Susceptibility to Five Antimicrobial Agents of Strains of the
Bacteroides fragilis Group Isolated in Brazil

ANTONIO EUGENIO C. C. DE ALMEIDA AND MILTON DE UZEDA*

Departamento de Microbiologia Médica, Instituto de Microbiologia, Universidade Federal do Rio de Janeiro,
21942 Rio de Janeiro, Brazil

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The in vitro activity of metronidazole, chloramphenicol, clindamycin, cefoxitin, and carbenicillin was tested
by an agar dilution method against 228 strains of the Bacteroides fragilis group isolated from human intestinal
microbiota during 1981 and 1982. All the strains were susceptible to metronidazole. Resistance rates for
chloramphenicol, clindamycin, cefoxitin, and carbenicillin were 2, 37, 21, and 13%, respectively.

Anaerobic bacteriology is not a routine method for the
laboratory diagnosis of infectious diseases in Brazil. The
isolation and identification of anaerobic bacteria from clinical
material and determination of their susceptibility to
antimicrobial agents is restricted to a few university hospi-
tals and research institutes (3). In most cases, treatment of
clinically suspected anaerobic infections is decided on the
basis of data found in the international scientific literature.
Unfortunately, we do not have a significant number of
clinical isolates of these microorganisms to evaluate their
susceptibility to antimicrobial agents. Considering the prev-
ance of the Bacteroides fragilis group in these anaerobic
infections, their endogenous origin, and the possibility that
regional differences occur among resistant strains (11), we
studied the susceptibility to five antimicrobial agents of
bacteria isolated from human intestinal microbiota. Metroni-
dazole, chloramphenicol, clindamycin, cefoxitin, and car-
benicillin were selected because they are usually recom-
med for the treatment of anaerobic infections in Brazil.

Microorganisms were cultivated from fecal samples ob-
tained from 60 individuals between the ages of 1 month and
65 years during 1981 and 1982. Forty individuals had no
antimicrobial therapy for at least 1 month before the sam-
ping, whereas the other twenty were patients with antimi-
crobial usage during the period of sampling and 1 week
before. Antimicrobial agents used in the treatment of these
patients were aminoglycosides, penicillins, cephalosporins,
erthyromycin, tetracycline, chloramphenicol, and metroni-
dazole. A total of 228 B. fragilis group strains was isolated
by using selective B. fragilis bile-esculin (BBE) medium (6)
incubated anaerobically in jars containing a gaseous mixture
(80% N₂, 10% CO₂, 10% H₂) at 37°C for 48 h. Four colonies
were picked from each sample and identified to the species
level. B. fragilis, Bacteroides thetaiotaomicron, Bacteroides
distasonis, Bacteroides ovatus, Bacteroides vulgatus, and
Bacteroides uniformis were characterized biochemically
(10). MICs of the antimicrobial agents were determined by
an agar dilution method with a Steers replicator (12). The
medium used was brain heart infusion agar supplemented
with defibrinated sheep blood (50 ml/liter), yeast extract (5
g/liter), hemin (0.005 g/liter), and menadione (0.005 g/liter).
Chloramphenicol (Sigma Chemical Co., St. Louis, Mo.) and
clindamycin (The Upjohn Co., Kalamazoo, Mich.) were
diluted into medium to concentrations of 0.5 to 512 μg/ml.
Cefoxitin (Merck Sharp & Dohme, Rahway, N.J.) and car-
benicillin (Pfizer Inc., New York, N.Y.) concentrations ranged from 2 to 512 μg/ml, and metronidazole (Rhodia S.A.
Div. Farmacéutica, São Paulo, Brazil) concentrations ranged
from 0.125 to 32 μg/ml, all in twofold serial dilutions.
Inoculum was prepared from a brain heart infusion-supplemented broth diluted to a density of 10⁶ CFU/ml; the
concentration of organisms deposited on the agar with the
Steers replicator was approximately 3 × 10⁸ CFU/ml. Ref-
ence strains of B. fragilis (ATCC 25285) and B. thetaiota-
omicron (ATCC 29741) were included in each experiment to
assess the reliability of the method. Inoculated plates were
incubated in anaerobic jars at 37°C for 48 h. After the
incubation period, the MIC for each strain tested was
recorded as the lowest concentration of antimicrobial agent
that prevented macroscopic growth.

