Susceptibility of the Bacteroides fragilis Group in the United States in 1981


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The minimal inhibitory concentrations of nine antimicrobial agents were determined for over 750 clinical isolates of the Bacteroides fragilis group of anaerobic bacteria collected from nine centers in the United States during 1981. High resistance rates were documented for cefoperazone, cefotaxime, and tetracycline. Cefoxitin had the best activity of the β-lactam antibiotics, whereas moxalactam and piperacillin had good activities. The resistance rate for clindamycin was 6%. There were no metronidazole- or chloramphenicol-resistant isolates encountered. There were significant differences in susceptibility among the various species of the B. fragilis group, particularly with moxalactam, cefotaxim, and clindamycin. Clustering of clindamycin-, piperacillin-, and cefoxitin-resistant isolates was observed at different hospitals. The variability of resistance rates with the β-lactam antibiotics and clindamycin indicates that susceptibility testing of significant clinical isolates should be performed to define local resistance patterns.

The Bacteroides fragilis group of organisms, consisting of Bacteroides fragilis, Bacteroides thetaiotaomicron, Bacteroides distasonis, Bacteroides ovatus, Bacteroides vulgatus, and Bacteroides uniformis, are recognized as important pathogens in suppurative diseases and other infections. These organisms are usually considered together on the basis of taxonomy and because of increased antibiotic resistance as compared with other anaerobic bacteria (1, 6, 7, 13, 24). Of the group, B. fragilis emerges as the most important pathogen; it has the highest frequency of isolation from serious infections and is the most common anaerobic bacterium that invades the bloodstream. However, other species of the group are equally pathogenic once they have invaded deep tissues.

The number of useful antimicrobial agents active against these organisms is relatively limited. Most strains of the B. fragilis group are resistant to penicillin G and cephalosporins, including cephalothin, cefazolin, and cefamandole, on the basis of the presence of constitutive β-lactamases (13, 14, 24, 25). Furthermore, the isolation rate of resistant strains seems to be increasing (4, 17). The change in the resistance rates may be related to genetic mechanisms such as the transferable antimicrobial resistance which has been described for clindamycin-erythromycin and tetracycline and for high levels of penicillin-ampicillin resistance (12).

Most clinical microbiology laboratories do not routinely test antimicrobial susceptibility of anaerobic bacteria. The choice of an appropriate therapeutic agent to treat infections involving B. fragilis group is usually based on published susceptibility data, even though these data show wide variations. This variability could be explained by regional differences in the frequency of resistant organisms, the selection of strains to be tested, or differences in the testing methods. Thus, there may be reported falsely high or low resistance rates that could lead to the selection of an ineffective drug or to the use of toxic drug combinations.

This study was designed to establish the current susceptibility rates for clinical isolates of the B. fragilis group from nine centers across the United States by standardized susceptibility methods performed in one laboratory. Preliminary results have been previously published (4).

**MATERIALS AND METHODS**

**Bacterial isolates.** All clinical isolates of the B. fragilis group collected during the 12-month period of January to December, 1981, were referred from the
nine study group hospitals to the Tufts-New England Medical Center, Anaerobic Bacteriology Laboratory, Boston, Mass. Only one isolate of the same species per patient was studied. The identification of the strains was confirmed by established methods (8).


Antibiotic susceptibility testing. The minimal inhibitory concentration (MIC) were determined by an agar-dilution method with a Steer's replicator by the anaerobic chamber techniques described previously (24).

Data analysis. All data analysis was performed on a TRS/80 model I computer (Radio Shack, Tandy Co., Fort Worth, Tex.) with a data base management and statistical package developed by G. J. Cuchural, Tufts-New England Medical Center, Boston, Mass.

RESULTS

There was a wide variation in the susceptibility of the Bacteroides strains to the nine antimicrobial agents with regard to the 50 and 90% MICs and to the percentage of resistant strains (Table 1). Two breakpoints for each drug were selected on the basis of achievable blood levels (the lower levels may be more relevant because the drugs must penetrate sites harboring Bacteroides species, namely, abscesses). Although somewhat arbitrary, the lower breakpoints generally agree with previously published data. Higher breakpoints are based on previously published guidelines (9). The β-lactam antibiotics demonstrated variable activity; cefoxitin was the most active, with a 90% MIC of 16 μg/ml. Piperacillin and moxalactam were the next most active β-lactam antibiotics. There was a high level of resistance with cefoperazone, cefotaxime, and tetracycline. Of the isolates studied, 6% were resistant to clindamycin at 4 μg/ml. No metronidazole- or chloramphenicol-resistant isolates were encountered.

