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NOTES

Activity of Cefazolin and Two β-Lactamase Inhibitors, Clavulanic Acid and Sulbactam, against *Bacteroides fragilis*

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One hundred clinical isolates of the *Bacteroides fragilis* group of bacteria were tested by agar dilution for susceptibility to cefazolin alone or in combination with clavulanic acid or sulbactam. For cefazolin, the MIC for 50% of the isolates (MIC_{50}) was 32 μg/ml, the breakpoint for susceptibility. With the addition of 0.5 μg of clavulanic acid per ml, the MIC for 90% of the isolates (MIC_{90}) was 8 μg/ml, well within the achievable range of concentrations in serum or tissue. Similarly, with the addition of 0.5 μg of sulbactam per ml, the MIC_{90} was 16 μg/ml. The addition of a higher concentration (4.0 μg/ml) of clavulanic acid or sulbactam resulted in MIC_{90}s which were fourfold lower than those with 0.5 μg/ml. A fixed ratio of cefazolin–β-lactamase inhibitor of 4:1 resulted in an MIC_{50} and MIC_{90} which were intermediate between the 0.5- and 4.0-μg/ml fixed concentration of β-lactamase inhibitor.

β-Lactamase-mediated antibiotic inactivation is the most common reason for penicillin and cephalosporin resistance among the *Bacteroides fragilis* group of bacteria. Many studies have shown that the addition of clavulanic acid or sulbactam, each of which inactivates many β-lactamases, can lead to a substantial reduction in the MICs of penicillins such as ampicillin (2, 6) or cephalosporins such as cephaloridine (6, 7) or cefoperazone (1, 4, 7). In fact, the β-lactam–β-lactamase inhibitor combinations are as potent as clindamycin or chloramphenicol against the *B. fragilis* group. Experimental animal studies have supported this observation by showing that these combinations are effective in protecting from or treating such infections (3).

Because of its reasonable cost, low toxicity, high levels in serum and tissues, and longer half-life compared with those of cephalosporins, cefazolin is one of the most widely used antibiotics in the United States. We added clavulanic acid or sulbactam to cefazolin to study their inhibitory effects on 100 clinical isolates of the *B. fragilis* group. We also compared a 4:1 fixed ratio of cefazolin to β-lactamase inhibitor (BLI) with combinations in which the concentration of the BLI was constant at 0.5 or 4.0 μg/ml and the concentration of cefazolin varied twofold from 0.5 to 32 μg/ml.

Drugs. The following antibiotic powders were gifts from the manufacturers: clavulanic acid (Beecham Laboratories, Bristol, Tenn.), sulbactam (Roerig Pharmaceuticals, New York, N.Y.), and cefazolin (Smith Kline & French Laboratories, Philadelphia, Pa.). All drugs were dissolved in the appropriate diluent, and they were always prepared fresh on the day of the test.

Bacterial strains. All strains of the *B. fragilis* group were collected over a 2-year period from the Clinical Microbiology Laboratory at Temple University Hospital, Philadelphia, Pa. All isolates were obtained from blood or deep-tissue sources. Identification was done in duplicate by using RapID-ANA panels (Innovative Diagnostic Systems, Inc., Atlanta, Ga.). The control strains (*B. fragilis* ATCC 25285 and *Clostridium perfringens* ATCC 13124) were obtained from the American Type Culture Collection, Rockville, Md. Breakdown by species was *B. fragilis*, 76 strains; *Bacteroides thetaiotaomicron*, 18 strains; *Bacteroides distasonis*, 1 strain; *Bacteroides vulgatus*, 1 strain; *Bacteroides ovatus*, 3 strains; and *Bacteroides uniformis*, 1 strain.

Susceptibility testing. Agar dilution MICs were determined by the method of the National Committee for Clinical Laboratory Standards (7a) for susceptibility testing of anaerobes. A suspension of 10^8 cells per ml was prepared by adjusting a broth incubated overnight to a 0.5 McFarland standard, and a Steers replicator was used to apply a final inoculum of 10^5 cells to a plate of Wilkins-Chalgren agar (Oxoid Ltd., London, England) containing antibiotic(s) (9). The plates were incubated at 35°C for 48 h in an anaerobic glove box with an atmosphere of 5% CO_2–10% H_2–85% N_2. The MIC was read as the lowest concentration of antibiotic for which there was no growth or only a faint haze. All MICs were determined in duplicate, and if there was a discrepancy, the higher value was used.

