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# CP-45,899 in Combination with Penicillin or Ampicillin Against Penicillin-Resistant *Staphylococcus*, *Haemophilus influenzae*, and *Bacteroides*

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CP-45,899 is a new, semisynthetic β-lactamase inhibitor. When tested alone, CP-45,899 displayed only weak antibacterial activity, with the notable exception of its potent action against penicillin-susceptible and -resistant *Neisseria gonorrhoeae*. A combination of 3.12 μg of CP-45,899 per ml with 3.12 μg of ampicillin per ml, tested in broth cultures, inhibited ca. 90% of resistant *Staphylococcus* and *Haemophilus influenzae* strains; similar data were obtained in a variety of media. The same combination of CP-45,899 with ampicillin or penicillin G inhibited 90% of *Bacteroides fragilis* as interpreted from agar dilution minimal inhibitory concentrations. Inhibitory concentrations of CP-45,899-ampicillin were bactericidal against *H. influenzae* strains and were as bactericidal as nafcillin or cephalothin against *S. aureus*. Ampicillin-resistant *S. aureus*, *H. influenzae*, and *B. fragilis* strains did not develop resistance to CP-45,899-ampicillin when transferred as many as six passages in the presence of a sublethal concentration of the combination.

CP-45,899 (penicillanic acid, 1,1-dioxide) is a chemically stable β-lactamase inhibitor, which synergistically increases the activity of β-lactam antibiotics against β-lactamase-producing gram-positive and gram-negative microorganisms (2). We have previously shown that ampicillin or penicillin G combinations with CP-45,899 were particularly potent against penicillin-resistant *Staphylococcus aureus*, *S. epidermidis*, *Haemophilus influenzae*, and *Bacteroides fragilis*. Good activity was observed with CP-45,899 as a single agent only against *Neisseria gonorrhoeae* (2). The purpose of the study reported below was to extend these findings with additional clinical isolates, measure bactericidal activity, assess the rate of resistance emergence, and examine the effect of growth media on the potency of CP-45,899-antibiotic combinations.

## MATERIALS AND METHODS

**Materials.** CP-45,899 was prepared in Pfizer Central Research Laboratories; ampicillin·$3H_2O$ and sodium penicillin G were supplied by the Quality Control Dept., Pfizer Inc. Amoxicillin·$3H_2O$ came from Carrera C. S. a. S., Milano. Sodium cephalothin and cephalexin·$H_2O$ were products of Eli Lilly & Co.; sodium cefoxitin was a product of Merck Sharp & Dohme. Sodium methicillin·$H_2O$ was a product of Bristol Laboratories, whereas sodium nafcillin·$H_2O$ came from Wyeth Laboratories.

Microorganisms used were recent clinical isolates obtained from hospitals in several geographical areas within the eastern United States. All of the penicillin-resistant strains of *H. influenzae* or *N. gonorrhoeae* produced a constitutive β-lactamase as determined by employing the chromogenic cephalosporin 87/312 substrate from Glaxo Research Ltd. (6). The penicillin-resistant strains of *Staphylococcus* were defined as resistant by the zone sizes observed around a 10-U penicillin disc in the standard disc susceptibility test (9).

In vitro susceptibility studies were performed in brain heart infusion (BHI) broth or agar as the basal medium. The medium was enriched with 5% Fildes (Difco) plus 2% IsoVitaleX (BBL Microbiology Systems) for *H. influenzae*; incubations were in an atmosphere of 5% $CO_2$, 10% $H_2$, 85% $N_2$, and a trace of $O_2$. Tests with *N. gonorrhoeae* were performed on gonococcus agar base (BBL Microbiology Systems) supplemented with hemoglobin and IsoVitaleX. Studies with *Bacteroides* were carried out in BHI media as described in the *Anaerobe Laboratory Manual* (5); incubation was in an 80% $N_2$, 10% $CO_2$, and 10% $H_2$ gas mixture either in an anaerobic chamber or in GasPak jars (BBL Microbiology Systems) equipped with gas-exchange capability.

**Methods. MIC.** Agar dilution minimal inhibitory concentrations (MICs) were determined by the method of Ericsson and Sherris (4) by using the multiple inoculator described by Steers et al. (10). Cultures grown overnight in BHI broth to $\geq 1 \times 10^9$ cells per ml were diluted 100-fold in BHI broth; thus ~20,000 cells of each strain were used as the inoculum.

