

## Comparison of the In Vitro Activity of Several Cephalosporin Antibiotics Against Gram-Negative and Gram-Positive Bacteria Resistant to Cephaloridine

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The in vitro activity of each of two oral [cefatrizine (BL-S640), cephalexin] and three parenteral (cefamandole, cefazolin, cephapirin) cephalosporin antibiotics was compared with that of cephalothin against 168 clinical isolates of gram-negative and gram-positive bacteria selected as resistant to 20  $\mu\text{g}$  of cephaloridine per ml on the basis of agar dilution susceptibility test data. Each of the five other cephalosporins inhibited a greater percentage of gram-negative bacillary isolates than did cephalothin or cephaloridine, with minimal inhibitory concentration values ranging 2- to 50-fold lower. Significant differences between minimal inhibitory concentrations of the compounds tested were also observed in tests against strains of *Streptococcus faecalis* and of methicillin-resistant *Staphylococcus aureus*. Potential advantages of including more than a single cephalosporin antibiotic in the panel of antibiotics used for routine susceptibility testing, suggested by these observations, are discussed.

The number of semisynthetic cephalosporin antibiotics approved or under development for use in clinical therapeutics continues to increase. Thus, chemical modification of the nucleus of cephalosporin C, 7-aminocephalosporanic acid, has yielded a family of potent antibacterial agents differing somewhat from each other with respect to certain clinically relevant properties such as potential local and systemic toxicity, pharmacokinetic characteristics, cost, and, to a lesser extent, antibacterial spectrum. However, with the possible exception of the nephrotoxicity of cephaloridine at doses above 4 g daily (3, 9), no generally accepted rationale exists at present that constitutes a compelling basis for the preferential selection of any of the existing compounds over the others. Indeed, no cephalosporin antibiotic is currently the drug of choice for any infection (7). The generally broad and apparently quite similar spectra of antimicrobial activity of the cephalosporanic acid derivatives that have been studied form the basis for the recommendation that, except under unusual circumstances, only a single cephalosporin, usually cephalothin, need be used in routine susceptibility testing procedures (2). In some laboratories cephaloridine is used for this purpose because of its greater chemical stability.

We report here data that suggest that the simultaneous use of more than one cephalosporanic acid derivative in routine clinical anti-

microbial susceptibility testing may result in more appropriate selection from among available antibiotics of a cephalosporin having potentially greater effectiveness for treatment of selected gram-negative or gram-positive bacterial infections.

### MATERIALS AND METHODS

**Bacteria.** One hundred forty-nine isolates of gram-negative bacteria and 13 isolates of *Streptococcus faecalis* recently obtained from clinical specimens submitted to the routine clinical bacteriology laboratory of the Bernalillo County Medical Center were collected, using resistance to at least 20  $\mu\text{g}$  of cephaloridine per ml in a standard agar dilution assay (6) as the criterion of selection. Seventy-eight strains of *Escherichia coli*, 47 of *Enterobacter*, 16 of *Klebsiella*, 6 of *Shigella*, and 2 of *Salmonella* comprised the gram-negative strains. All isolates were identified according to standard criteria (4) by or under the supervision of John A. Ulrich. Additionally, 8 strains of *S. aureus*, resistant to 12.5  $\mu\text{g}$  of methicillin/ml, collected from patients during an outbreak of surgical wound infections produced by this organism (8) were also included in these tests.

**Antibiotic susceptibility tests.** The minimal inhibitory concentration (MIC) of each antibiotic tested for each bacterial strain was determined by the agar dilution method recommended by the International Collaborative Study (6). Mueller-Hinton agar (Difco Laboratories, Detroit, Mich.) was the bacteriological medium used. The inoculum was 0.002 ml of an overnight broth culture containing approximately  $10^8$  organisms delivered by means of

a Steers replicator (10) (Melrose Machine Shop, Woodlyn, Pa.).

The antibiotics used were obtained as standard powders from their manufacturers: sodium cephalothin, cefamandole naphtate, cephalixin monohydrate, and sodium cefazolin from Eli Lilly and Co., Indianapolis, Ind.; and sodium cephalirin and cefatrizine (BL S640) from Bristol Laboratories, Syracuse, N.Y. The powders were stored at 4°C and diluted just before use in appropriate phosphate buffers before being added to the agar medium.

Except as indicated below, bacterial strains were defined as susceptible to a given concentration of antibiotic when fewer than 12 colonies were apparent after overnight (18 h, 35°C) incubation of the standard 0.002-ml inoculum. An identical simultaneous control inoculum included in each test for comparison yielded confluent bacterial growth.

