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Cefoxitin Activity Against Multiply Antibiotic-Resistant *Klebsiella pneumoniae* In Vitro

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Due to the emergence of cephalothin- and gentamicin-resistant *Klebsiella pneumoniae* at this institution, we investigated the in vitro activity of two new cephalosporin compounds—cefoxitin and cefamandole. Whereas both drugs were active against cephalothin- and gentamicin-susceptible isolates of *Klebsiella*, only cefoxitin exhibited significant activity against cephalothin- and gentamicin-resistant isolates. Seventeen of 20 (85%) of the cephalothin- and gentamicin-resistant isolates were inhibited by ≤12.5 μg of cefoxitin per ml. The minimum bactericidal concentration in broth of representative isolates equaled the agar dilution minimum inhibitory concentration. Kirby-Bauer disk susceptibility testing correlated well with the agar dilution minimum inhibitory concentration. Cephalothin and cefamandole resistance of isolates could be correlated with antibiotic degradation by β-lactamase. Cephalothin and cefamandole evoked only a transient decrease in viable bacterial cell count with rapid inactivation of antibiotics, and full regrowth of the organisms. Cefoxitin, on the other hand, was quite effective in vitro against multiply resistant *Klebsiella*. No β-lactamase degradation of cefoxitin was detected. Growth curves with antibiotics indicated rapid killing of cephalothin- and gentamicin-resistant isolates by cefoxitin.

Emergence of multiply antibiotic-resistant *Klebsiella pneumoniae* has become a serious therapeutic problem at this institution and at others in recent years (10). The ongoing epidemic of *Klebsiella* infections at this hospital with multidrug resistance, including cephalothin and gentamicin, is due to three serotypes: 2, 21, and 22. Because of the broad resistance of these organisms, we investigated the in vitro activity of two new cephalosporin compounds against these multiply resistant *Klebsiella*.

Cefoxitin, a cephamycin derivative structurally related to the cephalosporins, is active against *Klebsiella* and is resistant to β-lactamase degradation (8). Cefamandole, a new cephalosporin, is reported to show greater activity in vitro against *Klebsiella* than currently available cephalosporins (3). These new antibiotics were compared to cephalothin in vitro to assess their relative effectiveness against cephalothin- and gentamicin-susceptible and -resistant clinical isolates of *Klebsiella*.

MATERIALS AND METHODS

Bacterial isolates. Twenty consecutive *Klebsiella* isolates resistant to both cephalothin and gentamicin by the Bauer-Kirby method (1) and 10 consecutive isolates of *Klebsiella* susceptible to both cephalothin and gentamicin by the same method were collected from clinical laboratory specimens from the Nashville Veterans' Administration Hospital in March 1976. Most of the isolates were recovered from urine, but also from sputum, wound, and blood cultures.

Antibiotics. Cefoxitin was supplied as the sodium salt by Merck Sharp & Dohme Research Laboratories (Rahway, N.J.). Cefamandole was supplied as the lithium salt by Eli Lilly & Co. (Indianapolis, Ind.). The commercial preparation of cephalothin (Keflin, Lilly) was employed.

Disk susceptibility testing. Disks containing 30 μg of cefoxitin, cefamondole, or cephalothin were supplied by the manufacturers. Susceptibility testing was performed by the standard Bauer-Kirby method.

Agar dilution minimum inhibitory concentrations (MICs). Test organisms were allowed to grow overnight at 37°C in Mueller-Hinton broth to final concentrations of approximately 10^9 bacteria per ml. Antibiotics were added to Mueller-Hinton agar by serial twofold dilutions, achieving concentrations from 100 to 1.56 μg/ml. By using a calibrated loop to deliver 0.001 ml, the bacterial inoculum was streaked on the agar surface. After 18 h of incubation at 37°C, plates were examined for growth. The presence of less than five colonies was recorded as indicating growth inhibition >99.9% of the bacterial inoculum.

