antimicrob. Ag. Chemother.* 1970, p. 335-340.
 8. Moellering, R. C., Jr., C. Wennersten, and A. N. Weinberg. 1971. Studies on antibiotic synergism against enterococci. I. Bacteriologic studies. *J. Lab. Clin. Med.* 77:821-828.
 9. Moellering, R. C., Jr., C. Wennersten, and A. N. Weinberg. 1971. Synergy of penicillin and gentamicin against enterococci. *J. Infect. Dis.* 124:S207-S209.
 10. Rantz, L. A., and E. Randall. 1955. Use of autoclaved extracts of hemolytic streptococci for serological grouping. *Stanford Med. Bull.* 13:290-291.
 11. Shadomy, S., and C. Kirchoff. 1972. In vitro susceptibility testing with tobramycin. *Antimicrob. Ag. Chemother.* 1:412-416.
 12. Standiford, H. D., H. B. deMaine, and W. M. M. Kirby. 1970. Antibiotic synergism of enterococci. *Arch. Intern. Med.* 126:255-259.
 13. Watanakunakorn, C. 1971. Penicillin combined with gentamicin or streptomycin: synergism against enterococci. *J. Infect. Dis.* 124:581-586.
 14. Zimmermann, R. A., R. C. Moellering, Jr., and A. N. Weinberg. 1971. Mechanism of resistance to antibiotic synergism in enterococci. *J. Bacteriol.* 105:873-879.