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# Comparative Kill and Growth Rates Determined with Cefdinir and Cefaclor and with *Streptococcus pneumoniae* and β-Lactamase-Producing *Haemophilus influenzae*

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**The relationship between the growth rate and the kill rate was used to evaluate and to compare the in vitro bactericidal activities of cefdinir, a new oral cephalosporin, and cefaclor against *Streptococcus pneumoniae* and β-lactamase-producing strains of *Haemophilus influenzae*. These frequently encountered pathogens of community-acquired respiratory tract infections are usually susceptible to both drugs. The MIC ranges for cefdinir and cefaclor were, respectively, 0.03 to 0.06 and 0.25 to 0.5 μg/ml for *S. pneumoniae* and 0.25 and 4 to 8 μg/ml for *H. influenzae*. The colony counts (CFU per milliliter) measured after 6 h of exposure to a range of antibiotic concentrations in broth were plotted against the colony count of the control culture over the same period of time. Higher kill rates versus bacterial growth rates were noted for *S. pneumoniae* for both drugs (positive balance). Conversely, lower kill rates versus growth rates were noted for *H. influenzae* for both drugs (negative balance). In conclusion, the bactericidal activities of both drugs against *S. pneumoniae* and *H. influenzae* were similar when expressed by the relationship between the growth rate and the kill rate at 6 h, but cefdinir was more active at lower concentrations.**

The evaluation of the bactericidal activities of antibiotics against different bacterial species remains an unresolved problem in clinical microbiology. There is considerable controversy about the frequency of antibiotic tolerance (17) in different species of bacteria. Technical factors, such as antibiotic carryover, adherence to test tube walls, medium composition, and pH, etc., are known to influence bactericidal activities (3, 4, 6, 7, 10, 11, 18, 19). The organisms being tested should be in the logarithmic phase of growth, since most antibiotics act more rapidly on growing bacteria. On the other hand, the rapid autolysis of *Streptococcus pneumoniae* and the possible degradation of antibiotics during the first 24 h influence the conventional measurement of the MBC. Classically, the MBC is defined as the minimum concentration of drug required to kill 99.9% of the inoculum. Usually, only one measurement of the MBC is performed, after 24 h.

Cefdinir is a new oral beta-lactam that is stable against β-lactamase (14) and that should be useful for the treatment of upper and lower respiratory tract infections. We compared the bactericidal activity of cefdinir with that of cefaclor, one of the most commonly used oral cephalosporins in these clinical settings. The test model used in our laboratory examined kill rates in relationship to growth rates for strains of *S. pneumoniae* and *Haemophilus influenzae* after 6 h. This method reduced the influence of the rapid autolysis of *S. pneumoniae* on bactericidal activity measurements, the possible degradation of the test antibiotics in vitro, and the potential for strain-dependent growth rates.

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## MATERIALS AND METHODS

**Bacterial strains.** The bacteria used in this study were clinical isolates from material collected by endoscopy or transtracheal aspiration from patients with infections of the bronchopulmonary tract. They included five penicillin-susceptible strains of *S. pneumoniae* belonging to different serotypes (identification and serotyping of the strains were confirmed by the World Health Organization Collaborating Center for Reference and Research on Pneumococci, Statens Serum Institut, Copenhagen, Denmark [J. Henrichsen]) and five strains of β-lactamase-producing *H. influenzae* identified by conventional methods. β-Lactamase production was detected by the nitrocefin test (Cefinase; BBL Microbiology Systems, Cockeysville, Md.).

**Antibiotics.** Standard antimicrobial powders were provided by Parke-Davis Co., Ann Arbor, Mich. (cefdinir) and by E. Lilly & Co., Indianapolis, Ind. (cefaclor). The solutions of the antibiotics were prepared on the day of use.

**Culture medium.** Mueller-Hinton medium (Mueller-Hinton II; BBL) (MH) supplemented with 3% heat-inactivated horse serum was used for pneumococcal testing, and 3% Fildes enrichment (BBL) was used for *H. influenzae* testing. Fresh media were prepared on the day of use.

**Antimicrobial susceptibility testing.** The susceptibilities of the five isolates of each of the two bacterial species were determined by the broth macrodilution technique (8). Tubes (5 ml) containing twofold serial dilutions, from 32 to 0.008 μg/ml, of the compounds in MH broth (supplemented in accordance with the bacterial species) were inoculated with the organisms to yield final inocula of approximately $10^5$ CFU/ml. The tubes were incubated for 24 h at 35°C and inspected for a lack of turbidity.