The MIC range and the MICs for 50% (MIC_{50}) and 90% (MIC_{90}) of the isolates for all strains, antibiotics, and antibiotic combinations tested are shown in Table 1. Because of the preponderance of *B. fragilis* in our collection, there were not enough representatives of the other species in the group to tabulate susceptibility by species.

The synergism of both clavulanic acid and sulbactam with cefazolin was striking. The low concentration of clavulanic acid (0.5 μg/ml) lowered the median MIC 16-fold, and the high concentration (4.0 μg/ml) lowered it greater than 64-fold. Sulbactam lowered the MIC_{50} 8-fold at the low concentration (0.5 μg/ml) and 16-fold at the high concentration (4.0 μg/ml). The fixed ratio of 4:1 for cefazolin to BLI gave MIC_{50}s and MIC_{90}s at or between the two fixed concentrations for clavulanic acid or sulbactam.

The susceptibility pattern of the *B. fragilis* group at Temple University Hospital with regard to chloramphenicol,

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TABLE 1. Susceptibility of 100 strains of the *B. fragilis* group to selected antimicrobial agents

Antibiotic[a]	No. of strains tested	MIC (μg/ml)		
		Range	50%	90%
Cefazolin	100	4–>32	32	>32
+ CA (0.5 μg/ml)	100	1–>32	2	8
+ CA (4.0 μg/ml)	100	0.5–>32	≤0.5	2
+ CA (4:1 ratio)	100	1–>32	2	4
+ SUL (0.5 μg/ml)	100	1–>32	4	16
+ SUL (4.0 μg/ml)	100	1–32	2	4
+ SUL (4:1 ratio)	100	1–>32	2	8
CA	100	4–128	8	32
SUL	100	8–64	32	32
Cefoxitin	96	2–>32	8	32
Clindamycin	96	≤0.25–>32	0.5	4
Chloramphenicol	95	≤1–8	2	4

[a] CA, Clavulanic acid; SUL, sulbactam.

clindamycin, and cefoxitin parallels that at other U.S. hospitals in the 1980s (5). Chloramphenicol resistance is very rare in all studies. Resistance to cefoxitin and clindamycin is less than 10% in the other published studies and less than 4% in our series. Some of these differences may be the result of local or regional factors, and some may be the result of the species mix.

Although cefazolin is a very widely used cephalosporin, few data are available regarding its activity against *B. fragilis*. Because levels in serum and tissue are higher and the half-life in serum is longer for cefazolin than for other cephalosporins (8), its potential synergy with clavulanic acid and sulbactam should reflect both the peak levels achieved immediately after an infusion and the low levels of BLI as compared with those of cefazolin which exist at the end of the dosing interval. Even with maximum doses of clavulanic acid and sulbactam, the short half-life of these drugs in serum (1.25 to 1.5 h) results in levels in blood of approximately 0.5 μg/ml or less at the trough of an 8-h dosing interval.

For the most resistant strains (MIC, >16 μg of cefazolin component per ml), increasing the concentration of BLI eightfold from 0.5 to 4.0 μg/ml resulted in the inhibition of 4 of 10 strains by sulbactam and 3 of 8 strains by clavulanic acid. A further doubling of the concentration of BLI did not make any difference. Higher concentrations are not only clinically difficult to achieve in serum and tissue but are also inhibitory by themselves.

Thus, for most strains of *B. fragilis* in which cefazolin plus a BLI is likely to be an active combination, even the low concentration of BLI present at the end of the dosing interval should be synergistic for the inhibition of bacterial growth. The results of tests performed with a 4:1 fixed ratio of cefazolin to BLI do not simulate the ratios predicted for serum or tissue but approximate the results with a fixed concentration of 0.5 or 4.0 μg of BLI per ml. If resources for testing are limited, the former seems to be a reasonable substitute for the latter, because maintaining a fixed ratio is simpler when serial dilutions of the combined drugs are performed.

There are strains which are resistant to high concentrations of cefazolin-BLI combinations, and although they produce β-lactamase, the mechanism of the resistance is unknown. These strains are quite rare, but it will be important to monitor their frequency once these β-lactamase-inhibiting drugs come into widespread clinical use.

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