In Vitro Synergy Studies with Clostridium difficile

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Agar dilution anaerobic susceptibility studies using rifampin, vancomycin, metronidazole, and bacitracin individually and in combination were performed with Clostridium difficile. Fifty-five strains of C. difficile were studied. Eighty-five percent of strains tested (29 of 34) were synergistically inhibited by the combination of bacitracin and rifampin (fractional inhibitory concentration, ≤0.50).

Antimicrobial therapy is indicated in patients with Clostridium difficile-associated disease who do not respond to appropriate nonspecific treatment or have severe progressive disease. Oral vancomycin and metronidazole are the recognized therapeutic agents (6, 10, 11). In addition, oral bacitracin has recently been investigated with variable success (3, 13). Despite optimal therapy, up to 20% of patients develop relapses of diarrhea. Prompt repopulation of the gut with "normal flora" is considered the ideal goal by many, and therapeutic instillation of normal flora organisms has been proposed by some investigators (12).

The use of combination therapy with vancomycin and rifampin in patients with multiple relapses has shown some promise (1). In vitro studies of synergy of antibiotic combinations with C. difficile are lacking. We evaluated the in vitro activities of bacitracin, vancomycin, and metronidazole alone and in combination with rifampin against C. difficile. Rifampin is a very active antimicrobial agent against C. difficile, with excellent inhibitory activity (7).

(This study was presented in abstract form at the 1990 annual meeting of the American Society for Microbiology, Anaheim, Calif.)

C. difficile strains were isolated on CCFA agar from stool specimens of patients (2). Thirty strains were obtained from The University of Michigan Medical Center, and the remainder were obtained from patients at the Medical Center of Delaware. Organisms were identified by growth on CCFA agar, Gram stain, and An-IDENT or API 20A strips (API, Plainview, N.Y.). Clostridium perfringens ATCC 13124, Clostridium sporogenes ATCC 19404, and Peptostreptococcus magnus ATCC 29328 were used as controls.

Stock solutions (2,560 μg/ml) of rifampin (Merrell Dow Pharmaceuticals, Cincinnati, Ohio), vancomycin (Eli Lilly & Co., Indianapolis, Ind.), and metronidazole (Searle, Chicago, Ill.) and bacitracin (in units) (Sigma Chemical Co., St. Louis, Mo.) were prepared according to manufacturer’s directions and stored at −70°C until use.

MIC and antibiotic combination testing were performed by the agar dilution method (9). MICs were determined by using dilutions of individual antibiotics incorporated into Wilkins-Chalgren agar (Difco Laboratories, Detroit, Mich.). Seven doubling dilutions of each antibiotic were prepared and further diluted 1/20 in Wilkins-Chalgren agar to yield final concentrations in agar three dilutions above and below the mean MIC. A checkerboard plate, in which each dilution was combined with each rifampin dilution, was prepared. C. difficile isolates were subcultured from thioglycolate indicator supplemented with hemin and vitamin K or from chopped meat broth to preinoculated plates. Vancomycin susceptibility data were revealed with a 0.5 McFarland standard with preinoculated broth. Agar dilution plates were inoculated with a modified Steers replicator and incubated for 48 h in anaerobe jars (GasPak; BBL Microbiology Systems; with Scott Ana Pack). The presence of one colony or a slight haze was considered no growth.

Individual-drug MIC plates were run with each synergy test. Aerobic contamination, anaerobic growth control, and uninoculated sterile plates were included with each run, as were the control organisms. Determinations of all MICs for representative strains and combination testing were performed in duplicate. Antimicrobial interaction was quantified according to the fractional inhibitory concentration (FIC) and FIC index. The FIC index was interpreted as follows: ≤0.5, partial synergy; 0.51 to 0.75, partial synergy; and 0.76 to 1.25, additivity (4, 8).

Table 1 shows the MICs of each of the antimicrobial agents tested. All 55 strains tested were susceptible to rifampin at an MIC of 0.002 μg/ml or less. Almost 90% were inhibited at a concentration of 0.001 μg/ml. Vancomycin susceptibility data revealed all 47 strains to be susceptible at or below 1.6 μg/ml. Metronidazole MICs were from 0.8 to 3.2 μg/ml. All 34 strains tested were susceptible to bacitracin at or below 32 U/ml. Only 44% of our isolates were susceptible to bacitracin at an MIC of 16 U/ml.