When analyzed by species, B. distasonis was the most resistant to cefoxitin (Table 2). Moxalactam had a strikingly higher rate of resistance among the non-B. fragilis species. B. thetaiotaomicron and B. vulgatus were the most resistant to clindamycin.

There was variability in the resistance rates among the nine centers (Table 3). Clindamycin resistance was seen at several centers with the highest rate at the Jackson Memorial Hospital, whereas high cefoxitin resistance rates were encountered at the Danbury Hospital and the Tufts-New England Medical Center. High resistance rates to piperacillin were seen at several hospitals.

DISCUSSION

The results of our study indicate that several drugs were active against the B. fragilis group of organisms. Cefoxitin was the most active β-lactam antibiotic; the range of resistance rates was 3 to 17%. There was no high-level resistance to cefoxitin (MIC > 128 μg/ml), suggesting that the mechanism of resistance is more likely related to penetration of the compound into the bacterial periplasmic space rather than inactivation of the drug, as has been shown in Bacteroides isolates by investigators in Stockholm (5, 14).

The next most active β-lactam antibiotics were piperacillin and moxalactam. Some investigators believe that higher blood levels can be achieved with these two drugs. If 128 and 32 μg of drug per ml were used as the respective breakpoints, resistance rates for these two agents would be lower (Table 1). Piperacillin had the lowest incidence of β-lactam resistance other than cefoxitin. This may reflect the substrate specificity of the β-lactamases of Bacteroides species, which are primarily cephalosporinases (14, 25). Some centers had higher rates of piper-
acillin resistance, suggesting the presence of a new β-lactamase in *Bacteroides* species or the fact that the gene(s) coding for resistance have been amplified (12, 19) or both. On the basis of previously published data, piperacillin appears to be more active than ticarcillin and carbenicillin against the *B. fragilis* group of organisms (3, 28).

Chloramphenicol and metronidazole were the most active of the non-β-lactam antimicrobial agents, and there were no resistant isolates encountered. The lack of resistance to chloramphenicol is puzzling since there are at least two mechanisms by which *Bacteroides* species can inactivate the drug, acetylation and nitroreduction (2, 15). Although there are scattered reports of metronidazole-resistant *Bacteroides* species this phenomenon has not been documented in the United States. All putative metronidazole-resistant anaerobic bacteria referred to the Tufts Anaerobic Laboratory were actually susceptible or were contaminated with a microaerophilic or aerobic organism or *Propionibacterium acnes*.

Clindamycin remains a highly active agent; 94% of the isolates were inhibited at ≤4 μg/ml. Further analysis of the in vitro activity of this drug showed three classes of organisms: highly susceptible (MIC, ≤0.25 μg/ml); intermediate (MIC, 0.5 to 4 μg/ml); and resistant (MIC, >4 μg/ml), which confirms previous observations (21). Most of the resistant group had MICs of 128 to 512 μg/ml. The transfer of high-level clindamycin resistance has been demonstrated to be mediated by at least two transfer factors, pBF4 and pBF4 (pIP410) (12, 17, 26, 27, 29). These two transfer factors have common clindamycin resistance gene(s) as determined by DNA-DNA hybridization studies (20). These genes also code for erythromycin and streptomycin resistance (11, 17). Thus, the clinical use of clindamycin, erythromycin, or the streptomycins may increase the frequency of clindamycin resistance in *Bacteroides* species. The 6% resistance rate in this survey may reflect a real

### TABLE 2. Resistance rates of species of the *B. fragilis* group

<table>
<thead>
<tr>
<th>Species</th>
<th>(no. of isolates)</th>
<th>% Resistant isolates at lower breakpoints with:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cfx</td>
</tr>
<tr>
<td><em>B. distasonis</em></td>
<td>(57)</td>
<td>17</td>
</tr>
<tr>
<td><em>B. fragilis</em></td>
<td>(463)</td>
<td>7</td>
</tr>
<tr>
<td><em>B. ovatus</em></td>
<td>(150)</td>
<td>8</td>
</tr>
<tr>
<td><em>B. thetaiotaomicron</em></td>
<td>(63)</td>
<td>7</td>
</tr>
<tr>
<td><em>B. vulgatus</em></td>
<td>(60)</td>
<td>7</td>
</tr>
</tbody>
</table>

* Metronidazole and chloramphenicol are not included because there were no resistant strains. *B. uniformis* and *Bacteroides* species were not included because of a low number of isolates.

