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Study of Comparative Antipneumococcal Activities of Penicillin G, RP 59500, Erythromycin, Sparfloxacin, Ciprofloxacin, and Vancomycin by Using Time-Kill Methodology

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Time-kill studies were used to examine the in vitro activities of penicillin G, RP 59500, erythromycin, ciprofloxacin, sparfloxacin, and vancomycin against 10 pneumococci expressing various degrees of susceptibility to penicillin and erythromycin. RP 59500 MICs for all strains were 0.5 to 2.0 μg/ml, while erythromycin MICs were 0.008 to 0.06 μg/ml for erythromycin-susceptible strains and 32.0 to 64.0 μg/ml for erythromycin-resistant strains. Strains were more susceptible to sparfloxacin (0.125 to 0.5 μg/ml) than to ciprofloxacin (0.5 to 4.0 μg/ml), and all were inhibited by vancomycin at MICs of 0.25 to 0.5 μg/ml. Time-kill studies showed that antibiotic concentrations greater than the MIC were bactericidal for each strain, with the following exceptions. Erythromycin was bactericidal for one penicillin-resistant strain at 6 h, with regrowth after 12 and 24 h. Three penicillin-susceptible strains were bacteriostatically inhibited by erythromycin at concentrations greater than or equal to the MIC by 6 h. One penicillin-susceptible strain (penicillin MIC, 0.06 μg/ml) was bacteriostatically inhibited by penicillin G at 24 h at the MIC or at one-half the MIC; a bactericidal effect was found only with penicillin G at concentrations of ≥0.25 μg/ml. At 10 min after inoculation a 1- to 3-$\log_{10}$-unit reduction (90 to 99.9%) in the original inoculum was seen for 6 of 10 strains with RP 59500 at concentrations greater than or equal to the MIC. This effect was not found with any of the other compounds tested. A bactericidal effect was found at ≥6 h with RP 59500 at concentrations of one-half to one-quarter the MIC in 7 of 10 strains, and a bacteriostatic effect was found in 3 of 10 strains, with regrowth at 24 h. One penicillin-resistant strain was examined by the time-kill methodology at 0, 1, 2, and 3 h. RP 59500 at a concentration equal to the MIC was bactericidal within 1 h, and at a concentration of one-half the MIC it was bactericidal within 3 h. This phenomenon was not seen with the other antimicrobial agents tested. Regrowth of strains at ciprofloxacin concentrations equal to the MIC or at one-half to one-quarter the MIC was found. For sparfloxacin, three of four penicillin-susceptible strains and two of four penicillin-resistant strains were bacteriostatically inhibited by 6 h. Bactericidal effects were found at 6, 12, and 24 h with both intermediate-resistant, one penicillin-susceptible, and two penicillin-resistant strains. Complete killing was observed with vancomycin at concentrations greater than the MIC. Of the new compounds tested, RP 59500 and sparfloxacin show promise for the treatment of infections caused by penicillin-susceptible and -resistant pneumococci. The clinical significance of rapid killing by RP 59500 remains to be determined.

Streptococcus pneumoniae continues to be a significant cause of morbidity and mortality in humans and is the leading cause of bacterial pneumonia as well as an important cause of otitis media and meningitis. Although this organism was originally exquisitely susceptible to penicillin, the past two decades, and in particular the fast few years, have witnessed an alarming increase in the number of strains resistant to penicillin as well as other antimicrobial agents all over the world (2, 14, 18, 19).

Resistance to macrolides frequently occurs in pneumococci, and susceptibilities to all macrolides and azalides are similar: strains resistant to erythromycin are also resistant to azithromycin, clarithromycin, roxithromycin, and other members of this group (25). Likewise, strains susceptible to erythromycin are susceptible to all members of the class. RP 59500 (quinupristin, dalfopristin), a new streptogramin (3, 5, 6, 11, 13, 22–25), is the only member of the macrolide-lincosamide-streptogramin group which is active against both erythromycin-susceptible and erythromycin-resistant strains, with MICs for 90% of isolates tested of 1.0 to 2.0 μg/ml (11, 25).

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Among the quinolones, commercially available agents such as ciprofloxacin and ofloxacin are marginally active against pneumococci (MICs for 90% of isolates tested, 2.0 to 4.0 μg/ml) (14, 25). Sparfloxacin is a new fluoroquinolone (1, 7, 8, 17, 25) with improved activity against these strains (MICs, 0.125 to 0.5 μg/ml) (25). Vancomycin remains the only agent to which resistance has not developed, but it has toxicity and administration problems.

Most studies of pneumococcal susceptibility in vitro have been performed by determination of MICs. In the current study we used the time-kill methodology to examine the activities of penicillin G, RP 59500, erythromycin, ciprofloxacin, sparfloxacin, and vancomycin against 10 pneumococcal strains with various degrees of susceptibility to penicillin and erythromycin.

