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Synergism, Killing Kinetics, and Antimicrobial Susceptibility of Group A and B Streptococci

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The susceptibility of 110 group A and 179 group B streptococci to 25 antimicrobics was tested by broth microdilution and agar disk diffusion tests. Representative strains were used in killing kinetics, penicillin-gentamicin synergy, and minimal bactericidal concentration tests. Group A streptococci were more susceptible than group B streptococci to 17 of the 25 antimicrobics tested. Group A and B streptococci were killed at the same rate if the amount of penicillin used was equivalent to their respective penicillin minimal inhibitory concentrations. Synergism was demonstrated for both group A and B streptococci when penicillin was used at concentrations equal to each respective minimal inhibitory concentration and subinhibitory concentration of gentamicin. This synergy could be demonstrated best using minimal bactericidal concentrations obtained by culturing 3- and 6-h cultures from the microdilution checkerboard tests rather than from 24-h subcultures. A greater synergistic effect was achieved by adding penicillin first and then adding gentamicin rather than in the reverse order, or simultaneously.

Both group A and group B streptococci are generally considered to be universally susceptible to the penicillins. There is a difference in the level of susceptibility, however, with group A streptococci being more susceptible than group B (3, 10). It has also been reported that a longer period of time is required for penicillin to begin killing cultures of group B than of group A (13). These differences may be responsible for the higher clinical failure rates in patients with group B streptococcal infections who are treated with penicillin. These failures have subsequently led to changes in the therapy regimens required to effect cures in group B streptococcal infections (4, 11, 15). Increases in dosage of penicillin and combination therapy with penicillin and an aminoglycoside have been recommended for treatment of meningitis caused by group B streptococci (4, 15). Because serious group B streptococcal infections are being reported more frequently in adults and neonates, we are reporting our findings on minimal inhibitory concentrations (MICs), minimal bactericidal concentrations (MBCs), kill kinetics, and synergy studies for group A and B streptococci.

MATERIALS AND METHODS

Bacterial isolates. Antibiograms were determined for 179 group B and 110 group A streptococcal isolates that had been submitted to the Streptococcus Section of the Centers for Disease Control, Atlanta, Ga., for identification, grouping and typing. All strains used in this study were isolated from clinically important sources—blood, cerebrospinal fluid, fetal membranes or fluids, or gastrointestinal, respiratory, and urinary tracts. The strains were also isolated in a wide variety of geographic locations. The cultures had been preserved by sand desiccation (7) or by freezing in defibrinated animal blood at −70°C.

Culture media. Trypticase soy agar supplemented with 5% sheep blood (BBL Microbiology Systems) was used for subculturing the stored isolates. Trypticase soy broth was used to prepare the adjusted inoculum suspension. Broth microdilution susceptibility tests and kinetic kill tests were performed in Schaedler broth (BBL). The agar diffusion susceptibility tests were performed on Mueller-Hinton agar (BBL) supplemented with 5% lysed horse blood. Colony counts were done on either Trypticase soy agar supplemented with 5% sheep blood or Mueller-Hinton agar supplemented with 5% lysed horse blood.

Antimicrobial agents. The antimicrobial agents were supplied by the following organizations: penicillin, tetracycline, doxycycline, and vancomycin—Pfizer Inc., New York, N.Y.; methicillin and kanamycin—Bristol Laboratories, Syracuse, N.Y.; cephalothin, cefamandole, cephalexin, erythromycin, and neomycin—Eli Lilly & Co., Indianapolis, Ind.; cefuxorime—Glaxo Laboratories, Ltd., Greenford, Middlesex, England; cephradine—E. R. Squibb & Sons, Princeton, N.J.; cefoxitin—Merck & Co., Inc., Rahway, N.J.; lincomycin and clindamycin—The Upjohn Co., Kalamazoo, Mich.; josamycin—Endo Laboratories, Garden City, N.Y.; rosaramicin and gentamicin—Schering Laboratories, Bloomfield, N.J.; minocycline—Lederle Laboratories, Pearl River, N.Y.; colistin—Warner-Lambert Pharmaceutical Co., Morris Plains, N.J.; nalidixic

acid—Winthrop Laboratories, New York, N.Y.; nitrofurantoin—Norwich Pharmaceutical Co., Norwich, N.Y.; chloramphenicol—Parke, Davis & Co., Detroit, Mich.; and sulfamethoxazole-trimethoprim—Burroughs Wellcome Co., Research Triangle Park, N.C.

MIC tests. The MICs of 25 antimicrobial agents were determined by broth microdilution. Stock solutions of the antimicrobial agents were prepared with the appropriate diluent (1) to obtain solutions at 10 times each of the desired final concentrations. One part of the 10× stock solution was added to nine parts of broth to obtain each desired concentration and dispensed in 0.1-ml volumes into each well of microdilution trays with the MIC 2000 dispenser (Dynatech Laboratories, Inc., Alexandria, Va.). The microdilution trays were stored at −70°C for not longer than 3 to 4 weeks. Cultures to be tested were grown overnight at 35°C in a candle extinction jar. Suspensions of approximately 10^8 colony-forming units (CFU) per ml were prepared in Trypticase soy broth. Each adjusted suspension was then diluted to 10^7 CFU/ml in sterile distilled water and inoculated into the thawed antimicrobic microdilution trays using an automated inoculator (Dynatech). The final inoculum concentration was approximately 10^5 CFU/ml. Trays were incubated in candle extinction jars overnight at 35°C. The MIC was read as the least amount of antimicrobic which inhibited growth as observed with the unaided eye.