MBC in broth. Antibiotic concentrations from

100 to 1.56 μg/ml by serial twofold dilutions were prepared in Mueller-Hinton broth. After overnight growth, broth cultures were diluted 10^{-4} in broth, and 0.5 ml was added to each antibiotic dilution. The least concentration of antibiotic resulting in no visible growth after 18 h at 37°C was recorded as representing the tube dilution MIC. The minimum bactericidal concentration (MBC) was determined by subculturing 0.01 ml on antibiotic-free blood agar plates, and the least antibiotic concentration without visible growth after 18 h at 37°C was recorded as the MBC.

β-Lactamase assay. The Rosen (9) acidometric technique for assaying β-lactamase was used with cephalothin, cefamandole, or cefoxitin as a substrate. A 20-mg amount of each antibiotic was added to 10 ml of of distilled water containing 2 ml of 0.5% phenol red indicator. The pH was adjusted to 8.5 with 1 N NaOH added dropwise. *Klebsiella* isolates freshly grown on Mueller-Hinton agar were pressed into the tip of capillary tubes containing the antibiotic-indicator solution to form a plug. The capillary tubes were incubated vertically for 1 h at room temperature, and a change from violet to yellow color was interpreted as indicating a positive result. A group A *Streptococcus pyogenes* isolate and a noninoculated antibiotic indicator solution served as negative controls.

The microbiological method of Kjellander and Myrback as modified by Findell and Sherris (4), in which a β-lactamase-producing organism allows growth of a susceptible indicator organism around an antibiotic disk in a "cloverleaf" pattern, was employed. A swab of an 18-h broth culture of *Sarcina lutea* ATCC 9341 was used to inoculate a Mueller-Hinton agar plate. The *Klebsiella* isolates to be tested for β-lactamase were swabbed in the shape of a cross across the plate from an overnight broth culture. A 30-μg of disk of cephalothin, cefamandole, or cefoxitin was placed in the center of the cross and then incubated overnight at 37° and then at 22°C for another 24 h to allow full growth and yellow color of *S. lutea*. Patterns of *Sarcina* growth were read at 24 and 48 h and tracings were made. The cloverleaf pattern of growth of the susceptible indicator organism next to the *Klebsiella* indicated β-lactamase degradation of the antibiotic diffusing from the disk, whereas a circular zone of inhibition indicated absence of β-lactamase degradation.

Growth curves and antibiotic inactivation. Bacterial growth in the presence of cephalothin, cefamandole, or cefoxitin was determined for representative isolates of each of the three serotypes of cephalothin- and gentamicin-resistant *Klebsiella*. A total of 20 μg of each antibiotic per ml was added to a 10^{-4} broth dilution of an overnight growth of bacteria, and portions were sampled at 3, 6, 12, and 24 h for quantitative viable cell counts by the pour plate technique with appropriate dilutions.

Portions of each sample were membrane filtered (Millipore Corp., Bedford, Mass.; 0.45-μm pore size) and frozen at −70°C until the antibiotic assay. Before the assay, samples and antibiotic standards were heated at 56°C for 30 min to inactivate β-lactamase. A comparison of heated and nonheated samples indicated insignificant degradation (<10%) of any antibiotic. Assay of antibiotics was by the well diffusion method in brain heart infusion agar, with *Staphylococcus aureus* MB2786d (provided by C. Martin, Merck Sharp & Dohme) as the indicator organism.

Gentamicin resistance. For the purpose of this study, resistance to gentamicin was detected by confluent growth to 10-μg gentamicin disks by Bauer-Kirby testing. This was confirmed by growth from 10^4 to 10^8 organisms per ml in Mueller-Hinton broth containing 10 μg of gentamicin per ml, with viability subsequently confirmed by growth on antibiotic-free Mueller-Hinton agar. All isolates had gentamicin MICs >6.25 μg/ml, with the mean MIC to gentamicin of 18.7 μg/ml.

RESULTS

Cefamandole and cefoxitin inhibited the cephalothin- and gentamicin-susceptible isolates as shown in Fig. 1. All strains were inhibited in the agar dilution studies by ≤12.5 μg of each of the three cephalosporin compounds per ml. In Bauer-Kirby susceptibility testing, the zone of inhibition was >18 mm for all isolates with either cephalothin, cefamandole, or cefoxitin disks.