MATERIALS AND METHODS

Bacteria. Ten clinical isolates of *S. pneumoniae* obtained from blood, cerebrospinal fluid, nasopharynx, or sputum were tested. This group included four penicillin-susceptible (MICs, <0.1 μg/ml), two penicillin-intermediate-resistant (MICs, 0.1 to 1.0 μg/ml) and four penicillin-resistant (MICs, ≥2.0 μg/ml)

organisms. Strains were stored at −70°C in double-strength skim milk (Difco Laboratories, Detroit, Mich.) and were subcultured three times on Trypticase soy agar–5% sheep blood agar plates (BBL Microbiology Systems, Detroit, Mich.) before testing.

Antimicrobial agents. The antimicrobial agents used in the study were penicillin G (Sigma Scientific, St. Louis, Mo.), RP 59500 (Rhône-Poulenc Rorer, Paris, France), erythromycin (Abbott Laboratories, Chicago, Ill.), ciprofloxacin (Miles, Inc., West Haven, Conn.), sparfloxacin (Rhône-Poulenc Rorer, Collegeville, Pa.), and vancomycin (Eli Lilly & Co., Indianapolis, Ind.).

MIC and time-kill determinations. For the time-kill experiments, glass tubes containing 5 ml of cation-adjusted Mueller-Hinton broth (Difco) plus 5% lysed horse blood with doubling antibiotic concentrations were used. Antibiotic concentrations were chosen to be 3 doubling dilutions above and 4 doubling dilutions below the agar dilution MIC. Drug-free control tubes were included with each run. Time-kill studies of one strain (strain 228) were repeated to test for the reproducibility of the method.

Lysed horse blood was prepared by freezing and thawing horse blood (Cleveland Scientific, Bath, Ohio) eight times. Equal volumes of lysed blood and sterile deionized water were then mixed and centrifuged at 12,000 × *g* for 20 min. Appropriate amounts of 50% lysed blood were then added to the cation-adjusted Mueller-Hinton broth to yield a final concentration of 5% lysed horse blood (26). The bacterial inoculum was prepared by diluting a 16-h broth (medium as described above) culture in the same medium. Dilutions required to obtain the correct inoculum (10^5 to 10^6 CFU/ml) were determined by prior viability studies with each strain.

To inoculate each tube of serially diluted antibiotic, 50 μl of diluted inoculum was delivered by pipette beneath the surface of the broth, and the mixture was then vortexed and plated for viability counts (0 h). Only tubes containing an initial inoculum within the range of 10^5 to 10^6 CFU/ml were acceptable. Tubes were inoculated with organism suspensions (see above) and were incubated at 35°C in a shaking water bath.

Viability counts were performed in duplicate at 0, 6, 12, and 24 h by removing an aliquot and preparing 10-fold dilutions in Mueller-Hinton broth and plating 0.1-ml aliquots from each dilution onto Trypticase soy agar–5% sheep blood agar plates (BBL). Plates were incubated for up to 48 h, and colony counts were performed on plates yielding 30 to 300 colonies (16). In addition, one plate containing a penicillin-resistant strain (strain 228) was tested for viable bacteria after 0, 1, 2, and 3 h.

MICs and MBCs were determined from time-kill curves at 24 h. The MIC was defined as the lowest concentration of antibiotic which inhibited bacterial growth. Antibiotics were considered to be bactericidal at the lowest concentration that reduced the original inoculum by ≥3 $\log_{10}$ CFU/ml. Regrowth was defined as an increase of ≥2 $\log_{10}$ CFU/ml after ≥6 h. Viability counts for 10-min determinations were performed in the same time-kill experiments described above.

The breakpoints of the antibiotics, when available, were those recommended by the National Committee for Clinical Laboratory Standards (21), 0.5 μg/ml as follows: for erythromycin, 2.0 μg/ml for ciprofloxacin and sparfloxacin, 4.0 μg/ml for RP 59500, and 4.0 μg/ml for vancomycin.

Agar dilution MICs were determined by the method recommended by the National Committee for Clinical Laboratory Standards (21) for streptococci by using Mueller-Hinton agar (BBL) supplemented with 5% sheep blood, incorporating antimicrobial agents at concentrations from 0.0075 to 32 μg/ml in twofold increments. Suspensions with a turbidity equal to a 0.5 McFarland standard were prepared by suspending the growth from overnight Trypticase soy agar–5% sheep blood agar plates in 2 ml of Mueller-Hinton broth (Difco). Suspensions were further diluted in broth to obtain a final inoculum of approximately 10^4 organisms per spot. Plates were inoculated with a Steers replicator containing 3-mm inoculating pins and were incubated overnight in ambient air at 35°C. The lowest concentration of antibiotic in plates showing no growth, a faint haze, or a single colony was defined as the MIC. Standard quality control strains were included in each experiment.

