The aim of the present study was to investigate the activities of clindamycin, imipenem, metronidazole, and piperacillin-tazobactam against susceptible and resistant isolates of Bacteroides fragilis Evaluated by Kill Kinetics

Antimicrobial regimes for infections involving Bacteroides fragilis have generally been limited as B. fragilis strains are potentially resistant to a broad range of antibiotics (28). Drugs with known activity against B. fragilis are some β-lactams, nitroimidazoles such as metronidazole, certain newer quinolones, chloramphenicol, and clindamycin (17, 20, 23, 24, 28, 29). A diminution of susceptibility to clindamycin has been reported in various countries (1, 7, 21, 26). Resistance against metronidazole still seems to be rare (1, 3). Golan et al. found an increasing fluoroquinolone resistance among Bacteroides since 1994 (9). Conversely, Snyderman et al. reported in 2002 decreased geometric mean MICs among B. fragilis strains for piperacillin-tazobactam (26). Resistance to carbapenems can be found occasionally (28). Thus, there is a great need for knowledge of resistance patterns to accomplish an adequate prophylaxis and treatment of anaerobic or mixed aerobic/anaerobic infections. This seems even more important as there are great differences in the levels of antimicrobial resistance between certain geographic areas and even from one hospital to another (7, 10, 17). Kill kinetic curves over time provide more information than the widely used MIC determination and allow a comparison of different antimicrobial classes (16, 27). Thus, the aim of the present study was to investigate the in vitro activities of clindamycin, imipenem, metronidazole, and piperacillin-tazobactam against B. fragilis isolates by kill kinetics over time. The strains either were kindly provided by Elli Goldstein, R. M. Alden Research Laboratory, Santa Monica, CA, or were isolates from an international anaerobe study.

Brucella broth (Becton, Dickinson, Cockeysville, MD) supplemented with vitamin K₁ (Sigma Chemical Co., St. Louis, MO) and hemin (Serva Feinbiochemica, Heidelberg, Germany) was used as growth medium and is referred to below as supplemented brucella broth. Aliquots were plated on Columbia agar (Oxoid Ltd., Basingstoke, Hampshire, United Kingdom) supplemented with sheep blood (Oxoid GmbH, Wesel, Germany), vitamin K₁, and hemin; this mixture is referred to below as supplemented Columbia agar.

MIC values were determined by Etest (AB Biodisk, Solna, Sweden) for all selected B. fragilis strains and clindamycin, imipenem, metronidazole, and piperacillin-tazobactam according to the manufacturer’s instructions as described previously (25). For the B. fragilis strains with MICs of ≤8.0 µg/ml, the killing activities of clindamycin (Sigma Chemical Co.), imipenem (Merck & Co., Inc., West Point, PA), metronidazole (Sigma Chemical Co.), and piperacillin (Sigma Chemical Co., tazobactam (Otsuka Chemical Co., Ltd, Osaka, Japan) were assessed using 0.5 × 1×, 2×, or 4× MIC. In the case of strains with MICs of >8.0 µg/ml, concentrations at 0.5×, 1×, 2×, or 4× maximum concentrations of drug in serum (C_max) were employed. The indicated concentrations were used as C_max clindamycin, 16 µg/ml (8); imipenem, 32 µg/ml (22); metronidazole, 16 µg/ml (11, 13, 15); piperacillin, 60 µg/ml (2); and tazobactam, 25 µg/ml (12, 14, 19).

An assay with antibiotic-free growth control was performed parallel to each experiment. The final inocula contained approximately 1.5 × 10⁸ CFU/ml. At 0, 2, 4, 6, 12, and 24 h after incubation at 37°C, aliquots were plated on the supplemented Columbia agar. CFU were counted after 48 h of incubation. The detection limit was 10² CFU/ml. All experiments were carried out in an anaerobic chamber (Heraeus, Hanau, Germany) containing 5% H₂, 15% CO₂, and 80% N₂.

