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Susceptibility of *Haemophilus influenzae* Type b to Cefaclor and Influence of Inoculum Size

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Cefaclor appeared to be an effective antibiotic against both beta-lactamase-positive and beta-lactamase-negative strains of *Haemophilus influenzae* type b when tested with 10^5 colony-forming units per ml. With inocula in excess of 10^6 colony-forming units per ml, these organisms were neither inhibited nor killed at concentrations of 400 μg/ml. This inoculum effect was also demonstrated in time-kill curve studies.

Cefaclor, an oral cephalosporin derivative with antibacterial activity against *Haemophilus influenzae*, has become a popular therapeutic choice for otitis media and for systemic *H. influenzae* infection after initial parenteral treatment with other agents in children. This study demonstrates the in vitro activity of cefaclor against *H. influenzae* type b, including beta-lactamase-producing strains, the effect of inoculum size, and the growth kinetics of these organisms in the presence of cefaclor.

Thirty strains of *H. influenzae* type b, including fifteen beta-lactamase-positive strains, were tested for susceptibility to cefaclor. Twenty-nine of these isolates were obtained from blood or cerebrospinal fluid samples from children with infection, and one was cultured from an infected eye sample. The definitions of minimal inhibitory and bactericidal concentrations and the methodology used have been described previously (1). Minimal inhibitory and bactericidal concentrations were determined for three inoculum sizes, 10^4, 10^6, and 10^7 colony-forming units (CFU)/ml, prepared by diluting an overnight-growth suspension of the organisms adjusted to a McFarland no. 0.5 turbidity standard.

Time-kill curves were determined with cefaclor for five beta-lactamase-positive and five beta-lactamase-negative *H. influenzae* type b strains with both light (10^4 to 10^5 CFU/ml) and heavy (10^6 to 10^7 CFU/ml) inocula. The organisms were grown overnight in Mueller-Hinton broth with 4% Fildes enrichment. A concentration of 2 μg/ml was chosen for the cefaclor because it correlated with achievable middle-ear fluid levels obtained after oral therapy (5). Each test sample (total volume, 4 ml) was incubated at 37°C in a candle jar, and 0.5-ml portions were removed at 0, 4, 8, and 24 h. These were serially diluted and streaked onto Fildes-enriched Mueller-Hinton agar. Colonies were counted after 24 h of incubation. Specimens from antibiotic-free control tubes were sampled simultaneously, and colony counts were determined in the same manner.

With the standard inoculum (10^4 to 10^5 CFU/ml), 100% of both beta-lactamase-positive and beta-lactamase-negative strains were inhibited by 1.56 μg of cefaclor per ml, and 97% were killed with 3.12 μg/ml (Table 1). When the inoculum density was increased to 10^6 CFU/ml, there was little change in these results. When 10^7 CFU of *H. influenzae* type b per ml was used, not even 400 μg of cefaclor per ml could inhibit or kill the organisms whether beta-lactamase positive or negative.

Cefaclor at a concentration of 2 μg/ml was bactericidal (99.9% killing) within 24 h for both light and heavy inocula of beta-lactamase-negative *H. influenzae* type b strains in time-kill studies (Fig. 1). When beta-lactamase-positive *H. influenzae* type b strains were used, an initial decrease of greater than 2 logs occurred for both light and heavy inocula within 8 h of incubation (Fig. 2). When the light inoculum specimen was sampled at 24 h, complete killing was observed. However, the heavy inoculum of beta-lactamase-positive *H. influenzae* type b grew to achieve approximately the number of organisms of the antibiotic-free control tube after 24 h of incubation.

Susceptibility studies with *H. influenzae* type b strains are usually performed with an inoculum size of 10^5 CFU/ml (4, 6). Inoculum size has been shown to play an important role in determining the susceptibility of a given bacterium to an antibiotic. Although inoculum size has been shown to influence the susceptibility of *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae*, and other organisms to cefaclor

TABLE 1. In vitro activity of cefaclor against *H. influenzae* type b strains

H. influenzae strain	No. of strains tested	Inoculum size (CFU/ml)	Minimal inhibitory concn (μg/ml)	Minimal bactericidal concn (μg/ml)
Beta-lactamase negative	15	10^4	0.78–1.56	0.78–6.25
		10^6	1.56	1.56–3.12
		10^7	>400	>400
Beta-lactamase positive	15	10^4	0.78–1.56	0.78–3.12
		10^6	1.56	1.56–3.12
		10^7	>400	>400

(7), the effect of inoculum size on the susceptibility of *H. influenzae* to cefaclor remains controversial (2, 10).