RESULTS

MICs. MIC results from growth curves of the 10 strains tested are listed in Table 1. Penicillin MICs for susceptible strains were 0.015 to 0.06 μg/ml, those for intermediate-resistant strains were 0.25 to 0.5 μg/ml, and those for resistant strains were 2.0 to 4.0 μg/ml. All strains were susceptible to RP 59500, irrespective of their erythromycin susceptibility status (MICs, 0.5 to 2.0 μg/ml). All four penicillin-susceptible strains were susceptible to erythromycin (MICs, 0.008 to 0.06 μg/ml); however, one of the two penicillin-intermediate-resistant strains and three of the four penicillin-resistant strains were resistant to this compound (MICs, 32.0 to 64.0 μg/ml). The MICs of sparfloxacin (0.125 to 0.5 μg/ml) were 4- to 32-fold less than those of ciprofloxacin (0.5 to 4.0 μg/ml). All strains were susceptible to vancomycin (MICs, 0.25 to 0.5 μg/ml).

Agar dilution MICs were also determined (data not shown)

TABLE 1. Broth dilution MICs for organisms tested

Strain	MIC (μg/ml)					
	Penicillin G	RP 59500	Erythromycin	Ciprofloxacin	Sparfloxacin	Vancomycin
64	0.03[a] (S)[b]	0.5	0.03	2.0	0.5	0.5
294	0.015 (S)	0.5	0.015	0.5	0.125	0.5
60	0.03 (S)	0.5	0.008	1.0	0.5	0.5
2	0.06 (S)	2.0	0.06	2.0	0.5	0.5
5	0.5 (I)	0.25	32.0	2.0	0.25	0.25
114B	0.25 (I)	1.0	0.03	2.0	0.125	0.5
114	4.0 (R)	0.5	32.0	1.0	0.25	0.5
227	4.0 (R)	0.5	32.0	4.0	0.25	0.5
167	4.0 (R)	0.5	0.03	4.0	0.25	0.5
228	4.0 (R)	0.5	64.0	4.0	0.25	0.5

[a] Agar dilution MICs were as indicated for the following strains: 64, 0.015 μg/ml; 294, 0.015 μg/ml; 60, 0.015 μg/ml; 2, 0.06 μg/ml; 5, 0.25 μg/ml; 114B, 0.125 μg/ml; 114, 2.0 μg/ml; 227, 4.0 μg/ml; 167, 2.0 μg/ml; 228, 2.0 μg/ml.

[b] See Materials and Methods. S, susceptible; I, intermediate resistant; R, resistant.

