IN VITRO SUSCEPTIBILITY OF CEPHALOTHIN-RESISTANT *ENTEROBACTERIACEAE* TO CEFOXITIN AND BL-S786

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The *in vitro* antibacterial activity of two agents relatively resistant to beta-lactamases, BL-S786 and cefoxitin, was tested against 123 recent different clinical isolates of cephalothin-resistant *Enterobacteriaceae*. BL-S786 showed considerable activity against *Escherichia coli* and lesser activity against *Klebsiella pneumoniae* with, respectively, 68% and 41% inhibited at 32 μg/ml. Cefoxitin showed more activity *in vitro* against *E. coli, K. pneumoniae, Serratia marcescens* and *Providencia stuartii*. Cefoxitin appears to be a more promising agent for treating infections caused by cephalothin-resistant *Enterobacteriaceae*.

The increasing resistance of clinical isolates of *Enterobacteriaceae* to cephalothin and related cephalosporins has parallelled the use of these agents.[1,2] A variety of cephalosporin derivatives with more antimicrobial potency and a wider spectrum of activity *in vitro* than cephalothin has recently been made available for *in vitro* testing and, in a few instances, clinical trials. Cefoxitin is a semisynthetic cephamycin resistant to many of the beta-lactamases elaborated by gram-negative bacilli resistant to cephalothin.[3] It has been shown to be active *in vitro* against many gram-negative bacilli including those resistant to cephalothin but is not active against *Enterobacter* strains.[2,4~6] Likewise, cefoxitin has been a promising agent in early clinical experience.[7]

BL-S786, 7-[α-(2-aminomethylphenyl) acetamido]-3-[(carboxymethyltetrazol-5-5 ylthio) methyl]-3-cephem-4-carboxylic acid, is more resistant to hydrolysis by β-lactamases of gram-negative bacilli than cephalothin or cefazolin. It has a wide spectrum of *in vitro* antibacterial activity including cephalothin-resistant *Enterobacter* sp., indole-positive *Proteus* sp., and *Citrobacter* sp. as well as isolates susceptible to cephalothin.[8] It is also effective in the therapy of experimentally infected mice.[8] This study compares the *in vitro* activity of BL-S786 and cefoxitin which has been the most promising β-lactam agent with our isolates[2] against *Enterobacteriaceae* resistant to cephalothin. Cefamandole was not included for testing because its advantage among the *Enterobacteriaceae* appears restricted to *Enterobacter* sp.[2,5,6]

Materials and Methods

Different clinical isolates of *Enterobacteriaceae* resistant to cephalothin by standardized disk testing[9] in the Microbiology Laboratory of Wadsworth V.A. Hospital were collected from November 1974 to November 1976 and identified by standard criteria. All isolates within a genus were from different patients. *Serratia marcescens* was identified to species by the fermentation of arabinose. Organisms

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showing a zone size of $\leq$14 mm to a 30 μg cephalothin disk on repeated standardized disk testing[9] were tested by the agar plate dilution method[10] recommended by the International Collaborative Study of the World Health Organization (ICS–WHO). Approximately 10^4 organisms grown overnight at 37°C in MUELLER-HINTON broth culture were inoculated with a replicating device[11] onto media prepared from MUELLER-HINTON broth solidified with 1.5% Difco agar and 5% defibrinated sheep blood prepared to contain cephalothin, cefoxitin, BL-S786 in twofold dilutions from 128 to 1 μg/ml. Plates identical except for lack of antibiotic were used as controls. Cephalothin was supplied by R. S. GRIFFITH of Eli Lilly & Co., cefoxitin by C. MARTIN of Merck, Sharp and Dohme Research Laboratories and BL-S786 by E. YEVAK of Bristol Laboratories.

The minimal inhibitory concentration (MIC) was recorded as the lowest concentration of antibiotic showing only a haze, one colony or no growth after overnight incubation.[10] Reference strains of *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 were included in parallel tests. All determinations were made in duplicate or triplicate and the MIC's expressed as averages. Only organisms with an MIC$\geq$32 μg/ml to cephalothin in agar dilution testing were included. One hundred and twenty-three isolates fulfilled these criteria and were included. A wide variety of serotypes of *S. marcescens* and *Klebsiella pneumoniae* was included to avoid duplication of strains.[12,13]

Results

The data are summarized on Figs. 1 through 5.