However, cefoxitin was the only antibiotic with significant activity against cephalothin- and gentamicin-resistant isolates (Fig. 2). The agar dilution MIC was ≤50 μg of cefoxitin per ml for all isolates, and 17 of 20 (85%) isolates were inhibited by ≤12.5 μg of cefoxitin per ml. In Bauer-Kirby susceptibility testing, cefoxitin produced zones of inhibition of ≥18 mm against 18 isolates (90%), with an agar dilution MIC ≤25 μg/ml. In contrast, cefamandole and cephalothin inhibited only two isolates (10%) at ≤50 μg/ml, and the other isolates were not inhibited by 100 μg of either drug per ml. In Bauer-Kirby

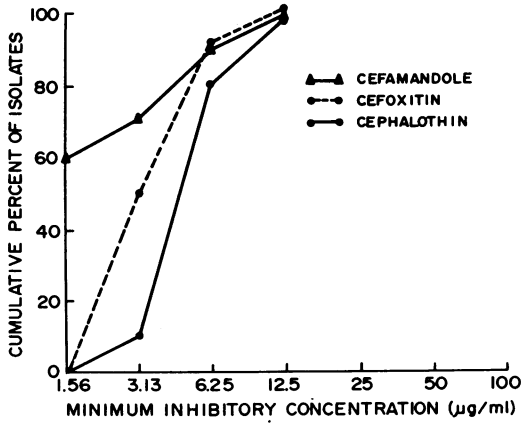

FIG. 1. *Comparative inhibition by cephalothin, cefamandole, and cefoxitin against cephalothin- and gentamicin-susceptible isolates of Klebsiella.*

testing neither cefamandole nor cephalothin produced zones of inhibition greater than 15 mm against any isolate.

Tube dilution MICs and MBCs were determined with cefoxitin for 10 of the isolates including all isolates having agar dilution MICs of >12.5 μg/ml. The tube dilution MIC was one dilution less than the agar dilution MIC in 4 of 10 isolates. In all isolates the MBC equaled the agar dilution MIC.

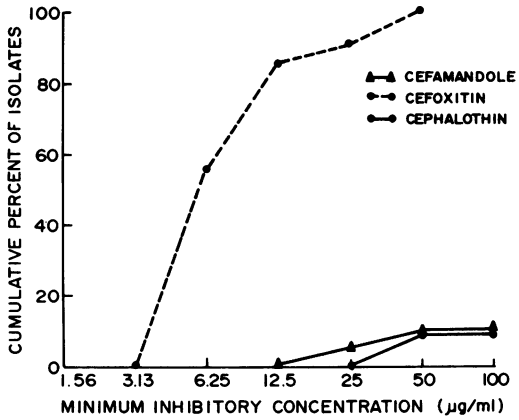

FIG. 2. *Comparative activity of cephalothin, cefamandole, and cefoxitin against cephalothin- and gentamicin-resistant isolates of Klebsiella.*

β-Lactamase assay. By the acidometric assay, none of 10 cephalothin-susceptible isolates or controls gave positive reactions for antibiotic degradation by β-lactamase. In contrast, all 20 cephalothin-resistant isolates gave positive reactions with cephalothin and cefamandole, but not with cefoxitin.

The microbiological assay demonstrated striking cloverleaf patterns with all 20 cephalothin-resistant isolates to cephalothin and cefamandole, but not cefoxitin. Eight of ten cephalothin-susceptible isolates also produced cloverleaf patterns (always to a less pronounced degree than the cephalothin-resistant isolates) with cephalothin and cefamandole. Again, zones around the cefoxitin disks were circular, indicating absence of β-lactamase degradation.