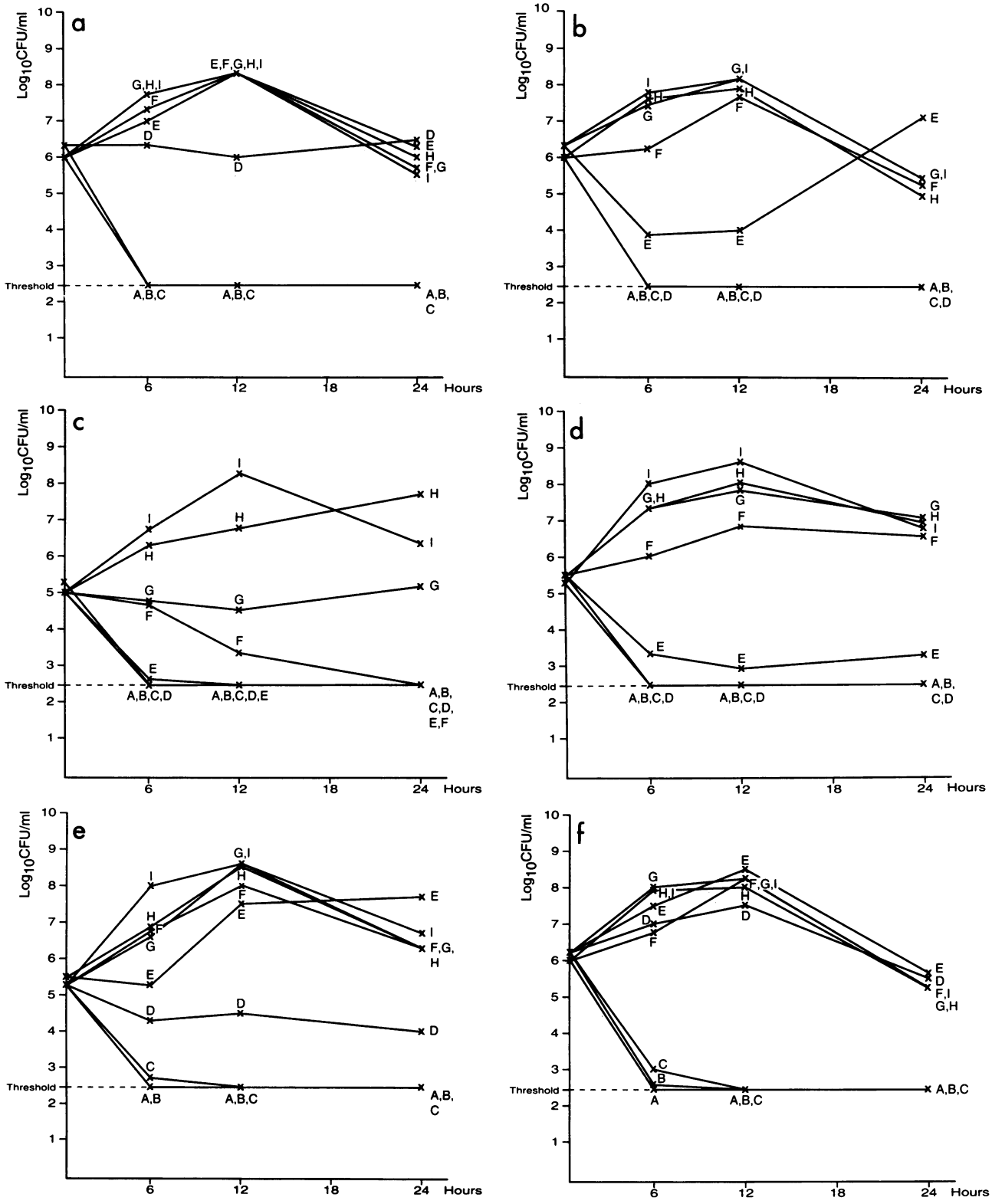

FIG. 1. Time-kill kinetics of penicillin G (a), RP 59500 (b), erythromycin (c), ciprofloxacin (d), sparfloxacin (e), and vancomycin (f) against a penicillin-susceptible pneumococcus (strain 60) at 0, 6, 12, and 24 h. (a) A, 0.125 μg/ml; B, 0.06 μg/ml; C, 0.03 μg/ml; D, 0.015 μg/ml (agar MIC); E, 0.08 μg/ml; F, 0.004 μg/ml; G, 0.002 μg/ml; H, 0.001 μg/ml; I, growth control. (b) A, 4.0 μg/ml; B, 2.0 μg/ml; C, 1.0 μg/ml; D, 0.5 μg/ml (agar MIC); E, 0.25 μg/ml; F, 0.125 μg/ml; G, 0.06 μg/ml; H, 0.03 μg/ml; F, growth control. (c) A, 0.5 μg/ml; B, 0.25 μg/ml; C, 0.125 μg/ml; D, 0.06 μg/ml (agar MIC); E, 0.03 μg/ml; F, 0.015 μg/ml; G, 0.008 μg/ml; H, 0.004 μg/ml; I, growth control. (d) A, 16.0 μg/ml; B, 8.0 μg/ml; C, 4.0 μg/ml; D, 2.0 μg/ml (agar MIC); E, 1.0 μg/ml; F, 0.5 μg/ml; G, 0.25 μg/ml; H, 0.125 μg/ml; I, growth control. (e and f) A, 2.0 μg/ml; B, 1.0 μg/ml; C, 0.5 μg/ml; D, 0.25 μg/ml (agar MIC); E, 0.125 μg/ml; F, 0.06 μg/ml; G, 0.03 μg/ml; H, 0.015 μg/ml; I, growth control.