MBC and MBC synergy. MBCs for penicillin, ampicillin, and vancomycin were determined for 15 strains each of group A and B streptococci. After the MICs were read, approximately 0.0015 ml of the culture mixture was removed from each well of the MIC plate (therefore from each antimicrobic concentration and from controls) at 24 h and at 48 h with the Dynatech inoculator and transferred onto Mueller-Hinton agar supplemented with 5% lysed horse blood. These plates were incubated for 48 h in a candle extinction jar at 35°C, and all of the MBCs were read at 48 h as the least concentration of antimicrobic which produced 99% kill.

Three sets of microdilution MIC checkerboard plates (1) were prepared for penicillin and gentamicin MBC synergy studies. Fifteen group A and 15 group B streptococci were tested using these plates. The penicillin concentrations ranged from 1.0 to 0.002 μg/ml, and the gentamicin concentrations ranged from 32 to 0.25 μg/ml. The first set of tests contained both antimicrobics at the time of inoculation. The second set contained only penicillin at the time of inoculation, and gentamicin was added after 3 h of incubation. The third set contained gentamicin only at the time of inoculation, and penicillin was added after 3 h of incubation. Aliquots for MBCs were removed from each well of the three sets of plates at 0, 3, 6, 24, and 48 h. The MBC tests were done as described above.

Antibiotic kill kinetics. Kill kinetics were done on one strain of group A and two strains of group B streptococci to determine the time required for killing and to determine synergy. Penicillin and gentamicin alone and in combination were used in these tests. Concentrations of penicillin G ranging from 0.25 to 0.004 μg/ml and with 0.5 or 5.0 μg of gentamicin per ml were prepared in 5 ml of broth. Gentamicin at concentrations of 0.5 and 5 μg/ml was also combined with penicillin at concentrations equal to the MIC, two dilutions greater than the MIC, and one dilution less than the MIC. These antibiotic concentrations and a growth control containing no antibiotic were inoculated with each strain at a final concentration of 10^5 CFU/ml. Aliquots from each antibiotic concentration and a growth control were removed at 0, 3, 6, and 24 h and plated to Trypticase soy agar supplemented with 5% sheep blood for viable organism counts. These plates were incubated in candle extinction jars for 48 h. The colonies were counted, and kill curves were plotted.

Agar disk diffusion tests. The agar disk diffusion test was performed by using a modification of the Bauer-Kirby technique (2). Suspensions of bacteria at 10^8 CFU/ml were prepared in Trypticase soy broth and streaked with a cotton swab onto the plates of Mueller-Hinton agar supplemented with 5% lysed horse blood. Antimicrobic disks (BBL) were placed onto the surface of the agar, and the plates were incubated overnight in a candle extinction jar at 35°C. Zone sizes were measured from the surface of the agar.

RESULTS

The 50% MIC values (MIC_{50}) and 90% MIC values (MIC_{90}) and ranges for the 25 antimicrobial agents for the group A and B streptococci are given in Table 1. For most drugs, MIC_{50} and MIC_{90} values were the same or within 1 $\log_2$ dilution of each other. Where these values were compared by drug for the group A and group B strains, the major differences were obtained with penicillin, the tetracyclines, and some of the aminoglycosides, with group A strains being more susceptible. The mean zone sizes for both groups of streptococci are shown in Table 2. The average zone sizes for most antimicrobics were approximately 5 mm larger for group A strains than for group B. A comparison of the penicillin zones for group A and group B streptococci is given in Fig. 1.

A comparison of geometric mean MICs and MBCs of group A and B streptococci for penicillin, gentamicin, and vancomycin is shown in Table 3. The geometric mean MBCs were lower than the geometric mean MICs for penicillin and gentamicin, but not for vancomycin. For individual strains, the MBCs for penicillin and gentamicin were the same or one dilution less than the MIC at 24 and 48 h for both group A and B cultures. Vancomycin MBCs at 24 and 48 h were essentially the same for group A, but were 1 dilution higher at 24 h than at 48 h for group B cultures.

The kinetics for killing and synergy for one group A and two group B strains are illustrated in Fig. 2, 3, and 4. Within the time frame of the study, the largest number of viable organisms for both groups of streptococci occurred after 6 h of incubation (although not shown in these