Growth curves and antibiotic inactivation. Cephalothin and cefamandole inhibited bacterial growth only transiently, with the antibiotic rapidly inactivated in the culture (Fig. 3). In

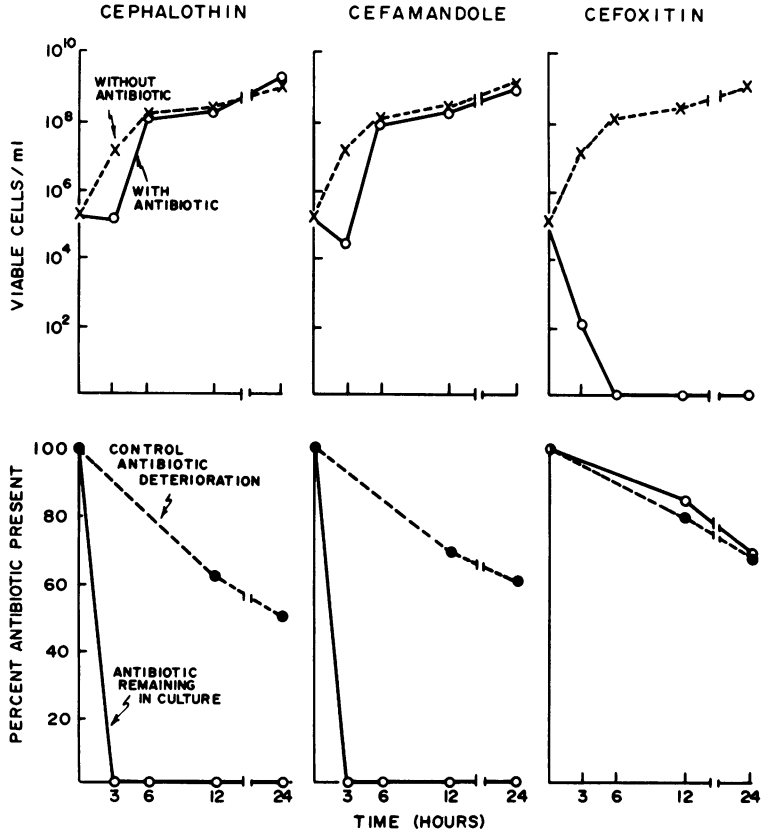

FIG. 3. *Growth curves of a cephalothin- and gentamicin-resistant Klebsiella strain in the presence of caphalosporin compounds (top), and antibiotic inactivation in Klebsiella cultures (bottom).*

contrast, cefoxitin caused a rapid decline in viable cell count with no significant antibiotic inactivation compared with control antibiotic deterioration in broth.

DISCUSSION

Cefoxitin is demonstrably effective in vitro when tested against our cephalothin- and gentamicin-resistant *Klebsiella*. Cefamandole appers to be ineffective in vitro against those same multiply resistant strains. Cefoxitin has previously been reported to be effective in vitro against cephalothin-resistant *Klebsiella* (7). Cefamandole has been reported to show promising in vitro activity against cephalothin-susceptible *Klebsiella*, but is only minimally effective against cephalothin-resistant isolates (7).

The indifference of cefoxitin to β-lactamase degradation appears to be the major factor contributing to the drug's efficacy against our multiply resistant *Klebsiella*. Cefamandole and cephalothin were readily degraded by the β-lactamase of our resistant isolates. In contrast, β-lactamase activity failed to significantly degrade cefoxitin according to the acidometric assay or during growth curves with the antibiotic. No β-lactamase degradation of cefoxitin occurred with the microbiological cloverleaf procedure to Findell and Sherris (4), which offers good conditions for enzyme induction due to exposure of organisms to graduations of the diffusing antibiotic.

Resistance to cefoxitin by *Klebsiella* is readily induced in vitro by exposure to subinhibitory concentrations in an incremental fashion. However, the resistance appears to be intrinsic in nature and not due to inactivation by β-lactamase (6).

Cefoxitin achieves serum and urine levels comparable to those of cephalothin, and toxicity appears minimal (5). If clinical trials in progress here confirm the efficacy of cefoxitin against our multiply antibiotic-resistant *Klebsiella*, cefoxitin will be a welcome new antibiotic for therapy of these troublesome infections.

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