

COMPARATIVE *IN VITRO* ACTIVITY OF CEPHALOSPORINS

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The *in vitro* activity of cephalixin, cephaloridine, cephalothin, cephapirin, cefoxitin, cephamycin C, cephradine, cephacetrile and cefazolin was determined against 443 isolates of bacteria. At a concentration of 12.5  $\mu\text{g/ml}$ , all of the cephalosporins inhibited more than 60 % of the isolates of *Klebsiella pneumoniae*. At the same concentration, cephalixin, cephaloridine, cephalothin, cephapirin, cephamycin C and cefazolin inhibited more than 90 % of isolates of *Proteus mirabilis*. All of the cephalosporins except cephalothin and cephapirin inhibited over 60 % of isolates of *Escherichia coli* at a concentration of 12.5  $\mu\text{g/ml}$ . Cefoxitin was the most active cephalosporin against gram-negative bacilli. There were substantial differences in the activity of cephalosporins against gram-positive cocci. Cephaloridine was the most active cephalosporin against these organisms. There was considerable fluctuation in the proportion of isolates of gram-negative bacilli susceptible to these cephalosporins from year to year, but there was no evidence to suggest that the number of resistant isolates was increasing.

Gram-negative bacilli are a common cause of infections in hospitalized cancer patients. Consequently, the discovery of new antibiotics with activity against these organisms are of major importance. Cephalosporins have proven to be significantly active against *Klebsiella pneumoniae*, *Escherichia coli*, *Proteus mirabilis* and most gram-positive cocci.<sup>1)</sup> Since the introduction of cephalothin, numerous derivatives have been synthesized which offer advantages such as oral administration, less toxicity and higher and more prolonged serum concentrations. This study was initiated to evaluate the comparative *in vitro* activity of the cephalosporins against gram-negative bacilli and gram-positive cocci and to determine whether the increased use of these antibiotics had resulted in the emergence of increasing numbers of resistant organisms.

### Materials and Methods

Susceptible testing was conducted on 443 isolates of gram-negative bacilli and gram-positive cocci using a dilution technique with an automatic microtiter system.<sup>2,3)</sup> The organisms were inoculated into MUELLER-HINTON broth (Difco) and incubated at 37°C for 18 hours. A 0.05-ml sample of 10<sup>-3</sup> dilution of the broth cultures of gram-negative bacilli and *Staphylococcus aureus* (approximately 10<sup>8</sup> colony forming units/ml) was used as the inoculum for the susceptibility tests. A 0.05-ml sample of a 10<sup>-2</sup> dilution of the broth cultures of *Diplococcus pneumoniae* and *Streptococcus pyogenes* (approximately 10<sup>8</sup> colony forming units/ml) was used as the inoculum.

All gram-negative bacilli were isolated from blood specimens of patients between 1967 and 1973. The majority of these patients were hospitalized at this institution and had underlying malignancies. A total of 160 isolates of *K. pneumoniae*, 130 isolates of *E. coli* and 88 isolates of *P. mirabilis* were tested.

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All gram-positive cocci were obtained from hospitalized patients, some of whom did not have cancer. A total of 15 isolates of *Diplococcus pneumoniae* and 50 isolates of *Staphylococcus aureus* were studied. Isolates of *S. aureus* were tested according to their susceptibility to penicillin G. Those 25 organisms inhibited by less than 0.10  $\mu\text{g/ml}$  were considered to be penicillin G susceptible and those 25 organisms resistant to more than 25  $\mu\text{g/ml}$  were considered to be penicillin G resistant.

The antibiotics, cephalothin, cephalexin and cephaloridine were supplied by Eli Lilly and Co., Indianapolis, Indiana. Merck, Sharp & Dohme supplied cephamycin C and cefoxitin. Cephradine, cephapirin, cefazolin and cephacetrile were obtained from the following companies, respectively: E. R. Squibb & Sons, Inc., Princeton, N. Y.; Bristol Laboratories, Syracuse, New York; Smith Kline & French, Philadelphia, Penn. and CIBA Pharmaceutical Co., Summit, N. J. Initial concentrations of 1,000  $\mu\text{g/ml}$  were prepared and appropriate dilutions from this stock concentration were made.