The procedure for broth MIC determinations was similar (3). The overnight culture was diluted 1,000-fold in BHI broth, and 0.5 ml of this dilution was added to each cup containing 0.5 ml of BHI broth plus antibiotics. Thus the final inoculum was $\leq 1 \times 10^6$ cells per ml. Incubations were at 37°C for 18 h with *S. aureus*, *S. epidermidis*, and *H. influenzae* and for 24 h with *Bacteroides* species. The *N. gonorrhoeae* inoculum was prepared by scraping growth from an agar plate and emulsifying it in 1 ml of BHI broth. Turbidity was adjusted to a no. 2 MacFarland standard. A loopful of inoculum was streaked over the agar surface followed by incubation at 37°C in a GasPak jar in the presence of 5% $CO_2$, 10% $H_2$, and 85% $N_2$ for 24 to 48 h. Synergy was defined as occurring when the MIC of each component in the combination was one-fourth (two $\log_2$ dilutions) or less its MIC as single agent. Antagonism was not observed.

**MBC.** Values for CP-45,899 in combination with ampicillin were measured by first determining MICs in broth medium. Cups containing broth lacking visible growth in tests for MICs were subcultured by streaking a loop calibrated to deliver 0.01 ml over the surface of antibiotic-free agar medium. The agar plates were incubated overnight (anaerobic techniques were used with *B. fragilis*). The minimal bactericidal concentration (MBC) was defined as the lowest concentration of antibiotic permitting growth of ≤5 colonies on subculture. Thus, the MBC indicates ≤500 colony-forming units (CFU) per ml or, as recommended, 99.95% kill (1). Bactericidal values for *H. influenzae* isolates were determined in enriched BHI broth as described above. MIC inoculum consisted of $\sim 5 \times 10^6$ CFU/ml; incubation was aerobic at 37°C for 24 h, and 0.01 ml was subcultured as described above. Thus, the MBCs for *H. influenzae* represent a 99.99% kill of the original MIC inoculum.

**Killing rates.** Experiments with CP-45,899 in combination with ampicillin or penicillin G were initiated by diluting an overnight culture in BHI broth (enriched for *B. fragilis*) containing an appropriate concentration of antibiotic. During incubation (on a shaker for *S. aureus*), aliquots were removed and diluted at 2-log intervals, and triplicate 0.1-ml portions of the appropriate dilutions were spread on BHI agar plates. After overnight incubation, colonies were counted and recorded as CFU/milliliter. All experimental procedures with *B. fragilis* were carried out in an anaerobic chamber.

**Resistance emergence studies: *S. aureus*.** After an initial MIC determination in BHI broth, the highest concentration supporting growth comparable to the antibiotic-free control was diluted 1,000-fold and used as inoculum in the next MIC determination. This procedure was repeated five times. The initial MIC of *H. influenzae* was measured in enriched BHI broth. The first dilution with good growth was diluted 10-fold and incubated overnight. This culture was diluted 10-fold for use as the inoculum in the next MIC determination. The average *H. influenzae* MIC inoculum was $\sim 3.7 \times 10^7$ CFU/ml. This procedure was repeated six times. Except for anaerobic techniques and the use of freshly prepared enriched BHI, the resistance emergence pattern of *B. fragilis* was studied in the same manner as was *S. aureus*.

**β-Lactamase studies.** Cell-free β-lactamase preparations of *S. aureus*, *S. epidermidis*, and *H. influenzae* were prepared as described previously (2). The *H. influenzae* and *B. fragilis* strains produced a constitutive enzyme. The *B. fragilis* culture, initially containing $\sim 10^7$ cells per ml, was grown for 5 h anaerobically, at which time 1 mM dithiothreitol and 0.01 M β-mercaptoethanol were added. The cells were then harvested, and the cell-free β-lactamase was prepared as described previously (2) with the addition of the above sulfhydryl reagents to all solutions. All cell-free extracts were stored at −76°C. The rate of hydrolysis was determined by the micro-iodometric assay of Zimmermann and Rosselet (11). Ampicillin at 30 μM (10.5 μg/ml) was the substrate, and incubation was at 37°C for 10 min. The *B. fragilis* enzyme was assayed with potassium penicillin G at 30 μM (12.5 μg/ml) as substrate, and incubation was for 20 min at 37°C. The amount of CP-45,899 required to produce 50% inhibition was estimated from plots of inhibition versus CP-45,899 concentration over a range of 30 μM (7.6 μg/ml) to 1 μM.