**Bactericidal activity determinations. (i) Preparation of inocula.** Portions of each of five colonies growing on MH agar were suspended in 20-ml screw-cap tubes, each containing 10 ml of MH broth (supplemented in accordance with the bacterial species). The tubes were placed in an incubator (test tube rotator; Cenco, Breda, The Netherlands), the

strains were allowed to grow until a turbidity equivalent to a 0.5 MacFarland standard was reached (model HF turbidimeter; Shaban Manufacturing Inc., Fredonia, N.Y.), and the cultures were diluted 100-fold.

Antibiotics were used at concentrations of 0.5, 1, 2, 4, 8, and 16 times the MIC for each strain. The initial bacterial concentration was approximately $10^6$ CFU/ml.

**(ii) Incubation.** All inoculated tubes, with and without antibiotics (final volume, 10 ml), were incubated on a test tube rotator at 35°C.

**(iii) Sampling and counting.** Colony counts were determined immediately after inoculation and vortexing and after 6 h with a Spiral inoculator system (Spiral Systems Inc., Cincinnati, Ohio). In this system, a variable cam-activated syringe dispenses the culture from the center to the edge of the plate in a logarithmically decreasing concentration in the form of an Archimedes spiral (5). With this system, only a limited number of dilutions are necessary to determine colony counts. The dispensed volume (0.05 ml) contained the undiluted bacterial culture and two samples of the original culture diluted 1/10 and 1/100 (at time zero and after 6 h). Large petri dishes (14-cm diameter) containing 30 ml of MH agar (supplemented in accordance with the bacterial species) were used. A Spiral 500 laser colony counter and a Casba 800 microprocessor (Spiral Systems Inc.) were used for bacterial counting. The lower limit of accuracy with this system is 30 colonies per plate (600 CFU/ml with a 0.05-ml inoculum). We selected 2.7 $\log_{10}$ CFU/ml as the lowest limit of accuracy for bacterial counts (22).

**Data presentation.** The decrease in growth (expressed as CFU per milliliter) after 6 h in antibiotic-containing medium was plotted against the increase in growth of the control culture over the same time period for each strain. This method of evaluation diminishes the effect of a slow-growing culture, which would be expressed as an artificially low bactericidal activity (20). A kill rate higher than the growth rate, i.e., a decrease in growth in the antibiotic-supplemented culture that is greater than the increase in growth in the control culture, was defined as a positive balance. A kill rate lower than the growth rate, i.e., a decrease in growth in the test culture that is smaller than the increase in growth in the control culture, was defined as a negative balance.

**Statistical analysis.** An index was defined to assess growth rate versus kill rate (Fig. 1). This index was defined as $b - a$. If the kill rate ($b_2$) was equal to the growth rate ($a$), the index was zero. If the kill rate ($b_3$) exceeded the growth rate, the index was defined as a positive balance. If the growth rate exceeded the kill rate ($b_1$), the index was defined as a negative balance. The Mann-Whitney test (15) was used to compare the differences between the effects of various concentrations of the same antibiotic on the 5 bacterial test strains and to compare differences between cefdinir and cefaclor at the same multiples of the MICs.

## RESULTS

The MIC ranges of cefdinir and cefaclor were, respectively, 0.03 to 0.06 and 0.25 to 0.5 μg/ml for the five *S. pneumoniae* strains and 0.25 and 4 to 8 μg/ml for the five *H. influenzae* strains. Cefdinir was more active in vitro than cefaclor against *S. pneumoniae* and *H. influenzae*.

The relationship between growth rate and kill rate, expressed as the change in log growth over 6 h, is presented in Fig. 2 for each strain of *S. pneumoniae*. For *S. pneumoniae*, the growth of the control at 6 h was increased by 2 to 3 $\log_{10}$ CFU/ml. At a concentration equal to the MIC, the growth

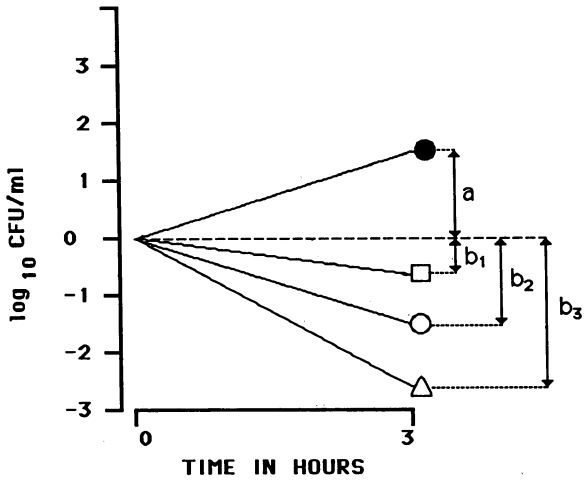


FIG. 1. Index of evaluation (change in colony counts plotted over time). Growth is indicated by a positive change in colony counts (●). Killing is indicated by a negative change in colony counts (□, ○, and △). Symbols: ●, $a$, growth rate; □, $b_1 - a$, negative balance; ○, $b_2 - a$, zero; △, $b_3 - a$, positive balance.

decreased by 2 to 3 $\log_{10}$ CFU/ml for three of five strains with cefdinir and four of five strains with cefaclor. For 2, 4, 8, and 16 times the MIC, the results were similar for cefdinir and cefaclor ($P > 0.05$). Overall kill rates were higher than overall growth rates over 6 h for *S. pneumoniae*. According to our definition, the index of bactericidal activity for both antibiotics was positive.