We have shown that at the higher inoculum of *H. influenzae* type b (10^7 CFU/ml), cefaclor did not appear to be either inhibitory or bactericidal whether or not the organism produced beta-lactamase.

Organisms are generally not readily seen by microscopic examination of clinical specimens until they reach a concentration of 10^5/ml or more (8). Colony counts of organisms in infected middle-ear fluid have not been performed, but the fact that Halsted et al. (3) found that organisms were usually identifiable in Gram stains of such fluid would imply that concentrations of 10^5 to 10^6 are common. Concentrations of organisms in infected urine also fall in this range (9). Specimens of pus will commonly contain concentrations of 10^7 organisms per ml (11). Although certain humoral factors other than inoculum size and drug concentration affect the efficacy of an antibiotic, insufficient clinical improvement in a situation in which large numbers of *H. influenzae* might be present should prompt the physician to try an alternate drug. Since the *H. influenzae* strains that cause otitis media are usually nontypable, our results must be considered as possibly, but not definitely, pertinent to the treatment of that infection.

Finally, the previous work reported from this laboratory (1) for the same strains of *H. influenzae* type b as were used in this study dealt with the interactions between cefamandole and *H. influenzae* type b strains. In comparison with that study, cefaclor was found to be less active than cefamandole was against strains of *H. influenzae* type b.

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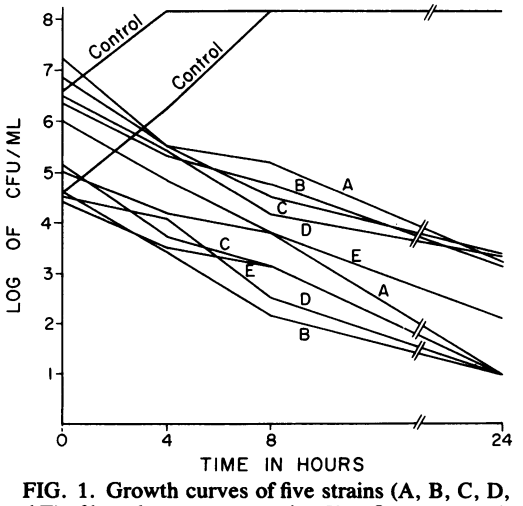

FIG. 1. Growth curves of five strains (A, B, C, D, and E) of beta-lactamase-negative *H. influenzae* type b at low (10^4 to 10^5) and high (10^6 to 10^7) inocula in cefaclor (2 μg/ml).

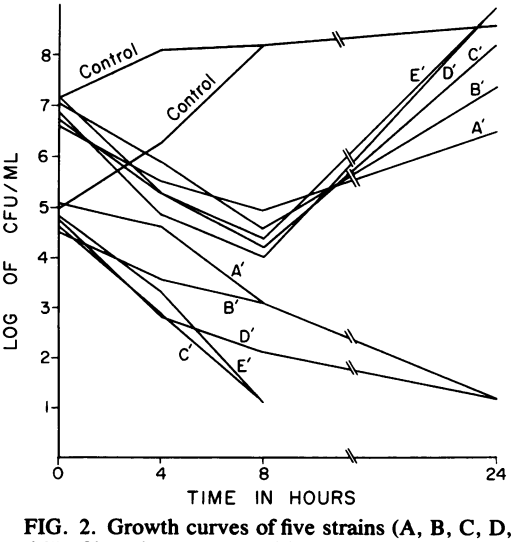

FIG. 2. Growth curves of five strains (A, B, C, D, and E) of beta-lactamase-positive *H. influenzae* type b at low (10^4 to 10^5) and high (10^6 to 10^7) inocula in cefaclor (2 μg/ml).

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