TABLE 1. *MIC values for 25 antimicrobial agents tested*

Drug	MIC (μg/ml)					
	Streptococci group A			Streptococci group B		
	Range	MIC_{50}[a]	MIC_{90}[b]	Range	MIC_{50}[a]	MIC_{90}[b]
Penicillin	≤0.01–0.12	≤0.01	≤0.01	≤0.01–0.12	0.06	0.06
Methicillin	0.12–4.0	0.5	1.0	0.5–16.0	2.0	2.0
Cefamandole	≤0.03–0.12	0.06	0.06	≤0.03–0.5	0.06	0.12
Cefoxitin	0.25–4.0	1.0	1.0	0.5–8.0	4.0	4.0
Cefuroxime	≤0.03–0.06	≤0.03	≤0.03	≤0.03–1.0	0.06	0.06
Cephradine	0.12–2.0	0.25	0.25	0.25–4.0	2.0	2.0
Cephalexin	0.12–4.0	0.25	0.5	0.5–8.0	4.0	4.0
Cephalothin	0.06–0.5	0.12	0.12	0.06–0.5	0.25	0.25
Erythromycin	0.03–> 0.5	0.03	0.06	≤0.01–0.25	0.06	0.06
Clindamycin	≤0.03–0.12	0.06	0.06	≤0.03–0.25	0.06	0.06
Lincomycin	0.06–0.25	0.12	0.12	0.03–0.25	0.12	0.12
Josamycin	0.06–0.5	0.12	0.25	0.12–1.0	0.25	0.5
Rosaramicin	0.12–0.5	0.12	0.25	0.12–0.5	0.25	0.5
Doxycycline	0.12–16.0	0.12	8.0	0.12–32.0	16.0	16.0
Minocycline	0.12–16.0	0.25	8.0	0.25–32.0	16.0	32.0
Tetracycline	0.12–32.0	0.25	16.0	0.12–>32.0	32.0	32.0
Gentamicin	1.0–8.0	2.0	2.0	1.0–>32.0	16.0	16.0
Kanamycin	8.0–>32.0	16.0	32.0	8.0–>32.0	>32.0	>32.0
Neomycin	4.0–>32.0	8.0	16.0	4.0–>32.0	32.0	>32.0
Chloramphenicol	1.0–4.0	2.0	2.0	1.0–4.0	2.0	4.0
Colistin	4.0–>32.0	>32.0	>32.0	8.0–>32.0	>32.0	>32.0
Nalidixic acid	>32.0	>32.0	>32.0	>32.0	>32.0	>32.0
Nitrofurantoin	2.0–16.0	4.0	8.0	8.0–32.0	16.0	32.0
Sulfa-trimethoprim	1.2/0.06–19/1	2.4/1.2	4.8/0.25	1.2/0.06–9.5/0.5	4.8/0.25	4.8/0.25
Vancomycin	0.25–0.5	0.5	0.5	0.5–2.0	0.5	0.5

[a] 50% of strains ≤ this MIC.
[b] 90% of strains ≤ this MIC.

TABLE 2. *Mean zone size: group A and B streptococci*

Drug	Zone size (mm)	
	Group A[a]	Group B[a]
Penicillin	37.8	29.3
Methicillin	24.5	19.9
Cefamandole	37.1	31.5
Cefoxitin	31.5	21.6
Cephalothin	38.0	30.8
Erythromycin	28.5	24.2
Clindamycin	26.5	21.7
Tetracycline	11.0/28.3[b]	10.9/23.6[b]
Gentamicin	15.7	6.0
Kanamycin	7.0	6.0
Neomycin	6.6	6.0
Chloramphenicol	27.6	23.4
Colistin	8.0	6.0
Nalidixic acid	6.9	6.0
Nitrofurantoin	30.1	21.7
Sulfa-trimethoprim	25.0[c]	24.2
Vancomycin	21.3	17.6

[a] n = 110 for group A and 179 for group B streptococci.
[b] Resistant strains/susceptible strains.
[c] Some strains had no zones.

figures, counts of growth controls at 12 h were similar to those at 6 h). The growth had slowed and numbers of viable organisms had started to decline at 24 h. This same general pattern also occurred if the penicillin concentration was less than the MIC, although the killing rate was slightly greater in some cases. With concentrations of penicillin equal to the MIC, the killing effect was greatest during the first 3 to 6 h of incubation. Killing at a slower rate continued to occur through 24 h, but complete sterilization was not achieved at 24 h with either group A or group B streptococci with penicillin alone. When subinhibitory concentrations of gentamicin alone (0.5 μg/ml) were added to the cultures, the viable organism counts were almost identical or very similar to the growth controls. When 5 μg of gentamicin alone per ml was added, however, the group A streptococcal culture was sterilized at 3 h. The counts on group B declined to approximately 10^3 CFU/ml at the end of 6 h, but then increased logarithmically to 10^8 CFU/ml at the end of 24 h. The gentamicin MIC for this group A strain was 4 μg/ml, and the MICs were 16 and 32 μg/ml for the group B strains. Com-

