

Augmentation of the In Vitro Activity of Azlocillin Against *Bacteroides fragilis* by Clavulanic Acid

MADHU B. BANSAL,^{1*} SEE-KEAN CHUAH,² AND HARAGOPAL THADEPALLI²

Division of Infectious Diseases, Departments of Medicine and Pathology,¹ Charles R. Drew Postgraduate Medical School, and University of California at Los Angeles School of Medicine,² Los Angeles, California 90059

Received 27 February 1984/Accepted 12 July 1984

Azlocillin was active against 90% of 154 strains of *Bacteroides fragilis* at a concentration of 64 µg/ml. Twenty-eight strains of *B. fragilis* with an azlocillin MIC of ≥8 µg/ml were retested with a combination of azlocillin plus clavulanic acid. Of these strains, 71% showed a 4- to 32-fold decrease in the MIC of azlocillin plus clavulanic acid.

The main cause of penicillin resistance in *Bacteroides fragilis* is the production of β-lactamase. Clavulanic acid (CA), a β-lactam antibiotic isolated from *Streptomyces clavuligerus*, is known to be a potent inhibitor of β-lactamase. The purpose of this study was to determine the in vitro activity of azlocillin alone and in combination with CA against *B. fragilis* isolated from clinical specimens.

Recent isolates of *B. fragilis* (154) obtained from various clinical infections during the past 3 years were used in this study. Each of them were earlier identified by methods described in the Virginia Polytechnic Institute *Anaerobe Laboratory Manual* (4) and were reidentified before actual testing in this study. Standard antibiotic powders of azlocillin and mezlocillin (Miles Laboratories, New Haven, Conn.) and piperacillin (Lederle Laboratories, Pearl River, N.Y.) were dissolved in distilled sterile water. CA (Beecham Laboratories, Bristol, Tenn.) was dissolved in sterile phosphate buffer (pH 6.0). Azlocillin and CA, when used in combination, were mixed in a ratio of 15:1.

The MICs of azlocillin, mezlocillin, and piperacillin for *B. fragilis* were determined by a serial agar dilution method (7) with decreasing antibiotic concentrations ranging from 128 to 0.25 µg/ml. Briefly, a twofold serial dilution of an antibiotic solution was mixed with brain heart infusion agar supplemented with hemin, vitamin K, and sheep blood. The plates were inoculated with a Steers replicator from a 24-h culture adjusted to match the turbidity of a McFarland Standard no. 1 to deliver approximately 10⁵ CFU per spot on the agar surface. Plates were incubated in an anaerobic glove box (1) (Coy Manufacturing Co., Ann Arbor, Mich.) for 48 h at 37°C. The lowest concentration of antibiotic that produced no growth or a barely visible haze on the agar surface was considered the MIC.

Twenty-eight strains of *B. fragilis* having azlocillin MICs of ≥8 µg/ml were tested for their MIC of CA at concentrations ranging from 0.625 to 16 µg/ml. These strains of *B. fragilis* were also resistant to mezlocillin, piperacillin, penicillin, cephalothin, and ticarcillin at 8 µg/ml. Other details were the same as described for the determination of the MIC. In addition, these strains were also tested for the ability to produce β-lactamase by a method in which a cefinase disk impregnated with the chromogenic cephalosporin nitrocefin (BBL Microbiology Systems, Cockeysville, Md.) is used. Each disk was moistened with 1 to 2 drops of sterile water, and the surface of the disk was smeared with

four to five colonies of the culture. A change in color of the disk from yellow to pink within 1 h was considered as a positive test for β-lactamase production.

Anaerobic blood agar plates were prepared with CA and azlocillin in serial dilutions ranging from 2 to 64 µg of azlocillin per ml mixed in a ratio of 1 part clavulanic acid to 15 parts azlocillin. The plates were inoculated and the incubations were done as described previously. The results were read after 48 h of incubation. The effect of CA on the activity of azlocillin was considered to be augmentation when the MIC of azlocillin in combination with CA decreased by fourfold or more.

The MICs of azlocillin, mezlocillin, and piperacillin for 50 and 90% of 154 strains of *B. fragilis* are shown in Table 1. Of the test organisms, 50% were inhibited by all drugs at 4 µg/ml.

All 28 selective strains of *B. fragilis* were resistant to 16 µg of CA per ml and were β-lactamase producers.

Figure 1 shows the MIC of azlocillin alone and in combination with CA against the 28 azlocillin-resistant strains of *B. fragilis*. The MIC₅₀ of azlocillin alone decreased from 32 to 4 µg/ml in the presence of CA. Similarly, the MIC₉₀ of azlocillin was decreased from 128 to about 16 µg/ml when CA was combined with azlocillin (Fig. 1). CA augmented azlocillin activity against 20 of 28 (71%) resistant strains of *B. fragilis*.

Previously published in vitro studies of azlocillin alone against a small number of strains of *B. fragilis* have found it to be effective, with MIC₉₀s of 50 µg/ml or less (3, 5, 10). In our study of 154 strains of *B. fragilis*, azlocillin by itself inhibited 90% of the strains at 64 µg/ml (Table 1).