## RESULTS AND DISCUSSION

CP-45,899 rapidly and potently inhibited β-lactamases from *S. aureus*, *S. epidermidis*, *H. influenzae*, and *B. fragilis* (Table 1). Of the three genera, the highest concentration of CP-45,899 required to produce 50% inhibition was about 3 to 5 μg/ml for the *Staphylococcus* enzymes. Because CP-45,899 is both a competitive and noncompetitive (irreversible) inhibitor, the extent of inhibition will increase with increasing incubation period (2; J. A. Retsema and W. U. Schelkly, Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, A27, p. 5).

This β-lactamase inhibition translates into potent synergistic antibacterial effects when CP-45,899 is combined with ampicillin, penicillin G, or amoxicillin as evidenced by the data in Tables 2 and 3. The CP-45,899-penicillin combinations inhibited 90% or more of the resistant strains at 3.12 plus 3.12 μg per ml, respectively. The antibiotic combinations were as potent as cephalexin against resistant *S. aureus* and more potent than cephalexin against *S. epidermidis*- and *H. influenzae*-resistant strains (Table 2). Against *Bacteroides* species (Table 3), the antibiotic combinations were more potent than cefoxitin.

The mechanism of methicillin resistance in *Staphylococcus* is not due to β-lactamase hydrolysis of methicillin; however, most of these strains do harbor a β-lactamase (7). Consequently, the penicillin-CP-45,899 combinations generally produced a pronounced synergistic effect and were significantly more potent than cephalexin (Table 4). Ratios of penicillin to CP-45,899 of 2:1 and 4:1 were more potent than a 1:1 ratio only with methicillin-resistant staphylococci (Table 4). This probably reflects the reduced importance of β-lactamase as a resistance determinant in these strains, i.e., approximately the same amount of β-lactamase inhibitor was required to inactivate the β-lactamase of a methicillin-resistant *Staphylococcus* as was required for a normally resistant *Staphylococcus*. Ampicillin-CP45,899 (1:1) has been shown to be effective in protecting mice infected with a methicillin-resistant *S. aureus* strain (2).

The type of growth media employed significantly affected the potency of amoxicillin when tested against 25 resistant *S. aureus* strains. In three different broth and agar media, the median MICs of amoxicillin varied from a low of 3.12 μg/ml on Trypticase soy agar (BBL Microbiology Systems) to a high of >200 μg/ml in BHI broth. However, the median MICs of amoxicillin-CP-45,899 (1:1) varied only from 0.78-0.78 to 1.56-1.56 μg per ml. The addition of 50% inactivated human serum to agar growth medium did not appreciably affect the potency of amoxicillin alone or amoxicillin-CP-45,899 (1:1).

TABLE 1. *Ability of CP-45,899 to inhibit β-lactamases from Staphylococcus, Haemophilus, and Bacteroides*

| Source of cell-free β-lactamase | μmol of substrate hydrolyzed/h per mg of protein | Concn of CP-45,899 required to decrease hydrolysis by 50% in 10-min assay (μM) |
|---|---|---|
| *S. aureus* | | |
| 01A400 | 2,270 | 11 |
| 01A109 | 1,430 | 9 |
| 01A137[a] | 1,480 | 18 |
| *S. epidermidis* | | |
| 01B087[a] | 180 | 21 |
| *H. influenzae* | | |
| 54A037 | 7,690 | 3.5 |
| 54A066 | 6,170 | 2 |
| 54A048 | 4,410 | <1 |
| *B. fragilis* | | |
| 78C049 | 430 | 7[b] |

[a] Strains which are also resistant to cefazolin, cephalexin (MIC, >50 μg/ml), and methicillin.
[b] Incubation period was 20 min.

The MICs of CP-45,899, as a single agent, ranged from 0.15 to 2.5 μg per ml against susceptible and resistant *N. gonorrhoeae* (Table 5). This suggests that CP-45,899 might be considered as a single agent in the therapy of *N. gonorrhoeae* infections. The combination ampicillin-CP-45,899 was two- to four-fold more active than CP-45,899 alone against the β-lactamase-producing strains tested (Table 5).