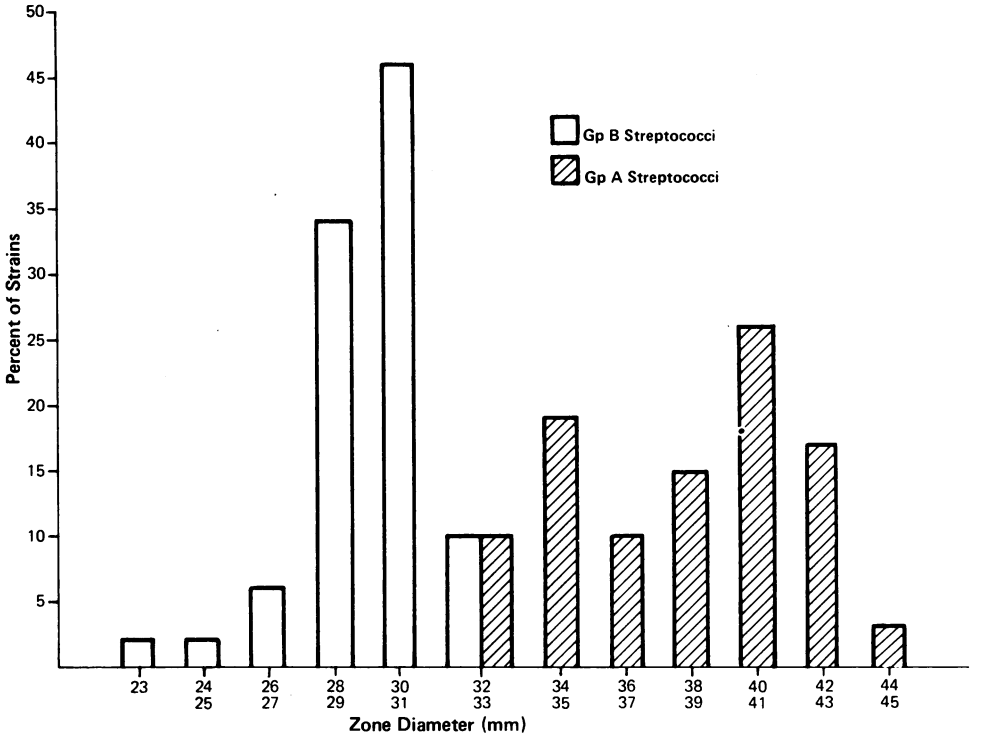

FIG. 1. *Distribution of penicillin zone diameters obtained with groups A and B streptococci.*

TABLE 3. *Geometric mean MICs and MBCs of penicillin, gentamicin, and vancomycin for strains of group A and B streptococci*

Drug	Group A			Group B		
	MIC, 24 h	MBC		MIC, 24 h	MBC	
		24 h	48 h		24 h	48 h
Penicillin	0.009[a]	0.006	0.004	0.045	0.028	0.017
Gentamicin	4.06	3.066	2.876	>32.0	31.008	28.0
Vancomycin	0.25	0.357	0.365	0.281	0.532	0.422

[a] All values are geometric means of the MICs and MBCs for 15 strains of each group.

binations of penicillin at the penicillin MICs (0.015 μg/ml for group A and 0.06 μg/ml for group B) and gentamicin at one-sixth to one-eighth of the gentamicin MICs produced complete sterilization of one culture of group B after 6 h and of the other group B and the group A strains within 24 h. When combinations of 0.5 μg of gentamicin per ml and 0.06 μg of penicillin per ml (the MIC) were tested with the group B strains, sterilization was obtained at 24 h with one strain but not with the other.

The action of penicillin and gentamicin in combination, obtained from the checkerboard studies, was demonstrated best with MBCs obtained with 6-h cultures as shown in Fig. 5 and 6. Data on the effect of the order of addition of the two drugs on synergism are also shown in these graphs. With the group A strain (Fig. 5), the best killing action was obtained when penicillin was added first and gentamicin was added later, and the curve for adding them simultaneously was not much different. But when gentamicin was added first, the penicillin had little influence on the 6-h gentamicin MBCs and vice versa. With the group B strains (Fig. 6), the greatest killing action was obtained when peni-

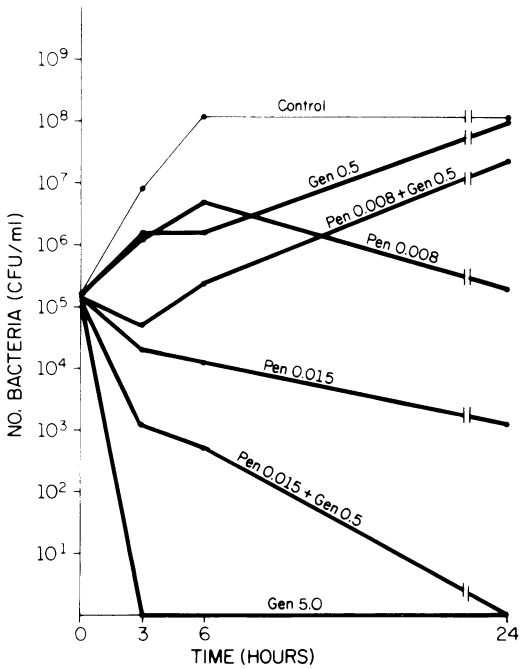

FIG. 2. *Kill curves for a strain of group A streptococcus. The penicillin MIC was 0.015 μg/ml, and the gentamicin MIC was 4.0 μg/ml.*