### Results

At a concentration of 12.5  $\mu\text{g/ml}$ , all of the cephalosporins except cephalothin and cephapirin inhibited over 60% of the isolates of *E. coli* (Fig. 1). Cephapirin and cephalothin inhibited only 6% and 38%, respectively. At the same concentration, all of the cephalosporins inhibited over 60% of the isolates of *K. pneumoniae* (Fig. 2). Cefoxitin inhibited 90%, but cephapirin inhibited only 65%. Cefoxitin, cephalothin and cefazolin were the most active cephalosporins against *P. mirabilis* (Fig. 3). At a concentration of 12.5  $\mu\text{g/ml}$ , cephalaxin, cephaloridine, cephalothin, cephapirin, cephamycin C and cefazolin inhibited

Fig. 1. Activity of cephalosporins against *Escherichia coli* (130 isolates tested).

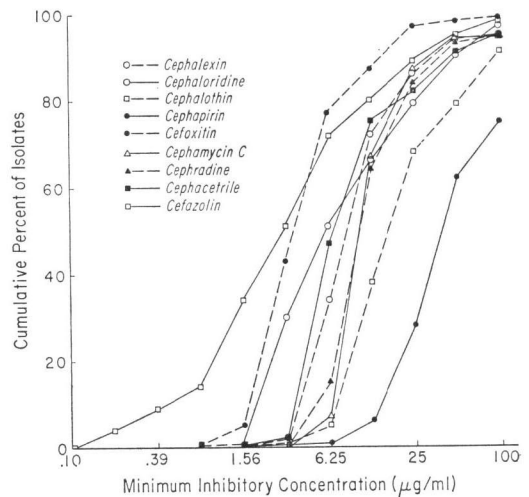


Fig. 2. Activity of cephalosporins against *Klebsiella* spp. (160 isolates tested).

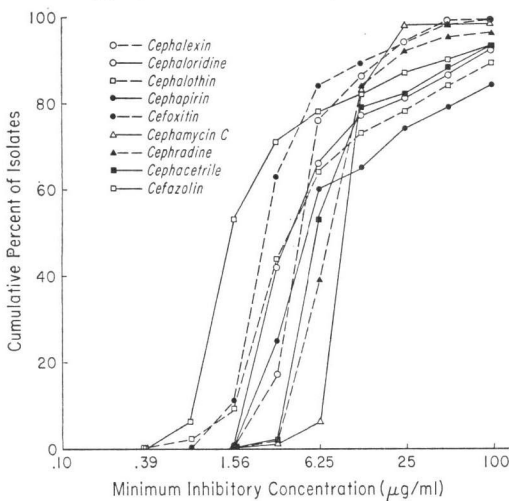


Fig. 3. Activity of cephalosporins against *Proteus mirabilis* (88 isolates tested).