**Bactericidal activity.** The MBCs of the ampicillin-CP-45,899 antibiotic combination against *S. aureus* were generally only one dilution higher than the MIC except for the β-lactam-tolerant strains (Table 6A). (Five of the *S. aureus* strains met the definition of β-lactam tolerance [8], i.e., had an MBC ≥64-fold higher than the MIC for nafcillin and cephalothin.) The MBCs of the combination antibiotic were equal to or lower than those observed with either nafcillin or cephalothin for many of the strains. In a killing-rate experiment, the combination of 3.12-3.12 μg per ml killed 99.9% of *S. aureus* cells

TABLE 2. *Concentrations of single β-lactam antibiotic and penicillin-CP45,899 combinations that inhibit 90% of resistant Staphylococcus and Haemophilus*[a]

| Organism | Concn (μg/ml) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | CP-45,899 | Ampicillin | Amoxicillin | Penicillin | Cephalexin | Ampicillin-CP-45,899 | Amoxicillin-CP-45,899 | Penicillin G-CP-45,899 |
| *S. aureus*[b] (45) | 200 | 200 | >200 (70) | >200 | 6.25 | 3.12–3.12 | 3.12–3.12[c] (70) | 3.12–3.12 |
| *S. epidermidis*[b] (16) | >50 | 12.5[c] | | 50 | 12.5[c] | 1.56–1.56[c] | | 3.12–3.12[c] |
| *H. influenzae*[d] (25) | 200 | 100[c](8) | | 50 | 100 | 3.12–3.12[c] (8) | | 3.12–3.12[c] |

[a] Numbers in parentheses are the number of isolates tested.
[b] Growth medium was BHI broth.
[c] This concentration also inhibited 100% of the strains tested.
[d] Growth medium was enriched BHI agar.

TABLE 3. *Concentrations of single β-lactam antibiotic or penicillin-CP45,899 combinations that inhibit 90% of the Bacterioides examined*

| Organism | Concn (μg/ml) | | | | | |
|---|---|---|---|---|---|---|
| | CP-45,899 | Ampicillin | Penicillin G | Cefoxitin[a] | Ampicillin-CP-45,899 | Penicillin G-CP-45,899 |
| *B. fragilis* (53 strains) | 200 | 200 | 200 | 12.5 | 3.12–3.12 | 3.12–1.56 |
| *B. thetaiotaomicron* (21 strains) | 50 | 25 | 25 | 25 | 1.56–1.56 | 1.56–0.39 |
| *B. vulgatus* (13 strains) | 100 | 25 | 50 | 25 | 3.12–3.12 | 3.12–0.78 |

[a] Cefoxitin evaluated against 25/53 *B. fragilis*, 6/21 *B. thetaiotaomicron*, and 7/13 *B. vulgatus* strains.

TABLE 4. *Effect of penicillin G-CP-45,899 combinations against methicillin-resistant Staphylococcus*

| Organism | % of strains inhibited | Inhibitory concn (μg/ml): Methicillin | Cephalexin | CP-45,899 | Ampicillin | Ampicillin-CP-45,899 1:1 | Ampicillin-CP-45,899 2:1 | Ampicillin-CP-45,899 4:1 | Penicillin G | Penicillin G-CP-45,899 1:1 | Penicillin G-CP-45,899 2:1 | Penicillin G-CP-45,899 4:1 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *S. aureus* (14 strains) | 50 | 12.5 | 100 | >400 | 200 | 12.5–12.5 | 12.5–6.25 | 12.5–3.12 | 100 | 12.5–12.5 | 12.5–6.25 | 12.5–3.12 |
| | 75 | 25 | 100 | | 200 | 12.5–12.5 | 12.5–6.25 | 12.5–3.12 | 200 | 12.5–12.5 | 12.5–6.25 | 25–6.25 |
| | 100 | 50 | 200 | | 400 | 12.5–12.5 | 25–12.5 | 25–6.25 | 400 | 25–25 | 25–12.5 | 25–6.25 |
| *S. epidermidis* (7 strains) | Median | 12.5 | 12.5 | >200 | 25 | 3.12–3.12 | 3.12–1.56 | 3.12–0.78 | 25 | 1.56–1.56 | 3.12–1.56 | 3.12–0.78 |