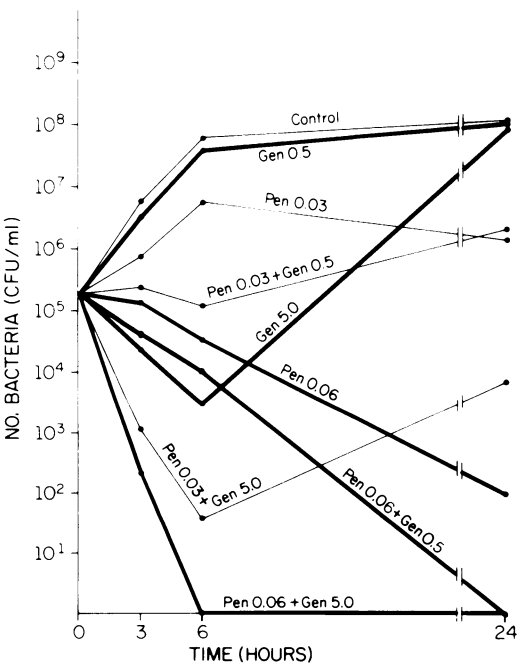

FIG. 3. *Kill curves for a strain of group B streptococcus. The penicillin MIC was 0.06 μg/ml, and the gentamicin MIC was 16 μg/ml.*

cillin was added first. When the two were added simultaneously or when the gentamicin was added first, the activity was only additive at best. However, these data show that the concentration of penicillin is very important, particularly with the group B strains, where antagonism was obtained with penicillin concentrations greater than 0.06 μg/ml or 0.25 μg/ml depending upon the order of addition of the drugs (antagonism indicated by the curves bending back to the right at the higher penicillin concentrations).

DISCUSSION

These data show that groups A and B streptococci vary in susceptibility to some antimicrobics. In general, group A streptococci are more susceptible to the penicillins, cephalosporins, aminoglycosides, and nitrofurantoin. They are essentially the same for the macrolides, sulfamethoxazole-trimethoprim, and vancomycin. Both groups varied in their susceptibility to tetracyclines and were resistant to colistin and nalidixic acid. We tested these strains against more drugs than have been reported previously, but for those drugs that had been tested by others, our results essentially agree (3, 6, 10, 12). However, we did not obtain penicillin MICs as high as the 0.2 and 0.8 μg/ml reported by Jokipii and Jokipii (9).

Many clinical bacteriology laboratories perform disk diffusion tests on group A and B streptococci using the Bauer-Kirby method, even though there is no recommendation that it should be done (2). In many instances, penicillin susceptibility is judged on the basis of the penicillin-staphylococcus interpretive standard, i.e., susceptible if the zone is ≥29 mm. This interpretation for penicillin appears to work for group A streptococci, but there is no indication that routine susceptibility tests should be performed on this group. When these zone interpretations are applied to group B streptococci, a significant number of strains would be judged intermediate, an interpretation that would be appropriate for strains from cases of meningitis. It is probable that the standard disk diffusion breakpoints will work for the other drugs shown in Table 2, with the exception of the aminoglycosides, which should not be tested. Our tentative recommendations for disk diffusion for these organisms are as follows. (i) Group A streptococci should not be tested by disk diffusion except when erythromycin is being considered as alternative therapy. (ii) If group B streptococci are tested for penicillin susceptibility by disk diffusion, use the Bauer-Kirby interpretative breakpoints for staphylococci; i.e., ≥29 mm indicates susceptible, 21 to 28 mm indicates intermediate, and 20 mm