### RESULTS

The inhibitory activity of each of the cephalosporins tested against 78 strains of *E. coli* is shown in Fig. 1. Only 8% of the strains tested were susceptible to 25 µg of cephalothin per ml and none was inhibited by lower concentrations. All other cephalosporins tested, except cephalirin, inhibited significantly larger percentages of *E. coli* strains. Cephalothin also exhibited considerably less inhibitory activity against the *Enterobacter* and *Klebsiella* strains tested than did the other cephalosporins (Fig. 2, 3). Cefamandole inhibited 79% of *Enterobacter* strains and 76% of *Klebsiella* strains at a concentration of 25 µg or less per ml. A similar percentage of *Klebsiella* strains was also inhibited by 25 µg or less of cefatrizine (75%) or

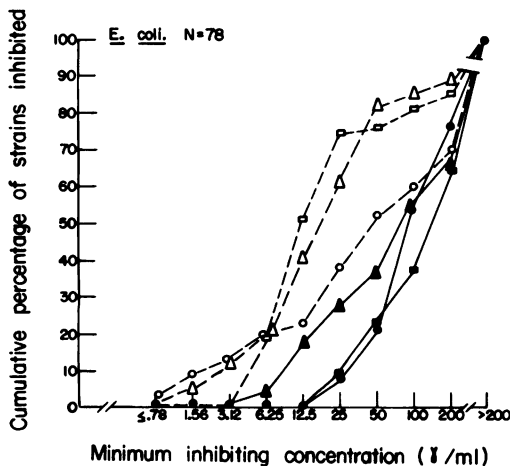


FIG. 1. Minimum inhibitory concentrations (micrograms per milliliter) of six cephalosporin antibiotics for 78 clinical isolates of *E. coli*. Symbols: ●, cephalothin; ○, cefamandole; ▲, cefatrizine (BL-S640); △, cefazolin; ■, cephalirin; □, cephalexin.

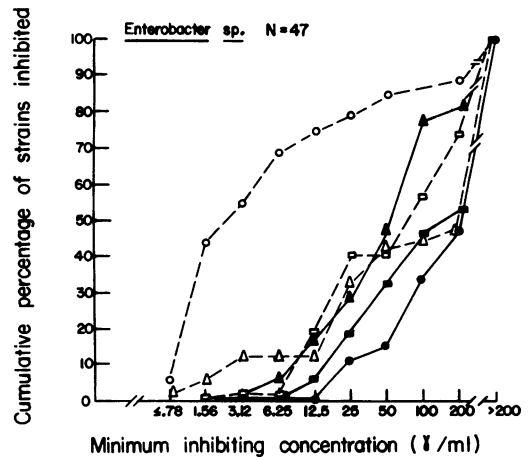


FIG. 2. Minimum inhibitory concentrations (micrograms per milliliter) of six cephalosporin antibiotics for 47 clinical isolates of *Enterobacter* species. Symbols: ●, cephalothin; ○, cefamandole; ▲, cefatrizine (BL-S640); △, cefazolin; ■, cephalirin; □, cephalexin.

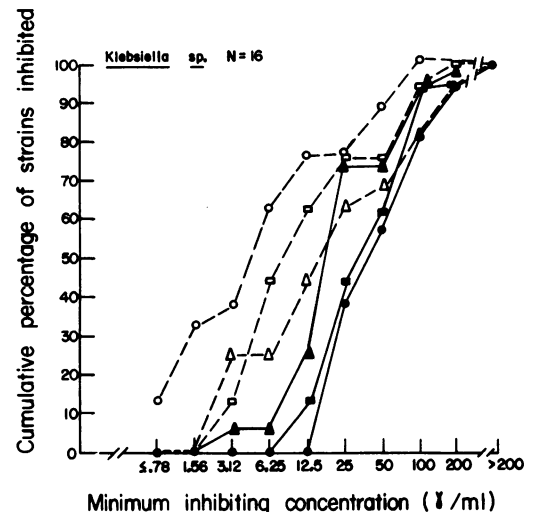


FIG. 3. Minimum inhibitory concentrations (micrograms per milliliter) of six cephalosporin antibiotics for 16 clinical isolates of *Klebsiella* species. Symbols: ●, cephalothin; ○, cefamandole; ▲, cefatrizine (BL-S640); △, cefazolin; ■, cephalirin; □, cephalexin.

cephalexin (76%) per ml. The latter drug alone of the compounds tested also inhibited all of six *Shigella* strains at a concentration of 12.5 µg or less per ml; only one of these was susceptible to 12.5 µg of cefatrizine, and another to 1.56 µg of cefamandole per ml. Neither of the two cephalothin-resistant *Salmonella* strains tested was inhibited by less than 25 µg of any other cepha-

losporin tested per ml, with the exception of the strain which was inhibited by 6.25  $\mu\text{g}$  of cephalixin per ml. Though none of the 13 *S. faecalis* strains that had been identified as resistant to 20  $\mu\text{g}$  of cephaloridine per ml was inhibited by 25  $\mu\text{g}$  or less of either cephalixin or cefatrizine per ml, 92 and 85% of these strains were inhibited by 25  $\mu\text{g}$  or less of cephalothin and cefamandole per ml, respectively, and all were susceptible to 25  $\mu\text{g}$  or less of cefazolin or cephapirin per ml (Fig. 4).