The kill rates for *H. influenzae* (Fig. 3) were lower ($P < 0.05$) than those for *S. pneumoniae* for each concentration from 1 to 16 times the MIC of both drugs. The index of bactericidal activity was negative. No concentration-dependent effects were noted for either drug ($P > 0.05$).

## DISCUSSION

*S. pneumoniae* and *H. influenzae* are frequently encountered in community-acquired upper and lower respiratory tract infections (9). Treatment is often begun empirically, and penicillin G, ampicillin, and cefaclor are all agents of choice. The bacteria in this study were actual clinical isolates from patients and included five penicillin G-susceptible *S. pneumoniae* and five β-lactamase-producing *H. influenzae* isolates. Exploring the true bactericidal activity of one drug versus another against various strains of bacteria poses some challenges.

Cefdinir was more active than cefaclor in vitro against *S. pneumoniae* and *H. influenzae*. However, the loss of cefaclor activity may be greater than 80% after 24 h (13). We have shown previously that the activity of this drug has been underestimated for this reason (24, 25). We attempted to minimize the possible degradation of antibiotic in the culture medium by making determinations at 6, rather than at 24 h. Additionally, evaluation of the bactericidal activities of beta-lactams against these bacteria has been difficult. Although there have been attempts to standardize the procedures used (12), various methods have been recommended for the preparation of the bacterial inoculum. Furthermore, normal, nontolerant organisms are killed more rapidly when they are exposed to beta-lactams during their logarithmic phase of growth than when they are tested after overnight incubation (10). Since growth curves are often species and

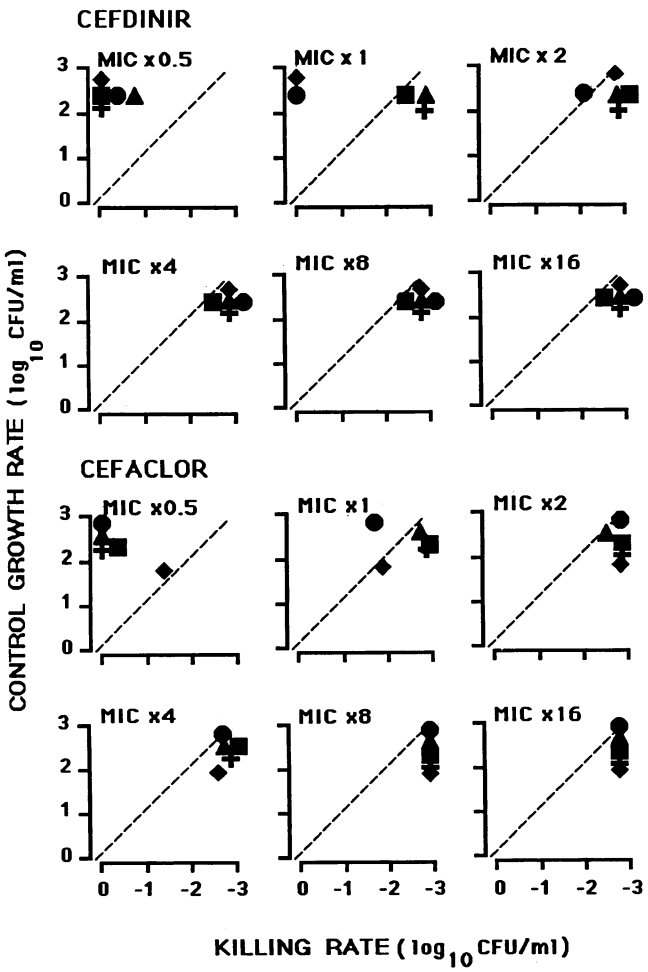


FIG. 2. Kill rates (decreases in $\log_{10}$ CFU per milliliter) over a 6-h period versus growth rates (increases in $\log_{10}$ CFU per milliliter) of the control cultures for *S. pneumoniae* (strains 1130 [+], 1900 [■], 1931 [●], 1537 [◆], and 1553 [▲]). The dotted line represents equivalent kill and growth rates for test and control cultures, respectively.