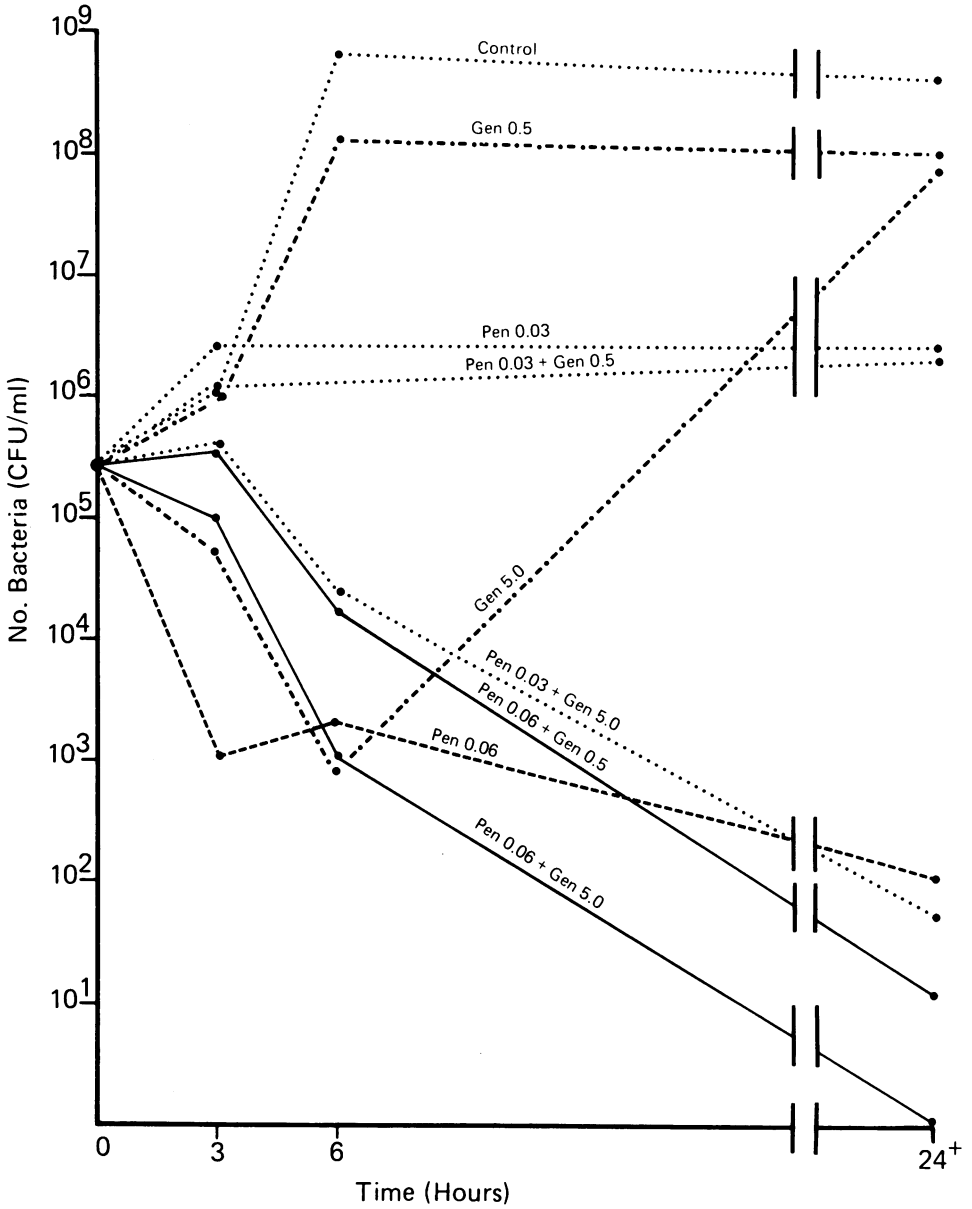

FIG. 4. *Kill curves for a strain of group B streptococcus. The penicillin MIC was 0.06 μg/ml, and the gentamicin MIC was 32 μg/ml.*

indicates resistant. Our reason for choosing this breakpoint is that many of the strains are from cases of meningitis, and some are better classified as intermediate. (iii) If group B streptococci are tested for susceptibility by disk diffusion, it appears that the present standards will adequately discriminate the categories of susceptibility.

It is our opinion, however, that group A and B streptococci should be tested by a dilution method, if possible.

The differences between the susceptibility of the two streptococcal groups to penicillins and aminoglycosides are important clinically, particularly in patients with meningitis caused by group B streptococci. Group B streptococci may have MICs equal to or slightly exceeding the levels of penicillin attainable in spinal fluid. This

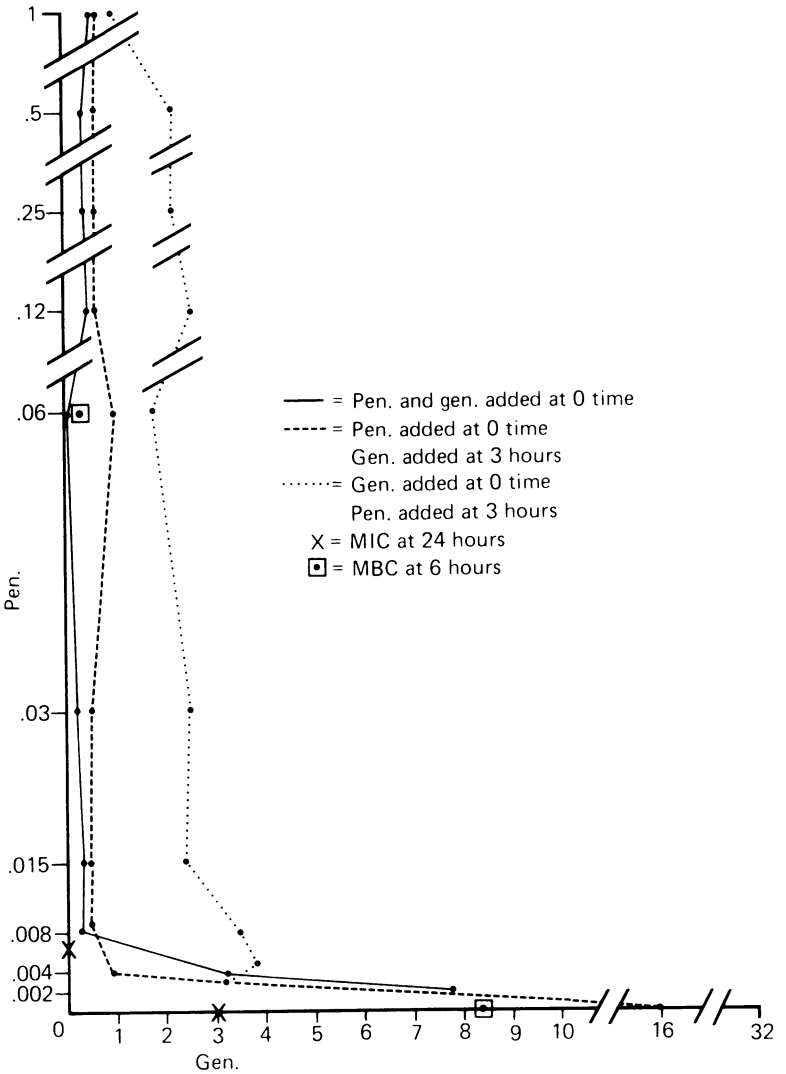

FIG. 5. *Six-hour geometric mean MBCs of penicillin and gentamicin for group A streptococci when the two antibiotics were added together and separately.*

presents therapeutic problems similar to those that may occur with pneumococcal meningitis (14). However, categorization of penicillin susceptibility of group B streptococci based on MICs will not be as easy as with pneumococci because the MICs of group B streptococci are at or near the MIC breakpoint used for pneumococci (14).