TABLE 5. *In vitro activity of CP-45,899 against N. gonorrhoeae*

| Strain no. | MIC (μg/ml): Ampicillin | CP-45,899 | Ampicillin-CP-45,899 |
|---|---|---|---|
| Susceptible strains | | | |
| F-18 CDC | 0.07 | 0.15 | 0.07–0.07 |
| G-9 | 0.09 | 0.31 | 0.09–0.09 |
| 66001 | 0.02 | 0.31 | 0.02–0.02 |
| 66008 | 0.02 | 0.15 | 0.02–0.02 |
| 66010 | 0.04 | 0.20 | 0.02–0.02[a] |
| 66011 | 0.02 | 0.31 | 0.01–0.01[a] |
| Resistant strains | | | |
| CDC 1 | >10 | 1.2 | 0.31–0.31[b] |
| CDC 2 | >10 | 2.5 | 0.9–0.9[a] |

[a] Additive response.
[b] Synergistic response.

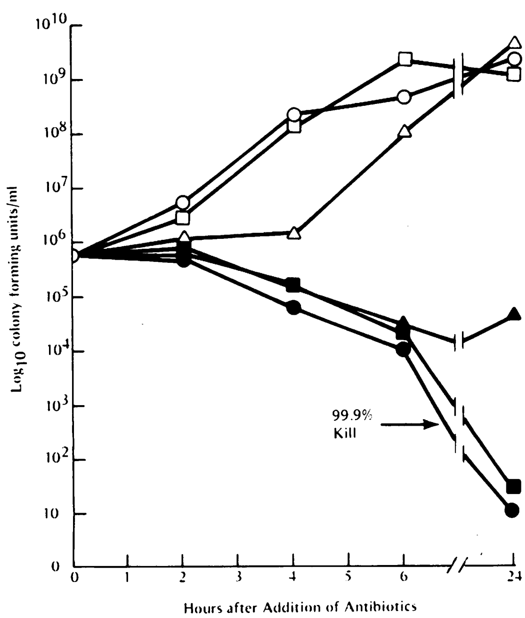


FIG. 1. *Killing rates with the ampicillin-CP-45,899 combination against an ampicillin-resistant S. aureus strain. Symbols: ○, Control, S. aureus 01A400 (MBC for ampicillin-CP-45,899 is 3.12-3.12 μg/ml); □, 10 μg of CP-45,899 per ml; △, 3.5 μg of ampicillin per ml; ●, ampicillin-CP-45,899, 1:1, 3.12-3.12 μg/ml; ▲, ampicillin-CP-45,899, 8:1, 6.25-0.78 μg/ml; ■, ampicillin-CP-45,899, 1:8, 0.78-6.25 μg/ml.*

in 24 h (Fig. 1). The combination of 6.25 μg of CP-45,899 per ml plus 0.78 μg of ampicillin per ml was also bactericidal, but the reverse ratio was not (Fig. 1). This indicates that sufficient β-lactamase inhibitor was required for maintaining bactericidal activity.

The ampicillin-CP-45,899 antibiotic combina-

TABLE 6. *Comparison of the bacteriostatic-bactericidal activity of a 1:1 combination of ampicillin-CP-45,899 against S. aureus, H. influenzae, and B. fragilis*[a]

| Organism | % of strains inhibited | Ampicillin | | CP-45,899 | | Ampicillin-CP-45,899 | | Cephalothin[c] | | Nafcillin | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | MIC | MBC[b] | MIC | MBC | MIC | MBC | MIC | MBC | MIC | MBC |
| (A) *S. aureus* (28 strains) | 50 | 100 | 200 | 200 | >200 | 1.56–1.56 | 3.12–3.12 | 0.10 | 3.12 | 0.2 | 0.78 |
| | 75 | 100 | >200 | | | 3.12–3.12 | 6.25–6.25 | 0.20 | 12.5 | 0.4 | 25 |
| | 90 | 200 | | | | 3.12–3.12 | 25–25 | 0.39 | 25 | 3.12 | 50 |
| | | | | | | | | Cephalexin MIC | | | |
| (B) *H. influenzae* (17 ampicillin-resistant strains) | 50 | 200 | >400 | 100 | 100 | 1.56–1.56 | 1.56–1.56 | 6.25 | | | |
| | 75 | 400 | | 100 | 200 | 1.56–1.56 | 3.12–3.12 | 50 | | | |
| | 100 | >400 | | 200 | 200 | 3.12–3.12 | 3.12–3.12 | >50 | | | |
| | | | | | | | | Cefoxitin MIC | | | |
| (C) *B. fragilis* (6 highly ampicillin-resistant strains) | 50 | 400 | >800 | 25 | 25 | 3.12–3.12 | 3.12–3.12 | 3.12 | | | |
| | 100 | 800 | | 50 | 100 | 12.5–12.5 | 12.5–12.5 | 12.5 | | | |