CA alone at the tested level (16 µg/ml) had no effect against 28 selected strains of *B. fragilis*. The combination of CA with azlocillin exhibited a marked augmentation against β-lactamase-producing strains of *B. fragilis*. Similar results have been reported when CA was used in combination with

TABLE 1. In vitro activity of azlocillin, mezlocillin, and piperacillin against 154 strains of *B. fragilis*

Antibiotic	MIC (µg/ml)		
	Range	50%	90%
Azlocillin	0.5->128	4	64
Mezlocillin	0.25->128	4	32
Piperacillin	0.5->128	4	64

* Corresponding author.

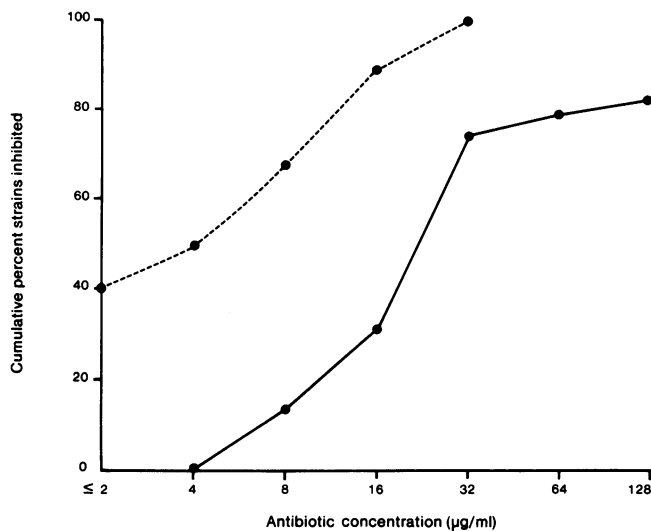


FIG. 1. In vitro susceptibilities of 28 azlocillin-resistant strains of *B. fragilis* to azlocillin alone (—) and azlocillin in combination with CA (---).

penicillin (8), cefoperazone (2), and mezlocillin (9) against *B. fragilis*. Combination of CA with amoxicillin has been found to be effective in the treatment of infections caused by gram-negative aerobic bacteria (16). In our study, the highly resistant strains of *B. fragilis* were the most susceptible to the augmenting effect of CA on azlocillin.

The fact that azlocillin, mezlocillin, and piperacillin inhibited 90% of 154 strains of *B. fragilis* at clinically attainable levels suggests that one of these penicillins may be tested as a single therapeutic agent in cases of mixed aerobic-anaerobic infections. The addition of CA may potentiate the

activity of azlocillin and thus decrease the daily dose required for treatment of such infections.

LITERATURE CITED

1. Aranki, A., S. A. Syed, E. B. Kenny, and R. Freter. 1969. Isolation of anaerobic bacteria from human gingiva and mouse cecum by means of a simplified glove box procedure. *Appl. Microbiol.* 17:568-576.
2. Crosby, M. A., and D. W. Gump. 1982. Activity of cefoperazone and two β -lactamase inhibitors, sulbactam and clavulanic acid, against *Bacteroides* spp. correlated with β -lactamase production. *Antimicrob. Agents Chemother.* 22:398-405.
3. Fu, K. P., and H. C. Neu. 1978. Azlocillin and mezlocillin: new ureido penicillins. *Antimicrob. Agents Chemother.* 13:930-938.
4. Holdeman, L. V., and W. E. C. Moore (ed.). 1977. Anaerobic laboratory manual, 4th ed. Virginia Polytechnic Institute and State University, Blacksburg.
5. Koning, H. B., K. G. Metzger, R. P. Murmann, H. A. Offe, P. Schacht, and W. Schrock. 1977. Azlocillin -ein neues Penicillin gegen *Pseudomonas aeruginosa* und andere gramnegative Bakterien. *Infection* 5:170-182.
6. Martinelli, R., A. A. da Silva Lopez, M. M. M. G. de Oliveira, and H. Rocha. 1981. Amoxicillin-clavulanic acid in treatment of urinary tract infection due to gram-negative bacteria resistant to penicillin. *Antimicrob. Agents Chemother.* 20:800-802.
7. Roy, I., V. Bach, and H. Thadepalli. 1977. In vitro activity of ticarcillin against anaerobic bacteria compared with that of carbenicillin and penicillin. *Antimicrob. Agents Chemother.* 11:258-261.
8. Wise, R. 1977. Clavulanic acid and susceptibility of *Bacteroides fragilis* to penicillin. *Lancet* ii:145.
9. Wise, R., P. Gillet, and J. M. Andrews. 1979. The *in vitro* activity of mezlocillin, when combined with cefoxitin or clavulanic acid. *J. Antimicrob. Chemother.* 5:301-306.
10. Wise, R., A. P. Gillett, J. M. Andrews, and K. S. Bedford. 1978. Activity of azlocillin and mezlocillin against gram-negative organisms: comparison with other penicillins. *Antimicrob. Agents Chemother.* 13:559-565.