Chloramphenicol is the alternative therapy in pneumococcal meningitis due to strains relatively resistant to penicillin (14). This alternative has not been generally considered for group B streptococcal meningitis, and the regimen most often used has been a combination of penicillin and an aminoglycoside (4, 15). McCracken, however, pointed out in an editorial that although the inclusion of an aminoglycoside with penicillin for treatment of group B meningitis may be clinically important, there is no clinical evidence to substantiate it (11).

In their study on the killing kinetics of groups A and B streptococci by antimicrobics, Schauf et al. reported a more rapid killing rate by ampicillin for group A than for group B, but they used the same concentration of ampicillin for both groups (13). It was our feeling that testing both groups at the same drug concentration without regard to difference in MICs put the

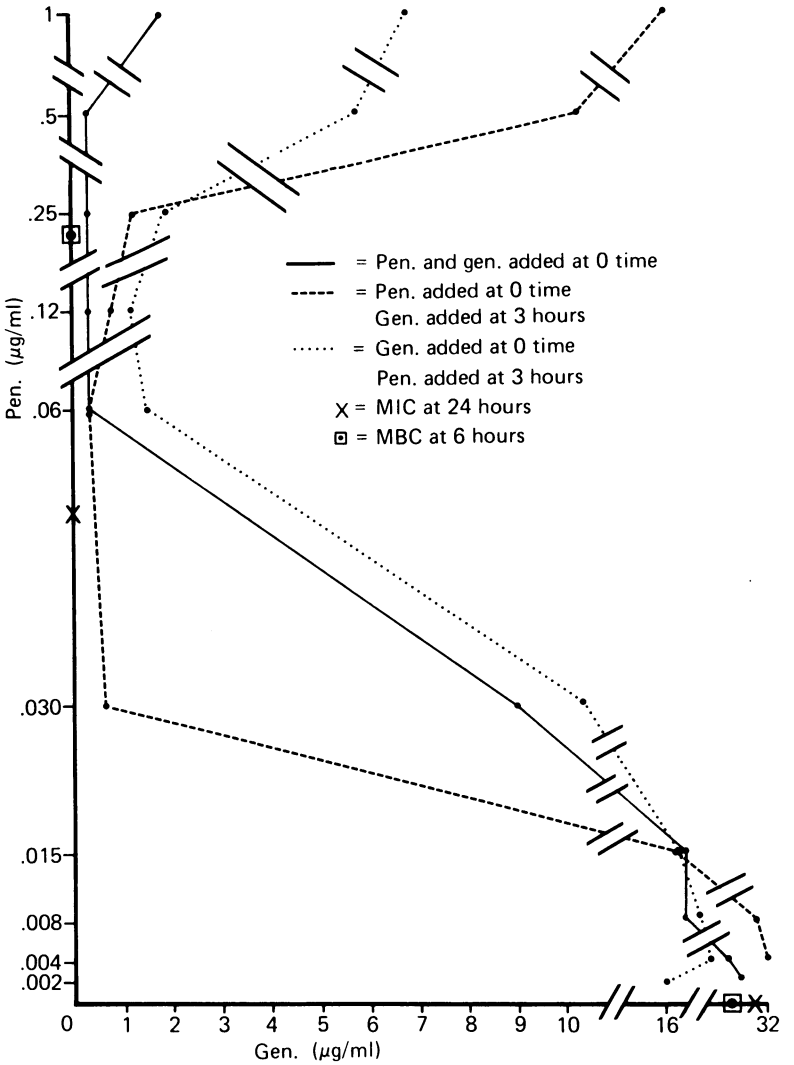

FIG. 6. *Six-hour geometric mean MBCs of penicillin and gentamicin for group B streptococci when the two antibiotics were added together and separately.*

group B strains at a distinct disadvantage since the amount of penicillin or ampicillin required to inhibit the group B strain is six to ten times that required for the group A strain. For this reason, we selected penicillin concentrations for each organism at the MIC of that strain and at 1 $\log_2$ dilution below its MIC to determine the killing rate. At the penicillin MIC for both groups of organisms, the viable numbers were reduced from 10^5 down to 10^3 to 10^4 CFU/ml after 6 h and down to 10^2 to 10^3 CFU/ml after 24 h.

Neither group of organisms was sterilized at this concentration after 24 h. Therefore the killing rate of penicillin is probably the same for both groups of organisms, but the amount of penicillin required to achieve killing is much greater for group B than for group A. Cooper et al. reported that for group B streptococci, the early bactericidal activity seen with a combination of gentamicin and ampicillin was due to the aminoglycoside (5). However, we feel that for this to occur, the penicillin must be present at levels equivalent to the penicillin MIC.