[a] Values are in micrograms per milliliter.

[b] MBC is equivalent to ≥99.9% kill of an inoculum of $\geq 10^6$ CFU/ml, except with *H. influenzae* with which the MBC is equal to ~99.99% kill of an inoculum of $5 \times 10^6$ CFU/ml.

[c] 17 strains tested instead of 28.

TABLE 7. *Lack of resistance emergence to ampicillin/CP-45,899 with resistant strains of S. aureus, H. influenzae, and B. fragilis*

| Organism | % of strains inhibited | Initial inhibitory concn (μg/ml) | | | Inhibitory concn after 6 and 7 transfers[a] in the presence of: | | |
|---|---|---|---|---|---|---|---|
| | | Ampicillin | CP-45,899 | Ampicillin-CP-45,899 (1:1) | Ampicillin | CP-45,899 | Ampicillin-CP-45,899 (1:1) |
| (A) *S. aureus* (16 strains) | 50 | 50 | 200 | 0.78–0.78 | 200 | 200 | 3.12–3.12 |
| | 75 | 50 | 200 | 1.56–1.56 | 400 | 200 | 3.12–3.12 |
| | 100 | 200 | 200 | 3.12–3.12 | >400 | 400 | 6.25–6.25 |
| | Median of 4 methicillin-resistant strains[b] | 200 | 400 | 3.12–3.12 | 400 | >400 | 9.38–9.38 |
| (B) *H. influenzae* (inoculum of ~4 × $10^7$ CFU/ml) | Median of 3 susceptible strains | 0.39 | 100 | 0.20–0.20 | 0.20 | 100 | 0.39–0.39 |
| | Median of 7 resistant strains | >400 | 50 | 6.25–6.25 | >400 | 100 | 6.25–6.25[c] |
| (C) *B. fragilis* (highly resistant to ampicillin) | Median of 7 strains | 400 | 25 | 3.12–3.12 | 400 | 25 | 6.25–6.25 |

[a] Cultures of *H. influenzae* and *B. fragilis* were transferred seven times and *S. aureus* was transferred six times in the presence of a sublethal amount of β-lactam antibiotic or combination.
[b] MIC of cefazolin and cephalexin against these strains was >50 μg/ml.
[c] The MIC of combination remained the same except for one strain in which it increased by one tube (3.12 to 6.25).

tion was exceptionally bactericidal for resistant *H. influenzae* strains (Table 6B). The MBC for all of the 17 strains tested was ≤3.12-3.12 μg/ml, employing a criterion of a 99.99% kill of the initial inoculum.

The MBC of ampicillin-CP-45,899 versus six strains of *B. fragilis* highly resistant to ampicillin was the same as the MIC (Table 6C). In a killing-rate experiment starting with a high inoculum ($5 \times 10^7$ cells per ml), penicillin G-CP-45,899 (3.12-3.12 μg/ml) killed >99.9% of the penicillin G-resistant *B. fragilis* cells within 6 h (Fig. 2).

**Resistance emergence studies.** When *S. aureus* strains resistant to ampicillin, but rather susceptible to the ampicillin-CP-45,899 antibiotic combination (MIC ≤0.78-0.78 μg/ml), were cultured in the presence of a sublethal concentration of the combination antibiotic, the MICs increased about two dilutions after three transfers. The MIC then remained constant at 3.12-3.12 μg/ml for three additional transfers (Table 7A). With *S. aureus* strains that had an initial MIC of 1.56-1.56 or 3.12-3.12 μg/ml for the combination, the MIC increased only one dilution after six transfers. The same type of relationship seemed to apply to the small sample of methicillin-resistant strains that were tested (Table 7A). The MIC of ampicillin-CP-45,899 remained the same after seven transfers of resistant *H. influenzae* strains in the presence of sublethal amounts of the combination antibiotic (Table 7B). The high inoculum used (~$4 \times 10^7$ CFU/ml) should have increased the probability of resistance emerging.