### TABLE 3. Resistance rates of the *B. fragilis* group by referral center

<table>
<thead>
<tr>
<th>Center</th>
<th>(no. of isolates)</th>
<th>% Resistant isolates at lower breakpoints with:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cfx</td>
</tr>
<tr>
<td>Albert Einstein Medical Center</td>
<td>(50)</td>
<td>12</td>
</tr>
<tr>
<td>Danbury Hospital</td>
<td>(47)</td>
<td>17</td>
</tr>
<tr>
<td>Duke University Medical Center</td>
<td>(136)</td>
<td>7</td>
</tr>
<tr>
<td>Jackson Memorial Hospital</td>
<td>(86)</td>
<td>5</td>
</tr>
<tr>
<td>Louisiana State University</td>
<td>(89)</td>
<td>11</td>
</tr>
<tr>
<td>Loyola University</td>
<td>(100)</td>
<td>5</td>
</tr>
<tr>
<td>University of Michigan</td>
<td>(101)</td>
<td>6</td>
</tr>
<tr>
<td>Tufts-New England Medical Center</td>
<td>(96)</td>
<td>14</td>
</tr>
<tr>
<td>Wadsworth Medical Center</td>
<td>(67)</td>
<td>7</td>
</tr>
</tbody>
</table>

* Metronidazole and chloramphenicol are not included because there were no resistant strains.

* Cfx, Cefoxitin; Mox, moxalactam; Cln, clindamycin; Ctx, cefotaxime; Cpz, cefoperazone; Pip, piperacillin; Tet, tetracycline.
increase over that reported in the early 1970s, when lower resistance rates were reported (1, 6, 10, 13, 16, 22, 23).

Our study confirms the widespread resistance to tetracycline in Bacteroides species that was first recognized in the 1960s (6, 10, 13). This high resistance rate may be explained by an efficient tetracycline resistance transfer system which is stimulated by the presence of low levels of the drug (12, 17, 25, 27). Tetracycline resistance has also been encountered in anaerobic cocci and clostridia. These observations relegate tetracycline to a drug of primarily historical interest in the treatment of anaerobic infections.

Analysis of resistance patterns at the referring hospitals indicated regional clustering of resistance to cefoxitin, clindamycin, and piperacillin. A mechanism for the clustering of clindamycin and piperacillin could be the presence of transferable genes coding for these resistances. These elements are known to exist in Bacteroides species. In addition, these organisms displayed a close relationship between tetracycline and clindamycin resistance. Previous studies have demonstrated that the transfer of these two resistances is associated (17, 27).

There were striking differences in susceptibility to moxalactam among B. fragilis and the other species. B. fragilis was the most susceptible, whereas high-level resistance was found in the other species. Some variation in species susceptibility was also encountered with cefoxitin, piperacillin, cefotaxime, and clindamycin. Although the non-B. fragilis species are less common in clinical specimens, indicating less virulence, once these organisms have invaded deep tissues, they should be considered pathogens, thus requiring appropriate treatment. It may be important to speculate the B. fragilis group as a guide to therapy and for epidemiological studies, as advocated by Rolfe and Finegold (18).

The regional differences in resistance patterns indicate that antimicrobial susceptibility studies should be performed on selected clinical isolates of the B. fragilis group. These include organisms isolated from blood cultures, from unusual sites such as bone, joint spaces, and spinal fluid, from very sick patients, and from other sites in patients who have failed to respond to appropriate therapy.

This study underscores the fact that anaerobic bacteria, like aerobic bacteria, have changed their susceptibility to a number of antimicrobial agents. The clustering of resistances at various hospitals indicates that we can no longer rely on published data to choose antimicrobial therapy. Microbiological laboratories will have to develop their own data base from which clinicians can make rational therapeutic decisions.

ACKNOWLEDGMENTS

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