Serratia marcescens. Only cefoxitin showed any appreciable activity with 17 of 31 isolates (54.8%) inhibited at $\leq$32 μg/ml (Fig. 1). BL-S786 was inactive even at 128 μg/ml. (Fig. 1).

Klebsiella pneumoniae. BL-S786 showed a modicum of activity; 12 of 31 isolates (41.3%) were inhibited at 32 μg/ml but cefoxitin was considerably more active with 96.5% inhibited at the same level (Fig. 2).

Escherichia coli and *Citrobacter* sp. These organisms were combined for analysis because of their taxonomic relationship. Cefoxitin was active *in vitro* with 19 of 25 isolates (76%) inhibited at 32 μg/ml.

Fig. 1. Antibiotic susceptibility patterns of 31 different clinical isolates of cephalothin-resistant *S. marcescens.*
CF, cephalothin; CFX, cefoxitin; 786, BL-S786.

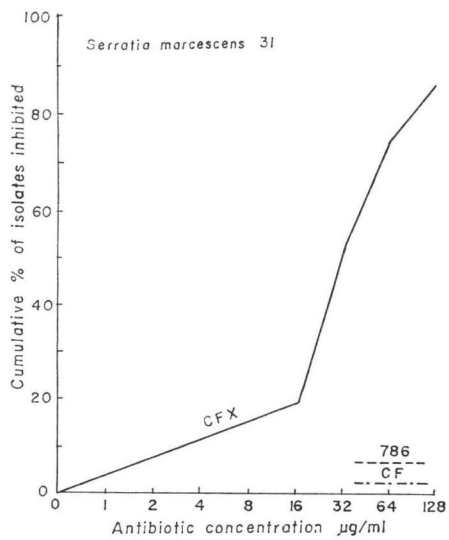


Fig. 2. Antibiotic susceptibility patterns of 29 different clinical isolates of cephalothin-resistant *K. pneumoniae.*
CF, cephalothin; CFX, cefoxitin; 786, BL-S786.

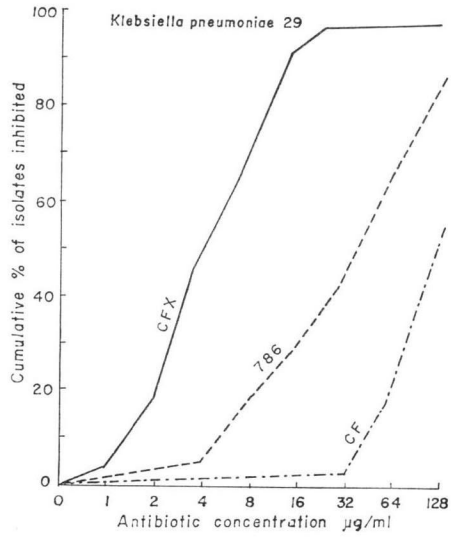


Fig. 3. Antibiotic susceptibility patterns of 19 different clinical isolates of *E. coli* and 6 of *Citrobacter* sp.
CF, cephalothin; CFX, cefoxitin; 786, BL-S786.

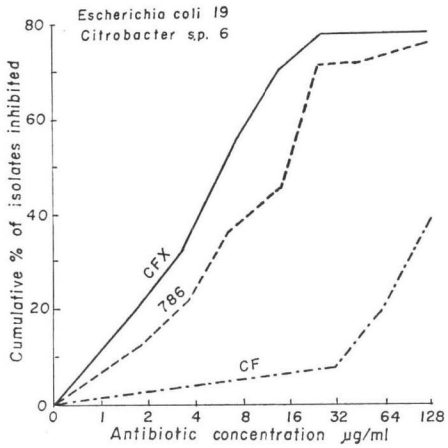


Fig. 4. Antibiotic susceptibility patterns of 19 different clinical isolates of *Enterobacter* sp.
CF, cephalothin; CFX, cefoxitin; 786, BL-S786.