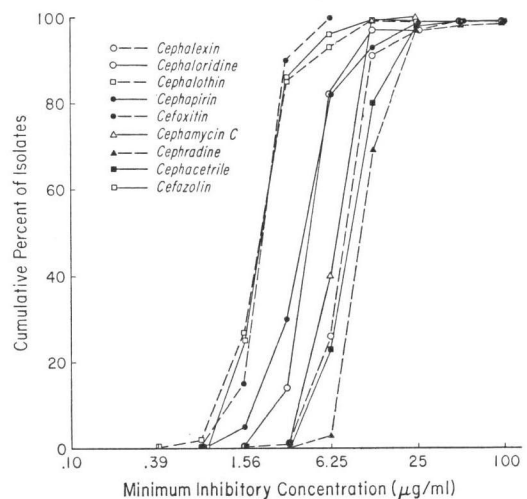
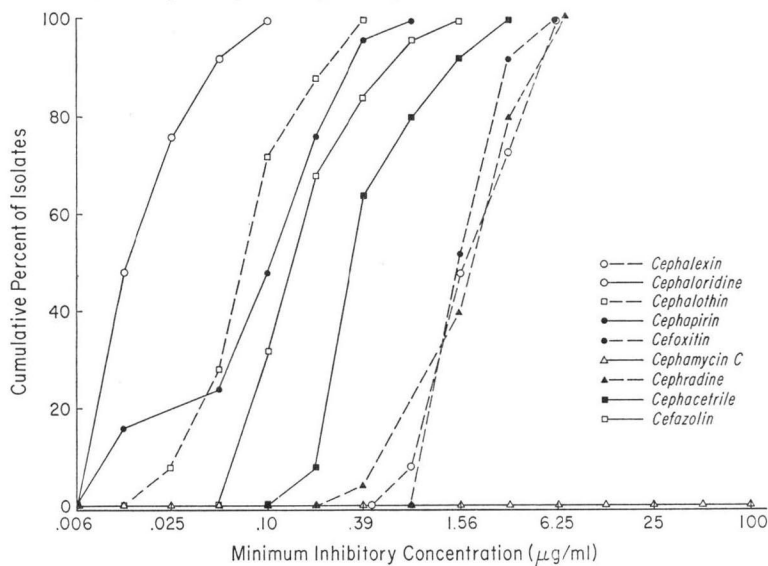
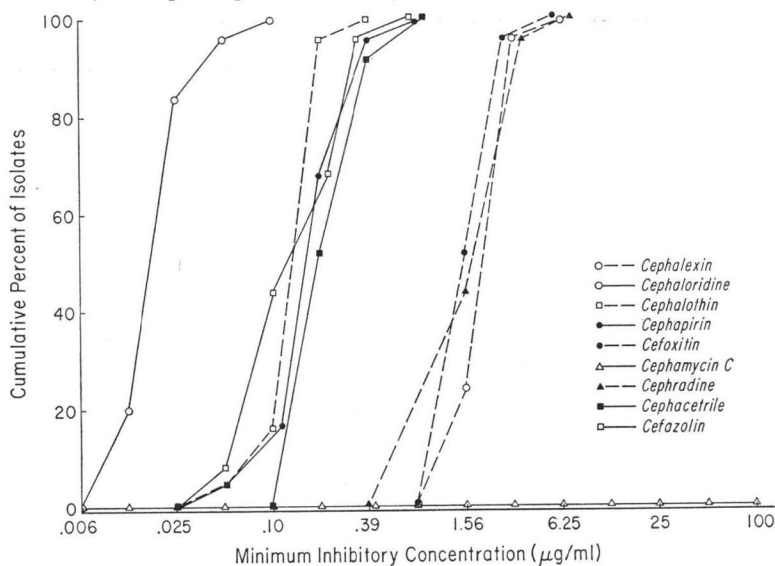


Fig. 4. Activity of cephalosporins against penicillin G resistant *Staphylococcus aureus*.Fig. 5. Activity of cephalosporins against penicillin G sensitive *Staphylococcus aureus*.

more than 90 % of these isolates.

Cefoxitin was consistently the most active cephalosporin against gram-negative bacilli. It inhibited 87 % of isolates of *E. coli* at a concentration of 12.5  $\mu\text{g/ml}$ . At a concentration of 6.25  $\mu\text{g/ml}$ , it inhibited 84 % of isolates of *K. pneumoniae* and 100 % of isolates of *P. mirabilis*.

There were substantial differences in the *in vitro* activity of the cephalosporins against penicillin G sensitive and resistant *S. aureus* (Figs. 4 and 5). Cephaloridine was the most active against both groups of *S. aureus*. Cephalothin, cefazolin, cephacetrile and cephapirin were intermediate in activity, and cefoxitin, cephalexin and cephradine were least active. Cephamycin C was inactive against both penicillin G sensitive and resistant *S. aureus*. There were

inhibited only 47 % at a concentration of 6.25  $\mu\text{g/ml}$ .