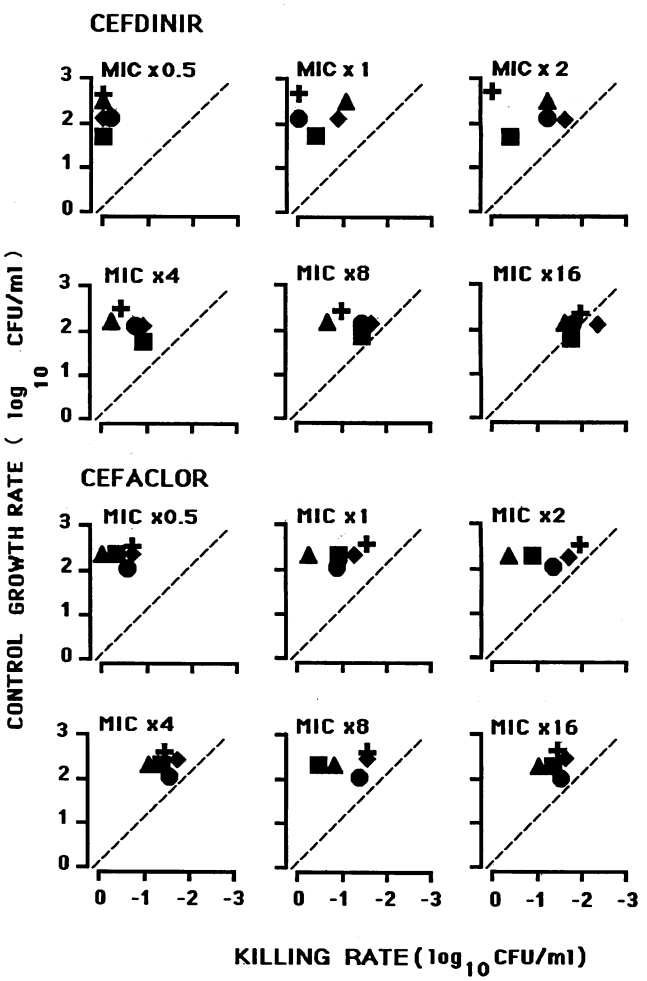


FIG. 3. Kill rates (decreases in $\log_{10}$ CFU per milliliter) over a 6-h period versus growth rates (increases in $\log_{10}$ CFU per milliliter) of the control cultures for *H. influenzae* (strains 894 [+], 31526 [■], 31794 [●], 2409 [◆], and 31219 [▲]). The dotted line represents equivalent kill and growth rates for test and control cultures, respectively.

strain dependent and it is difficult to consistently determine the mid-logarithmic phase of growth unless growth curves are continuously recorded, we attempted to minimize these experimental variables by comparing kill rates with growth rates simultaneously. Moreover, several investigators have pointed out the danger of using only one 24-h pattern to assess bactericidal action and also the relative inaccuracy of using an arbitrary breakpoint (such as 0.1% survival) or artificial indices (such as the MBC or the MBC/MIC ratio) to assess bactericidal effect (4, 6, 19). The conditions used to assess the tolerance of clinical isolates have varied considerably, and some investigators have been able to demonstrate the phenomenon only with stationary-phase cultures (10). Because of all these factors, our experimental model explored the kill rate for each strain relative to the growth rate of the control strain with the same inoculum after a shorter period, 6 h, to avoid the influence of the rapid autolysis of pneumococci and the degradation of the antibiotic that may occur after 24 h. A 6-h period was also convenient from a technical point of view, as it fit the laboratory technicians' normal working hours.

This study showed clear-cut differences in kill rates for *S. pneumoniae* and *H. influenzae*. Indeed, a positive balance (higher kill rates than growth rates) was observed for *S. pneumoniae* whereas a negative balance was observed for *H. influenzae* with both cephalosporins. No concentration-dependent effects were noted. Additional observations made by phase-contrast microscopy for *H. influenzae* (data not shown) confirmed the presence of large spheroplasts within the cultures containing antibiotics (1, 2, 16, 21, 23).

The overall results showed that the MIC ranges of cefdinir were lower than those of cefaclor for the strains of *S. pneumoniae* and *H. influenzae* tested. The relative stability of the molecules in broth medium may have influenced these values, as described above. Multiples of MICs were used to define the effective antibiotic concentrations in our study. Expressed in micrograms per milliliter, the mean effective concentrations for four times the MIC in this study were more than 10 times higher for cefaclor than for cefdinir. In conclusion, cefdinir had similar kill rates for *S. pneumoniae* and *H. influenzae* and was more active than cefaclor on an antibiotic-weight basis.

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