In Vitro Susceptibility of *Clostridium difficile* Isolates to Cefotaxime, Moxalactam, and Cefoperazone

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The in vitro susceptibility of 20 isolates of *Clostridium difficile* to cefotaxime, moxalactam, and cefoperazone was determined by a standard agar dilution method. The median minimal inhibitory concentrations were 64, 32, and 32 μg/ml for cefotaxime, moxalactam, and cefoperazone, respectively.

*Clostridium difficile*-induced diarrhea is a serious complication of antimicrobial chemotherapy. Many antimicrobial agents have been associated with the disease, with clindamycin and ampicillin most commonly cited (7). In addition, several recent reports have emphasized cephalosporin-associated diarrhea and colitis induced by *C. difficile* (2, 3, 12, 17). Ebright et al. (6) have demonstrated that eight first- or second-generation cephalosporins can cause hemorrhagic cecitis associated with a toxin neutralized by *Clostridium sordellii* antitoxin, and thus presumably due to *C. difficile*, in a hamster model. The development of new third-generation cephalosporins, a report of pseudomembranous colitis associated with moxalactam therapy (16), and the frequent observation of diarrhea in patients treated with cefoperazone (10) prompted this study of the in vitro susceptibility of *C. difficile* to three new cephalosporins.

Twenty strains of *C. difficile* were studied. All were obtained from stools of patients suspected of having antibiotic-associated diarrhea or colitis and therefore submitted to the Wisconsin State Laboratory of Hygiene for evaluation for the presence of *C. difficile* and its toxin. There was no discernible clustering of sources of these specimens. The initial stool samples were cultured by modification of a previously described technique on CCFA agar (9). Organisms with the typical colonial morphology of *C. difficile* were isolated and identified by a standard technique (11). All isolates were found to produce a cytopathic toxin that was neutralized by *C. difficile* antitoxin (purchased from T. Wilkins, Virginia Polytechnic Institute) when tested by a modification of the method of Aswell et al. (1) with human fetal lung fibroblasts (WiscL cells; Wisconsin State Laboratory of Hygiene, Madison). A stock culture of each organism in chopped meat-carbohydrate broth was stored at −70°C.

In vitro antimicrobial susceptibility testing was performed by a standard agar dilution method (National Committee for Clinical Laboratory Standards, *Proposed Reference Dilution Procedure for Antimicrobial Susceptibility Testing of Anaerobic Bacteria*, 1979). Serial twofold dilutions (from 128 to 0.12 μg/ml) of cefotaxime sodium (Hoechst-Roussel, Somerville, N.Y.), moxalactam sodium (Eli Lilly & Co., Indianapolis, Ind.), and cefoperazone sodium (Pfizer Pharmaceuticals, New York, N.Y.) were used. Two control organisms were also tested: *Escherichia coli* ATCC 25922 and *Bacteroides fragilis* ATCC 25285.

The results of the in vitro susceptibility tests are shown in Table 1. The minimal inhibitory concentrations of cefotaxime, moxalactam, and cefoperazone were 0.12, 0.25, and 0.25 μg/ml, respectively, for *E. coli* ATCC 25922 and 32, 0.25, and 32 μg/ml, respectively, for *B. fragilis* ATCC 25285.

As shown in Table 1, 70 to 80% of isolates were inhibited by concentrations of 32 μg of moxalactam or cefoperazone per ml, with 100% inhibited by a concentration of 64 μg/ml. Similar percent inhibition required a twofold-higher concentration of cefotaxime. These findings agree within one dilution with a previous report of the 50% minimal inhibitory concentrations of 128, 64, and 32 μg of cefotaxime, moxalactam, and cefoperazone per ml, respectively (15), for *C. difficile* and a report of a single isolate from a patient with pseudomembranous colitis with a minimal inhibitory concentration of 50 μg of moxalactam per ml (16).

If a minimal inhibitory concentration of 16 μg/ml is used as the cutoff point for susceptibility to these drugs, then essentially all isolates tested would be considered either intermediate sensitive or resistant to all three of these drugs. This would seem to predict that none of these agents
TABLE 1. Susceptibility of 20 isolates of C. difficile

<table>
<thead>
<tr>
<th>Drug</th>
<th>Cumulative % inhibited at concn (µg/ml) of:</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>16</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>0</td>
</tr>
<tr>
<td>Moxalactam</td>
<td>0</td>
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<tr>
<td>Cefoperazone</td>
<td>5</td>
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</table>

would inhibit C. difficile growth, so that toxin elaboration and production of diarrhea or colitis (or both) would be possible. However, as has been previously noted (5, 8), the mechanism of production of C. difficile-induced diarrhea is more complicated than a simple superinfection with drug-resistant C. difficile.

Furthermore, susceptibility to a given drug is generally based on achievable serum levels, whereas susceptibility to fecal drug levels may be more important in the pathogenesis of C. difficile-induced diarrhea (5, 8). To our knowledge, there are no available published data on fecal levels of these drugs. Pharmacokinetic differences among these agents (4, 13, 14) predict higher fecal concentrations of cefoperazone with similar parenteral dosage, because biliary excretion is the primary route of elimination. We have recently found fecal concentrations of cefoperazone up to 160 µg/g in patients with impaired biliary clearance of the drug (Craig, unpublished observations). Thus, inhibitory levels of cefoperazone may be achieved in feces.

Further studies are needed to clarify the relationship between the minimal inhibitory concentration of newer antimicrobial agents for C. difficile and the propensity of these agents to produce C. difficile-induced diarrheal diseases.

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LITERATURE CITED