The MIC with gentamicin for group B streptococci is at least eight times that for group A streptococci. For our studies, we selected two concentrations of gentamicin—one that was sub-

inhibitory for each group (0.5 μg/ml for group A and 5 μg/ml for group B)—but we tested both group A and B streptococci with both concentrations. For the group A strain with 0.5 μg of gentamicin per ml, there was no increase in numbers of organisms between 3 and 6 h (the growth control grew logarithmically), but then it began to grow again. On the other hand, 5 μg of gentamicin per ml rapidly sterilized the culture. With the group B strains, the response was dramatically different. Gentamicin at a concentration of 0.5 μg/ml had no effect on the growth of the organism, but with 5 μg/ml there was an initial decrease in numbers of approximately 2 $\log_{10}$ at 6 h, and then the organism began to grow logarithmically. In the presence of 0.06 μg of penicillin per ml, the two group B strains reacted differently. One strain was sterile after 6 h of incubation, but the other strain required 24 h of incubation to become sterile. We do not know if this difference in killing rate was due to the difference in gentamicin MICs for these strains, but this was the only difference we observed. The difference in killing effectiveness may be explained by difference in cell wall permeability to gentamicin (16). Some organisms may be better able to prevent entrance of the drug into the cells than do others, but if this is true, it is not an all-or-none phenomenon since some but not all of the cells in the culture were killed at 0.5 μg/ml for group A and 5 μg/ml for group B. The cells which survived the initial contact with gentamicin were able to reproduce rapidly. The initial killing and subsequent growth were also borne out in the microdilution tests with gentamicin. In wells where visible growth was seen (indicating approximately 10^7 organisms per ml), the actual counts were about 1 $\log_{10}$ less than the turbidity would suggest. The ability to reproduce after an initial reduction in number was not due to inactivation of the drug because assays showed that the drug remained at the same level that was put into the culture initially. It was also not due to selection of resistant bacteria since the susceptibility of surviving cells was not different from that in the original culture.

In these studies, the MBCs for penicillin and gentamicin were usually less than the MICs (Table 3). Although this finding seemed improbable at first, it was quite reproducible. This finding can be explained by the difference in the apparent number of cells (indicated by turbidity) and the actual number (by count).

Synergy between penicillin and gentamicin was difficult to demonstrate with the streptococci used in these studies, as it is with other bacteria that are quite susceptible to penicillin. Synergistic activity could be demonstrated with these organisms if an MBC was done from a subculture at 6 h rather than at 24 h. At 6 h penicillin alone had not yet killed the organisms, and thus the activity of the gentamicin could be demonstrated. These 6-h MBC tests were used not only to demonstrate synergy but also to determine whether or not adding the drugs at different times had an effect on activity. With group A streptococci, synergy was readily demonstrated when the two drugs were added at the same time or gentamicin was added 3 h later, but not if penicillin was added 3 h after the gentamicin. With group B streptococci, the best results were obtained when penicillin was added first.

The reason for not obtaining synergy when gentamicin was added first may be that the organisms were inhibited by the gentamicin during the first 6 h of incubation and, therefore, were not dividing when the penicillin was added at 3 h. Under these conditions, penicillin may not bring about lysis and killing. Our data confirm the report by Gutschik et al. (8) that subinhibitory levels of gentamicin alone reduce the number of viable bacterial cells 10-fold to 100-fold, but the culture eventually overcomes this inhibition and begins to multiply logarithmically. If penicillin is added after 3 h of incubation with gentamicin, penicillin kills the cells which begin to multiply but does not kill the stationary cells; therefore, the culture is not sterilized.

Extrapolating in vitro results into therapeutic strategy is often difficult and must be confirmed by careful clinical studies. However, these data suggest some points for consideration. The first is that, for appropriately selected patients, chloramphenicol might be considered for treatment of meningitis caused by group B streptococci. The MICs are usually 2 μg/ml, which is similar to those obtained for pneumococci, and pneumococcal meningitis responds quite well to chloramphenicol therapy. If, however, chloramphenicol is not acceptable, and combination therapy with penicillin and an aminoglycoside is indicated, the following points should be considered. Penicillin should be administered first and in amounts to attain a concentration of at least the MIC. If the penicillin level is less than the MIC, it is possible that the aminoglycoside would reduce rather than enhance killing. If the penicillin concentration is at the MIC level, subinhibitory levels of gentamicin will bring about more rapid killing. If the penicillin concentration is considerably larger than the MIC, the possibility of antagonism exists. Such levels are unlikely, however, in the spinal fluid, even with inflamed meninges. It is more likely that the

major difficulty would be in attaining and maintaining an adequate level of penicillin in the spinal fluid.

Although we were able to demonstrate synergism with penicillin and gentamicin using 6-h subcultures for MBCs, we would not recommend this as a routine procedure. With organisms such as group B streptococci, it is probably more clinically useful to determine the MICs to both drugs. With the MIC data, a decision can be made as to the likelihood of obtaining adequate levels of each drug to get the more rapid killing described above.

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