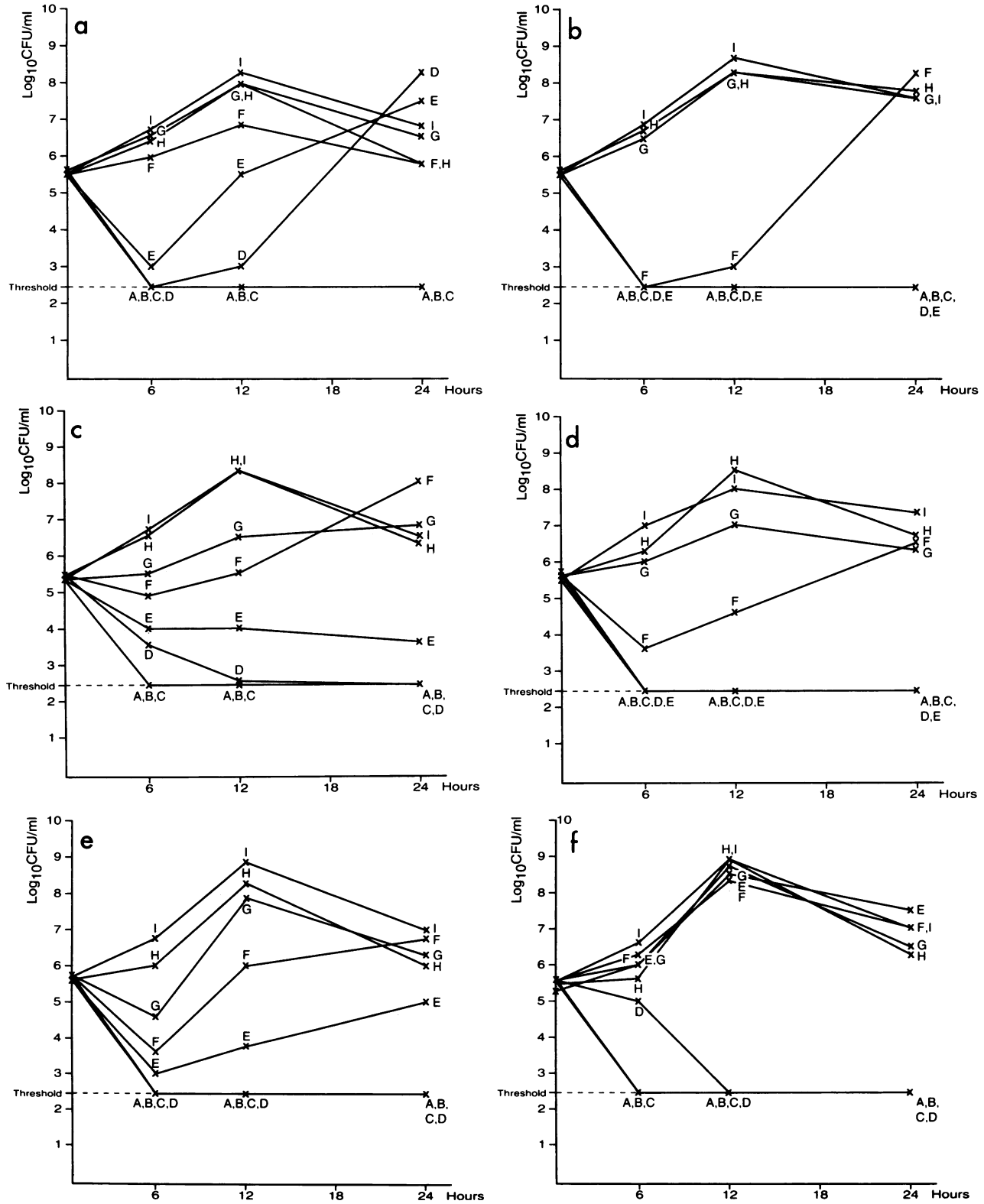

FIG. 2. Time-kill kinetics of penicillin G (a), RP 59500 (b), erythromycin (c), ciprofloxacin (d), sparfloxacin (e), and vancomycin (f) against a penicillin-intermediate-resistant pneumococcus (strain 5) at 0, 6, 12, and 24 h. (a, e, and f) A, 2.0 μg/ml; B, 1.0 μg/ml; C, 0.5 μg/ml; D, 0.25 μg/ml (agar MIC); E, 0.125 μg/ml; F, 0.06 μg/ml; G, 0.03 μg/ml; H, 0.015 μg/ml; I, growth control. (b) A, 4.0 μg/ml; B, 2.0 μg/ml; C, 1.0 μg/ml; D, 0.5 μg/ml (agar MIC); E, 0.25 μg/ml; F, 0.125 μg/ml; G, 0.06 μg/ml; H, 0.03 μg/ml; I, growth control. (c) A, 256 μg/ml; B, 128 μg/ml; C, 64 μg/ml; D, 32 μg/ml (agar MIC); E, 16 μg/ml; F, 8.0 μg/ml; G, 4.0 μg/ml; H, 2.0 μg/ml; I, growth control. (d) A, 32 μg/ml; B, 16 μg/ml; C, 8.0 μg/ml; D, 4.0 μg/ml (agar MIC); E, 2.0 μg/ml; F, 1.0 μg/ml; G, 0.5 μg/ml; H, 0.25 μg/ml; I, growth control.