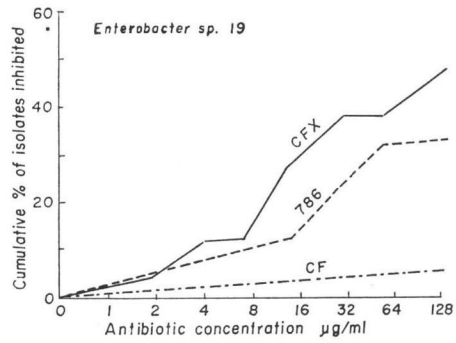
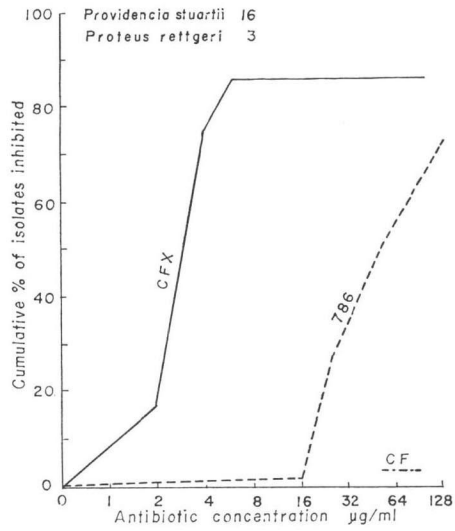


Fig. 5. Antibiotic susceptibility patterns of 16 different clinical isolates of *P. stuartii* and three of *P. rettgeri.*
CF, cephalothin; CFX, cefoxitin; 786, BL-S786.



The activity of BL-S786 was similar with 17 isolates inhibited at the same level; these isolates inhibited were *E. coli.* Inhibition of a considerable number of isolates by either cefoxitin or BL-S786 at the lower levels was noted (Fig. 3). None of the 6 *Citrobacter* sp. were inhibited at 128 μg/ml of BL-S786.

Enterobacter sp. Neither cefoxitin nor BL-S786 showed appreciable activity except for a few of the isolates (Fig. 4).

Providencia stuartii and *Proteus rettgeri.* Likewise, these isolates were combined for taxonomic reasons. Sixteen of 19 isolates were inhibited by cefoxitin at 8 μg/ml but only 5 of 19 (26.3%) by BL-S786 at 32 μg/ml. None of the three *P. rettgeri* were inhibited at a concentration of 128 μg/ml of BL-S786.

Discussion

Our results show that cefoxitin continues to show a high degree of *in vitro* activity against our cephalothin-resistant clinical isolates of *Enterobacteriaceae* and is more active overall than is BL-S786. There appears to be incomplete cross-resistance between the two compounds as some isolates not inhibited by BL-S786 at 64 or 128 μg/ml were inhibited by cefoxitin at lower concentrations; the converse was also noted.

Our results show that BL-S786 shows noteworthy activity against many cephalothin-resistant isolates of *E. coli, K. pneumoniae* and a smaller number of *Enterobacter* sp. and *P. stuartii.* The activity against *Enterobacter* sp. and *P. stuartii* agrees with the findings of LEITNER *et al.*[8] Our results of little activity against *Citrobacter* sp. and *P. rettgeri,* however, differ from those of LEITNER *et al.*[8] The small

number of isolates tested in either study prevents drawing any broad conclusions.

A major nosocomial problem has been noted with *S. marcescens* and *K. pneumoniae* resistant to cephalothin and many of the aminoglycosides.[12,13] Cefoxitin was more active than BL-S786 against these two genera.

The exact breakpoints for MIC's are not clearly defined for either cefoxitin or BL-S786. A value of 32 μg/ml for cefoxitin was chosen here based on mean peak values of 32 μg/ml 30 minutes after a one-gram dose given intravenously although a mean of 72 μg/ml 30 minutes after a two-gram dose has been found.[7] BL-S786 has a longer half-life in mice than cephalothin or cefazolin. A peak concentration of 36 μg/ml was found after a dose of 20 mg/kg.[8] Thus, 32 μg/ml was chosen as a breakpoint for an MIC with BL-S786 based upon reasonable expectations of blood levels in humans. Peak values with either drug may be transiently higher.

Further *in vitro* susceptibility studies from diverse centers, comparative studies of susceptibility to β-lactamases, pharmacokinetic data and clinical trials are necessary to determine the exact role of either cefoxitin or BL-S786. Cefoxitin appears to be more promising than BL-S786 in treating infections caused by our isolates of *Enterobacteriaceae* resistant to cephalothin.

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