By the standard definition of antibiotic susceptibility used in these studies and stated above, none of six strains of *S. aureus*, all of which were resistant to 12.5  $\mu\text{g}$  of methicillin per ml, was identified as resistant to 12.5  $\mu\text{g}$  of either cephalothin, cephapirin, or cefamandole per ml; four of six appeared resistant to cefazolin and five of six to both cefatrizine and cephalixin. Using a more stringent criterion of susceptibility, i.e., no detectable growth whatsoever after 24 h of incubation at 37°C, all six strains were identified as resistant to cefatrizine and cephalixin; all six appeared susceptible to cefamandole and cephapirin, five of six appeared susceptible to cephalothin, and two of six appeared susceptible to cefazolin.

## DISCUSSION

The data presented here indicate that a substantial percentage of clinically isolated strains of gram-negative and gram-positive bacteria that are resistant in vitro to concentrations of cephaloridine and of cephalothin ordinarily achieved in the serum of patients receiving recommended dosages of these antibiotics may be susceptible, under identical in vitro test conditions, to concentrations of other cephalosporins that are readily attainable in the urine and in the serum of patients after administration of commonly used dosage schedules. Thus, for example, nearly 80% of 47 *Enterobacter* strains resistant to 12.5  $\mu\text{g}$  of cephalothin per ml were susceptible to 12.5  $\mu\text{g}$  or less of cefamandole per ml, and 75% of 16 similarly resistant *Klebsiella* strains were inhibited by the cephalosporin compound cefatrizine at similar concentrations. Table 1 presents a summary of the comparative data obtained. The magnitude of the observed MIC differences among the compounds tested varied widely over a range of 2- to 50-fold. Susceptibility differences of this magnitude possess obvious potential relevance to the design of clinical antimicrobial therapy of infections due to organisms designated "resistant" on the basis of routine antimicrobial susceptibility testing by the agar dilution tech-

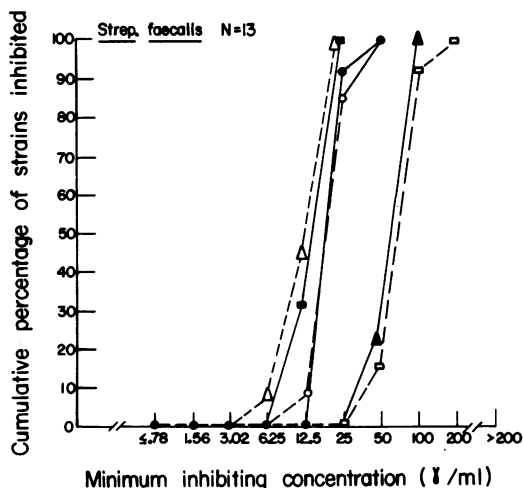


FIG. 4. Minimum inhibitory concentrations (micrograms per milliliter) of six cephalosporin antibiotics for 13 clinical isolates of *S. faecalis*. Symbols: ●, cephalothin; ○, cefamandole; ▲, cefatrizine (BL-S640); △, cefazolin; ■, cephapirin; □, cephalixin.

TABLE 1. Percentage of strains inhibited by 25  $\mu\text{g}$  of antibiotic per ml

Antibiotic	<i>E. coli</i> (78) <sup>a</sup>	<i>Enterobacter</i> (47)	<i>Klebsiella</i> (16)	<i>S. faecalis</i> (13)
Cephalothin	8	11	38	92
Cefamandole	39	79	76	85
Cefatrizine (BL-S640)	27	28	75	0
Cefazolin	61	33	63	100
Cephapirin	9	19	44	100
Cephalixin	75	40	76	0

<sup>a</sup> Number of strains tested.

nique using cephalothin or cephaloridine as the only prototype cephalosporin compound tested.

The significance of the apparent differences between cephalosporin MICs for the strains of methicillin-resistant *S. aureus* tested is uncertain. That these bacteria may appear falsely susceptible to cephalosporin antibiotics when standard in vitro susceptibility test procedures are used is well known in the case of cephalothin and cephaloridine (1, 5), and the therapeutic use of cephalosporins for the treatment of infections caused by methicillin-resistant *S. aureus* strains is not warranted.

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