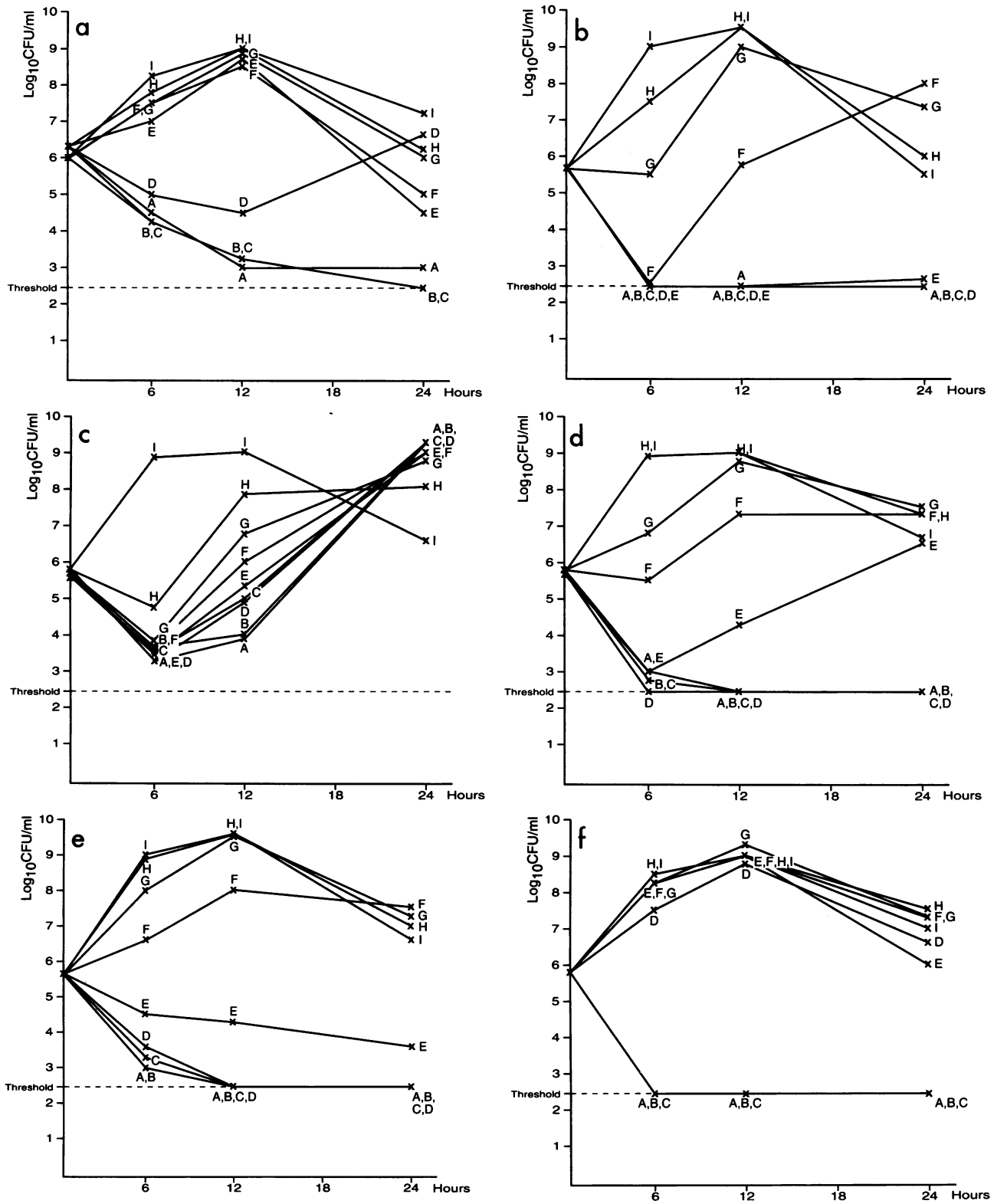

FIG. 3. Time-kill kinetics of penicillin G (a), RP 59500 (b), erythromycin (c), ciprofloxacin (d), sparfloxacin (e), and vancomycin (f) against a penicillin-resistant pneumococcus (strain 228) at 0, 6, 12, and 24 h. (a) A, 16.0 μg/ml; B, 8.0 μg/ml; C, 4.0 μg/ml; D, 2.0 μg/ml (agar MIC); E, 1.0 μg/ml; F, 0.5 μg/ml; G, 0.25 μg/ml; H, 0.125 μg/ml; I, growth control. (b) A, 8.0 μg/ml; B, 4.0 μg/ml; C, 2.0 μg/ml; D, 1.0 μg/ml (agar MIC); E, 0.5 μg/ml; F, 0.25 μg/ml; G, 0.125 μg/ml; H, 0.06 μg/ml; I, growth control. (c) A, 64.0 μg/ml; B, 32.0 μg/ml; C, 16.0 μg/ml; D, 8.0 μg/ml (agar MIC); E, 4.0 μg/ml; F, 2.0 μg/ml; G, 1.0 μg/ml; H, 0.5 μg/ml; I, growth control. (d) A, 32.0 μg/ml; B, 16.0 μg/ml; C, 8.0 μg/ml; D, 4.0 μg/ml (agar MIC); E, 2.0 μg/ml; F, 1.0 μg/ml; G, 0.5 μg/ml; H, 0.25 μg/ml; I, growth control. (e and f) A, 2.0 μg/ml; B, 1.0 μg/ml; C, 0.5 μg/ml; D, 0.25 μg/ml (agar MIC); E, 0.125 μg/ml; F, 0.06 μg/ml; G, 0.03 μg/ml; H, 0.015 μg/ml; I, growth control.