Table 1 lists the proportion of isolates of *E. coli* and *K. pneumoniae* sensitive to 12.5  $\mu\text{g/ml}$  of each cephalosporin by year. The susceptibility of these gram-negative bacilli has fluctuated substantially from year to year. Cephalothin has been used most extensively at this institution during this period. The proportion of isolates of *E. coli* susceptible to this antibiotic has varied from 16 % to 64 % whereas the proportion of susceptible *K. pneumoniae* has varied from 44 % to 92 %. Some of these differences are statistically significant. For example, the differences in susceptibility of isolates of *E. coli* to cephalothin in 1968 and 1973 compared to 1971 are statistically significant ( $p=.003$ ). Likewise, the differences in susceptibility of isolates of *K. pneumoniae* to cephalothin, cephalixin, cephaloridine, cephapirin and cefazolin in 1970 compared to 1968 are statistically significant ( $p=.001\sim.007$ ).

### Discussion

This *in vitro* data indicates that the cephalosporin antibiotics have a broad spectrum of activity against gram-negative bacilli and gram-positive cocci. Cefoxitin was the most active antibiotic against isolates of gram-negative bacilli. WALLICK and HENDLIN found similar results with their susceptibility testing of cefoxitin.<sup>4)</sup> Cefazolin inhibited 78 % of the *Klebsiella* spp. isolates at a concentration of 6.25  $\mu\text{g/ml}$ . TURCK *et al.* found similar results with cefazolin. In their study, more than 80 % of isolates were inhibited by a concentration of 5  $\mu\text{g/ml}$  or less.<sup>5)</sup> Cephapirin exhibited the least activity against isolates of *E. coli* and *Klebsiella* spp. whereas cephradine was least active against *P. mirabilis*.

*S. aureus* isolates were most sensitive to cephaloridine and cephalothin, and the results were similar regardless of their susceptibility to penicillin G. Other investigators found that cefazolin was equally active against penicillin G sensitive and resistant *S. aureus*.<sup>6)</sup> However, in this study, cefazolin was less active against penicillin G resistant isolates of *S. aureus*. At a concentration of 0.20  $\mu\text{g/ml}$ , cefazolin inhibited 68 % of penicillin G sensitive isolates, but only 24 % of penicillin G resistant isolates. Cefoxitin, cephalixin and cephradine were the least active cephalosporins against gram-positive cocci, except for cephamycin C which was inactive against *S. aureus* and only marginally active against *D. pneumoniae*. In NEU's study, cefoxitin had similar activity as cephalixin.<sup>6)</sup> In the study of BERGERON *et al.*, cefazolin and cephaloridine were more active than cephalothin and cephalixin against *D. pneumoniae*.<sup>7)</sup> However, cephaloridine is not as consistently active as cephalothin when a high inoculum of *S. aureus* is used.

The susceptibility of isolates of *E. coli* and *K. pneumoniae* to the cephalosporins has fluctuated substantially during the years from 1968 to 1973. Some of the most striking differences were observed with isolates of *K. pneumoniae*, comparing 1968 with 1970, differences which were statistically significant. It was not possible to correlate the dramatic decrease in cephalosporin susceptibility in 1970 to any change in antibiotic usage in this institution. Fortunately, since that time, the number of cephalosporin resistant isolates has decreased, although cephalothin usage was not altered. An explanation for this fluctuation in susceptibility is not readily apparent. Cefoxitin susceptibility fluctuated the least and it was the most active of the cephalosporins. This cephalosporin is resistant to hydrolysis by gram-negative bacilli. Some of the variability in the sensitivity of *E. coli* to cephalosporins may be due to the fact that *E. coli* vary in their ability to produce  $\beta$ -lactamases which results in their resistance. Another factor which may play a role is the degree of cell permeability to the antibiotics. Although *K. pneumoniae* seldom produce cephalosporinases, it is possible that the production of cephalosporinases may explain the changing susceptibility of these organisms to cephalothin.<sup>1)</sup>

*In vitro* evaluation of the cephalosporins has indicated that they have substantial differences in activity against gram-positive cocci and gram-negative bacilli. Consequently, use of a single

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*In vitro* evaluation of the cephalosporins has indicated that they have substantial differences in activity against gram-positive cocci and gram-negative bacilli. Consequently, use of a single

cephalosporin for routine *in vitro* susceptibility testing is probably inadequate. However, selection of the appropriate cephalosporin as therapy also depends on other factors such as their relative potential for causing thrombophlebitis and nephrotoxicity and their pharmacological disposition which may offer the advantage of higher serum concentrations or better tissue penetration.

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