The susceptibility of the 228 bacterial strains to the five
drugs are shown in Table 1 as the MICs for 50 and 90% of
isolates tested (MIC₅₀ and MIC₉₀, respectively), the ranges
of MICs observed, and the percentages of resistant bacteria.
Resistance to metronidazole was not observed, and few
strains showed resistance to chloramphenicol. As previously
described, metronidazole resistance of the B. fragilis
strain was reported by Ingham et al. (5), who isolated the
microorganism from the feces of a patient submitted to
long-term therapy with the drug for Crohn’s disease. How-
ever, a significant number of our bacteria showed resistance
to clindamycin. The MICs for some strains reached levels
higher than 512 μg/ml. The occurrence of resistance to
clindamycin in the B. fragilis group has been reported by
many others (2, 4, 7, 9, 11), and it is known that the clinical
use of clindamycin or erythromycin may increase the fre-
cency of clindamycin resistance among Bacteroides spe-
cies, because the genes coding for this resistance also code
for erythromycin resistance (13). The frequent prescription
of erythromycin in Brazil could explain the high percentages
of clindamycin-resistant strains we found, because we
observed that half of the microorganisms tested were simulta-
neously resistant to both the drugs. Although cefoxitin has
been considered resistant to bacterial beta-lactamase (1), in
our study a significant number of strains showed resistance
to this cephamycin. The MIC of cefoxitin reached 128 μg/ml,
and the MIC₉₀ for the 228 strains tested was 64 μg/ml.
Although results may vary with different laboratories and
different studies, noteworthy is the fact that the MIC₉₀ of
cefoxitin for isolates of the B. fragilis group in the United
States during 1981 and 1982 was only 16 μg/ml (2). We also

* Corresponding author.
TABLE 1. Comparative in vitro activity of metronidazole, chloramphenicol, clindamycin, cefoxitin, and carbenicillin against species of the B. fragilis group

<table>
<thead>
<tr>
<th>Organism (no. of isolates)</th>
<th>Antimicrobial agent</th>
<th>MIC (µg/ml)</th>
<th>% Resistant*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>50%</td>
<td>90%</td>
</tr>
<tr>
<td>Bacteroides fragilis (94)</td>
<td>Metronidazole</td>
<td>1–16</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Chloramphenicol</td>
<td>2–16</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Clindamycin</td>
<td>1-&gt;512</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Cefoxitin</td>
<td>4–128</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Carbenicillin</td>
<td>8-&gt;512</td>
<td>64</td>
</tr>
<tr>
<td>Bacteroides vulgatus (21)</td>
<td>Metronidazole</td>
<td>1–16</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Chloramphenicol</td>
<td>4–8</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Clindamycin</td>
<td>1-&gt;512</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Cefoxitin</td>
<td>8–128</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Carbenicillin</td>
<td>4-&gt;512</td>
<td>32</td>
</tr>
<tr>
<td>Bacteroides distasonis (41)</td>
<td>Metronidazole</td>
<td>1–16</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Chloramphenicol</td>
<td>2–16</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Clindamycin</td>
<td>1-&gt;512</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Cefoxitin</td>
<td>2–128</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Carbenicillin</td>
<td>8-&gt;512</td>
<td>32</td>
</tr>
<tr>
<td>Bacteroides ovatus (25)</td>
<td>Metronidazole</td>
<td>4–8</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Chloramphenicol</td>
<td>4–32</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Clindamycin</td>
<td>1-&gt;512</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Cefoxitin</td>
<td>8–128</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>Carbenicillin</td>
<td>16-&gt;512</td>
<td>64</td>
</tr>
<tr>
<td>Bacteroides uniformis (17)</td>
<td>Metronidazole</td>
<td>2–8</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Chloramphenicol</td>
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<td>8</td>
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<td></td>
<td>Clindamycin</td>
<td>2-&gt;512</td>
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<tr>
<td></td>
<td>Cefoxitin</td>
<td>4–64</td>
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</tr>
<tr>
<td></td>
<td>Carbenicillin</td>
<td>32–128</td>
<td>32</td>
</tr>
<tr>
<td>Bacteroides the-</td>
<td>Metronidazole</td>
<td>1–8</td>
<td>4</td>
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<tr>
<td>taiotaomicron (30)</td>
<td>Chloramphenicol</td>
<td>4–32</td>
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<tr>
<td></td>
<td>Clindamycin</td>
<td>1-&gt;512</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Cefoxitin</td>
<td>4–128</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>Carbenicillin</td>
<td>32–512</td>
<td>128</td>
</tr>
<tr>
<td>All Bacteroides fragilis group (228)</td>
<td>Metronidazole</td>
<td>1–16</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Chloramphenicol</td>
<td>2–32</td>
<td>8</td>
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<td>Clindamycin</td>
<td>1-&gt;512</td>
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<td>Carbenicillin</td>
<td>4-&gt;512</td>
<td>64</td>
</tr>
</tbody>
</table>

* Numbers are the percentages of resistant strains at breakpoints as follows: metronidazole, 16 µg/ml; chloramphenicol, 16 µg/ml; clindamycin, 4 µg/ml; cefoxitin, 32 µg/ml; carbenicillin, 128 µg/ml.

We believe that regional differences in resistance patterns that occur in the B. fragilis group (11) are an important argument to motivate laboratories to start developing anaerobic bacteriology in Brazil. They also should stimulate the establishment of a reference center for the study of anaerobic bacteria, because there are already such centers for streptococci, members of the family Enterobacteriaceae, and Corynebacterium diphtheriae.

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LITERATURE CITED