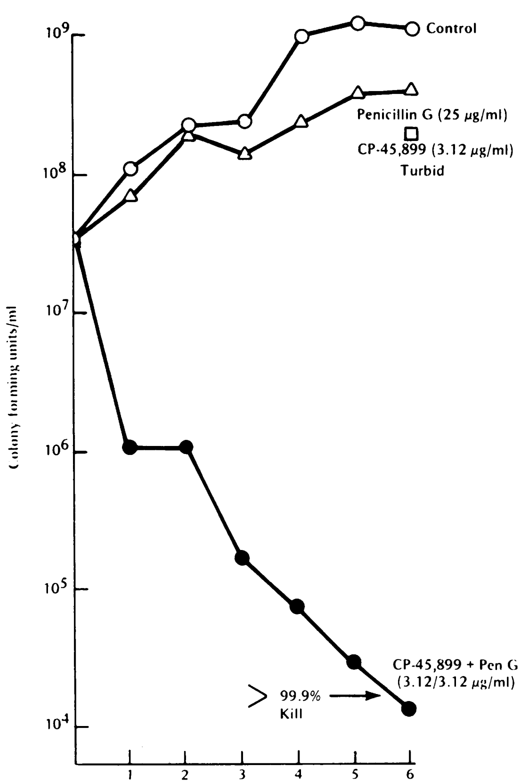


FIG. 2. *Killing rates with the penicillin G-CP-45,899 combination against a penicillin-resistant B. fragilis strain. Symbols:* ○, *control, B. fragilis 78C004 (MIC for penicillin G is 200 μg/ml, that for CP-45,899 is 25 μg/ml, and that for pencillin G-CP-45,899 is 1.56-1.56 μg/ml);* □, *3.12 μg of CP-45,899 per ml;* △, *25 μg of pencillin G per ml;* ●, *pencillin G-CP-45,899 at 3.12-3.12 μg/ml.*

The median MICs of antibiotic combinations increased only one dilution after seven transfers with *B. fragilis* strains highly resistant to ampicillin (Table 7C).

Thus, penicillin-resistant *S. aureus*, *H. influenzae*, and *B. fragilis* strains could not readily adapt (synthesizing more β-lactamase, increasing cell wall permeability barriers, etc.) to the ampicillin-CP-45,899 combination and counteract it. Also, if a few highly resistant cells existed in the initial population of the cultures tested, these cells were not able to become dominant in the culture after six or seven transfers in the presence of the antibiotic combination.

The 1:1 combinations of ampicillin-CP-45,899 and penicillin G-CP-45,899 demonstrated potent bactericidal activity against batteries of clinical isolates of ampicillin-resistant *S. aureus*, *H. influenzae*, and *B. fragilis*. CP-45,899, as a single agent, was active against susceptible and resistant *N. gonorrhoeae*. The potency of the antibiotic combinations is such that >90% of the resistant strains of *S. aureus*, *S. epidermidis*, *H. influenzae*, and *Bacteroides* species were inhibited by ≤3.12-3.12 μg/ml. The pharmokinetics properties of CP-45,899 (G. H. Foulds et al., Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 19th, Boston, Mass., abstr no. 312, 1979) are such that this potency is sufficient to allow the penicillin G-CP-45,899 and ampicillin-CP-45,899 antibiotic combinations to have value as chemotherapeutic agents. Ampicillin-CP-45,899 has been shown to be active in several mouse infection models (2; A. R. English et al., Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 18th, Atlanta, Ga., abstr. no. 147, 1978).

In vitro potencies similar to those reported here for ampicillin-CP-45,899 and penicillin G-CP-45,899 combinations have been observed by other investigators (C. Elster et al., abstr. no. 146; W. H. Khan et al., abstr. no. 145; N. Aswapokee et al., abstr. no. 144; and C. N. Baker et al., abstr. no. 293, all papers in Program Abstr.

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