TABLE 2. Effect of RP 59500 on 10 pneumococci within 10 min of addition of antimicrobial agent to organisms

Drug concn	Log_{10} colony count (CFU/ml) for the following strains[a]:									
	Penicillin susceptible				Penicillin intermediate resistant			Penicillin resistant		
	294	64	60	2	114B	5	114	227	167	228
8× MIC	−3	−2	−2	0	−1	−4	0	0	0	−3
4× MIC	−3	−2	−2	0	0	−4	0	0	0	−3
2× MIC	0	−2	−2	0	0	−3	0	0	0	−2
MIC	0	0	0	0	0	−3	0	0	0	0
0.5× MIC	0	0	0	0	0	0	0	0	0	0

[a] Log_{10} colony count lower than that at time zero without the antimicrobial agent. −1, Δ1 log_{10} CFU/ml = 90% killing; −2 = Δ2 log_{10} CFU/ml = 99% killing; −3 = Δ3 log_{10} CFU/ml = 99.9% killing.

and compared with the MICs from the growth curves. Of the 60 organism-antimicrobial agent combinations tested by the agar and broth dilution methodology, MICs were within one doubling dilution in 57 instances. The three instances in which there was disagreement were with erythromycin: for strains 60 and 294 agar were MICs 0.06 μg/ml, whereas growth curve MICs were 0.008 and 0.015 μg/ml, respectively. For strain 228 the agar MIC was 8.0 μg/ml, whereas it was >64.0 μg/ml in broth. The latter strain showed an inducible pattern of erythromycin resistance, with viability rapidly decreasing at 6 h but then rapidly increasing by 24 h, which is in contrast to the case for the drug-free control, which grew rapidly for 6 h but was in the decline phase at 24 h (see Fig. 3).

Time-kill experiments. MICs and MBCs were determined from growth curves (see Materials and Methods). In a few instances in which the initial inocula were between 1×10^5 and 3×10^5 cfu/ml, a 3-log_{10}-unit decrease in the viable count was just below the viability count detection limit of 300 CFU/ml (16); this was regarded as bactericidal because the counts remained below 300 CFU/ml from 6 h onward.

The results of the time-kill experiments for three strains (one penicillin-susceptible, one penicillin-intermediate-resistant, and one penicillin-resistant strain) are shown in Fig. 1 to 3, respectively. The one strain for which the time-kill experiments were repeated yielded similar results on both occasions. Time-kill studies showed that antibiotic concentrations greater than the MIC were bactericidal for each strain, with the following exceptions. Erythromycin was initially bactericidal for one penicillin-resistant and erythromycin-resistant strain at 6 h, but rapid regrowth then occurred (Fig. 3). Three penicillin-susceptible strains were bacteriostatically inhibited by erythromycin at concentrations greater than or equal to the MIC by 6 h. One penicillin-susceptible strain (penicillin MIC, 0.06 μg/ml) was bacteriostatically inhibited by penicillin G at 24 h at or at one-half the MIC; a bactericidal effect was found only with penicillin G at concentrations of ≥0.25 μg/ml.

At 10 min after inoculation a 1- to 3-log_{10}-unit reduction (90 to 99.9%) in the original inoculum was seen for 6 of 10 strains with RP 59500 at and above the MIC (Table 2). This effect was not found with any of the other compounds tested at any concentration. A bactericidal effect was found at ≥6 h with RP 59500 at concentrations of one-half to one-quarter the MIC in 7 of 10 strains, and a bacteriostatic effect was found in 3 of 10 strains, with regrowth by 24 h. One penicillin-resistant strain was examined by the time-kill methodology at 0, 1, 2, and 3 h. RP 59500 at a concentration equal to the MIC was bactericidal within 1 h, and at a concentration of one-half the MIC it was bactericidal within 3 h. This phenomenon was not seen with the other antimicrobial agents tested.

Regrowth of strains at ciprofloxacin concentrations equal to or at one-half to one-quarter the MIC was found. For sparfloxacin, three of four penicillin-susceptible strains and two of four penicillin-resistant strains were bacteriostatically inhibited by 6 h. Bactericidal effects at 6, 12, and 24 h were found with both intermediate-resistant, one penicillin-susceptible, and two penicillin-resistant strains. Complete killing was observed with vancomycin at concentrations greater than the MIC, with regrowth occurring at lower concentrations.

DISCUSSION

Time-kill studies are useful methods for examining the kinetic interaction between bacteria and antimicrobial agents. The viable count threshold of a 0.1-ml aliquot placed on a plate is theoretically 10 CFU/ml if one colony grows; however, for statistical accuracy the lower limit has been set at 30 colonies (300 CFU/ml, as in our study) (16), and this threshold was therefore used in Fig. 1 to 3. The problem of drug carryover was also addressed. We believe that spreading 0.1 ml of undiluted broth onto a plate containing 25 ml of medium would dilute the drug 1:250; further 10-fold dilutions would dilute drugs 1:2,500, 1:25,000, etc. With the concentrations of drugs used, only undiluted inocula would have had any potential for drug carryover, and only plates with low counts (<1,000 CFU/ml) would be likely to be affected. We therefore feel that drug carryover was not a confounding factor in the data generation. Irreversible drug binding could have played a role, but it would have occurred regardless of the method used. Because of the correspondence between agar dilution and growth curve MICs in the current study, we feel confident of the accuracy of our MIC methodology.