The results of synergy studies are shown in Table 2. Only 1 of 47 strains tested (2.1%) revealed synergy (FIC ≤0.50) for the combination of rifampin and vancomycin. None of the 42 strains were synergistically inhibited by metronidazole and rifampin. Partial synergy was demonstrated in 16 of 42 strains tested (38%) versus metronidazole and rifampin (FIC = 0.51 to 0.75). Of 34 strains, 29 (85%) demonstrated synergistic inhibition with the combination bacitracin and rifampin on the basis of FICs of ≤0.50. Partial synergy was found with the remaining five isolates.

Only 5 of 43 total duplicate FIC determinations (11.6%) fell into different synergy categories. In no instance was there a discrepancy of synergy as opposed to additivity.

The optimal therapy for C. difficile diarrhea and colitis would be one that allows for the prompt repopulation of the gut with normal colonic flora, most simply discontinuing the

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offending agent. Antimicrobial therapy directed at *C. difficile* is indicated in severe disease or in the setting of multiple relapses. Oral vancomycin is considered by some to be the drug of choice (6, 10). Metronidazole has been shown to have efficacy equal to that of vancomycin in mild-to-moderate disease (11). More recently, oral bacitracin has been tried with success (3, 13). Combination therapy with rifampin and vancomycin was recently reported to be successful in the setting of multiple relapses (1).

There have been no in vitro studies that suggest a combination of agents that is synergistic against *C. difficile*. It appeared that bacitracin and rifampin were synergistic (85% of strains) or partially synergistic (15% of strains) against all strains of *C. difficile* tested in our study. The combination of rifampin with either vancomycin or metronidazole was not synergistic in vitro. All of our strains of *C. difficile* were susceptible to vancomycin, metronidazole, and rifampin tested individually. Bacitracin susceptibility of our isolates reflected a somewhat higher MIC than is felt to be desirable, as some authors suggest that over 20 U/ml indicates relative resistance (5). However, prior studies of *C. difficile* isolates from patients with disease treated successfully with bacitracin have shown similar inhibitory concentrations of 16 to 32 U/ml (7). Limited data exist on stool concentrations of bacitracin following oral administration. In six patients studied (22 samples), the fecal concentration following administration of 80,000 U/day orally ranged from 80 to 200 U/ml (13). The toxicity of bacitracin is minimal in view of its negligible absorption, even in individuals with inflamed colonic mucosa (3). The combination of bacitracin and rifampin in patients with *C. difficile* colitis or diarrhea should be studied further.

### REFERENCES


### TABLE 1. Susceptibility of *C. difficile* to bacitracin, metronidazole, rifampin, and vancomycin

<table>
<thead>
<tr>
<th>Antibiotic (no. of strains)</th>
<th>Cumulative % of strains susceptible at the following MIC*:</th>
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<tbody>
<tr>
<td></td>
<td>0.001</td>
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<tr>
<td>Rifampin (55)</td>
<td>89</td>
</tr>
<tr>
<td>Vancomycin (47)</td>
<td>79</td>
</tr>
<tr>
<td>Metronidazole (42)</td>
<td>93</td>
</tr>
<tr>
<td>Bacitracin (34)</td>
<td>44</td>
</tr>
</tbody>
</table>

* Micrograms per milliliter for vancomycin, metronidazole, and rifampin; units per milliliter for bacitracin.

### TABLE 2. FIC ranges for *C. difficile*

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>No. of strains with the following FIC:</th>
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<tr>
<td></td>
<td>≤0.50</td>
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<tr>
<td>Vancomycin-rifampin</td>
<td>1</td>
</tr>
<tr>
<td>Metronidazole-rifampin</td>
<td>0</td>
</tr>
<tr>
<td>Bacitracin-rifampin</td>
<td>29</td>
</tr>
</tbody>
</table>

* Synergy.

* Partial synergy.

* Additivity.