Although many studies of the in vitro susceptibilities of pneumococci to various agents by MIC techniques have been published, few have studied this parameter by the time-kill methodology. Yourassowsky and coworkers (27, 28) have reported rapid killing rates for cefdinir and cefaclor against penicillin-susceptible pneumococci. Jacobs et al. (15) have reported a paradoxical increase in viability count for four of eight strains tested with ciprofloxacin and with temafloxacin for two of eight strains tested. This phenomenon was not observed in our study. Barakett et al. (4) have documented the good bactericidal activity of imipenem by the time-kill methodology against penicillin-susceptible and -resistant pneumococci.

RP 59500 is a parenterally administered combination of RP 57669 and RP 54476, which are semisynthetic, water-soluble derivatives of pristinamycin I_A and pristinamycin II_A, respectively (23). Although each precursor molecule is bacteriostatic, the combination is bactericidal against gram-positive cocci by binding to bacterial ribosomes (3). The very rapid killing by RP 59500 may have therapeutic implications. Fremaux et al. (11) have reported a 4- to 5-log_{10}-unit decrease in colony counts of erythromycin-susceptible and -resistant strains of pneumococci with RP 59500 at 0.5- to 4.0-fold the MIC. Similarly, we have found that RP 59500 has excellent bactericidal activity at or below the MIC by 6 h, irrespective of the strain's susceptibility to erythromycin, as described previously (11, 25). An additional feature of RP 59500 was the very rapid bactericidal activity within 10 min in 6 of 10 of the strains tested. This was not found with any of the other compounds tested. Berthaud and colleagues (5), using an in vitro pharmacokinetic model, have reported a similar rapid decrease of 3.9 to 4.3 log_{10} CFU/ml within 2 h for three pneumococcal strains (one

erythromycin resistant, one penicillin intermediate resistant, and one erythromycin and penicillin susceptible).

It is interesting that the rapid killing by RP 59500 was seen in all three fully penicillin-susceptible pneumococci, whereas it was seen in only three of the six penicillin-intermediate-resistant and -resistant strains. This rapid killing effect may be related to the presence of autolysins in penicillin-susceptible strains. A loss of autolysins is usually seen in penicillin-resistant strains (20). There was no correlation between the bactericidal or the bacteriostatic activity of RP 59500 with the erythromycin susceptibilities of the strains.

For all pneumococci tested in the present study ciprofloxacin MICs were clustered around the breakpoint. This phenomenon, which has been described before (14, 25), together with the regrowth of strains in the presence of ciprofloxacin concentrations equal to or one-half to one-quarter the MIC, detracts from the value of this compound in the treatment of pneumococcal infections. Additionally, several case reports of overwhelming pneumococcal infections in patients while on ciprofloxacin therapy have appeared in the literature (9, 10, 12).

Although sparfloxacin was active against all strains, with MICs ranging between 0.125 and 0.5 μg/ml, this agent was bactericidal for only 5 of the 10 organisms studied. Sparfloxacin appears to have two mechanisms of bactericidal activity, one which is dependent on protein and RNA syntheses as well as bacterial cell division and a second which is independent of the former two factors (17). The antipneumococcal activity of sparfloxacin in our study was superior to that of ciprofloxacin by both the MIC and the time-kill methodologies. Since this in vitro system lacks many host parameters such as host defenses, clinical studies are required to delineate the in vivo significance (if any) of the regrowth-bacteriostatic phenomena.

The bacteriostatic activity of erythromycin was corroborated by the results of time-kill experiments, in which regrowth occurred after 12 to 24 h in one strain bactericidally inhibited at 6 h and three strains were bacteriostatically inhibited by 6 to 24 h. Vancomycin was very active against all strains by both the MIC and the time-kill methodologies.

The higher penicillin concentrations required for bactericidal activity relative to the MIC for the penicillin-susceptible strain (MIC, 0.06 μg/ml), together with the high MICs of other β-lactams and the presence of abnormal penicillin-binding proteins sometimes seen in these strains (14), is indicative of the fact that many strains in this group are probably, in reality, better classified as intermediately resistant to penicillin. For many of these strains the oxacillin zone diameter is <20 mm (14).

The results of the present study indicate that, of the new compounds tested, both RP 59500 and sparfloxacin show promise for use in the therapy of nonmeningitic infections caused by penicillin-susceptible and -resistant pneumococci. The significance of the very rapid bactericidal activity of RP 59500 deserves further study and warrants clinical evaluation.

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