For all strains and their respective antimicrobial agents, the mean value and standard deviation were calculated. Statistical analysis was done with SPSS software. In those cases where the number of strains exceeded 3, the paired-sample Wilcoxon signed-rank test was employed to identify significant differences. In each case, at t = 6 h and t = 24 h differences were calculated. A P value of <0.05 was considered to be significant.

Table 1 shows the MIC values for the tested B. fragilis strains for the respective antimicrobial agent and the breakpoints according to EUCAST (6). The investigated strains were divided by a cutoff at 8.0 µg/ml into two groups: the susceptible/wild-type group and the resistant group, respectively. For clindamycin, the same cutoff

In Vitro Activities of Clindamycin, Imipenem, Metronidazole, and Piperacillin-Tazobactam against Susceptible and Resistant Isolates of Bacteroides fragilis Evaluated by Kill Kinetics

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is used independently of the breakpoint (4 μg/ml) according to EUCAST (6). The chosen cutoff at 8.0 μg/ml also separates two different groups, the wild-type and the resistant groups.

The pooled kill kinetic curves for *B. fragilis* strains with MICs of ≥8.0 μg/ml are shown in Fig. 1. At concentrations above the MIC, clindamycin showed bactericidal activity against only 5 out of 10 strains. Imipenem was bactericidal against 8 out of 9 strains, and metronidazole was bactericidal against 10 out of 10 strains. Piperacillin-tazobactam showed bactericidal activity against 6 out of 8 strains investigated. Piperacillin-tazobactam was the only antibiotic regime in which statistically significant differences were found after 6 h of incubation. The use of 4× MIC resulted in a higher killing rate than the use of 1× MIC (P < 0.05). A significantly higher killing rate using 1× MIC or 4× MIC instead of 0.5× MIC and clindamycin or imipenem, respectively, occurred at t = 24 h (P < 0.05). Between 1× MIC and 4× MIC, no statistical significances were found for clindamycin or imipenem, respectively. In contrast, increasing concentrations of metronidazole or piperacillin-tazobactam resulted in significantly higher killing rates after 24 h (P < 0.05).

The pooled kill kinetic curves for *B. fragilis* strains with MIC values of >8.0 μg/ml are shown in Fig. 2. The two metronidazole-resistant strains were effectively killed by metronidazole when concentrations of \( C_{\text{max}} \) (16 μg/ml) or more were used. Also, two of three imipenem-resistant strains were killed by imipenem with concentrations of \( C_{\text{max}} \) (32 μg/ml) or more. Piperacillin-tazobactam showed activity against 3 of the 4 piperacillin-tazobactam-resistant strains when concentrations of \( C_{\text{max}} \) (piperacillin, 60 μg/ml, and tazobactam, 25 μg/ml) or higher were used. In contrast, clindamycin did not inhibit the bacterial growth of the clindamycin-resistant strains even at concentrations of 4× \( C_{\text{max}} \) (64 μg/ml). Due to the limited number of strains with MICs of ≥8 μg/ml, statistical analysis could be performed only for piperacillin-tazo-

### TABLE 1 MICs (μg/ml) of the *B. fragilis* strains tested and breakpoints according to EUCAST (6)

<table>
<thead>
<tr>
<th><em>B. fragilis</em> strain</th>
<th>Clindamycin, ≤4 (s)/&gt;4 (r)</th>
<th>Imipenem, ≤2 (s)/&gt;8 (r)</th>
<th>Metronidazole, ≤4 (s)/&gt;4 (r)</th>
<th>Piperacillin-tazobactam, ≤8 (s)/&gt;16 (r)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WAL 13174</td>
<td>0.03</td>
<td>0.5</td>
<td>&gt;256</td>
<td>2</td>
</tr>
<tr>
<td>RMA 5935</td>
<td>0.03</td>
<td>&gt;32</td>
<td>0.25</td>
<td>&gt;256</td>
</tr>
<tr>
<td>RMA 5120</td>
<td>1</td>
<td>0.25</td>
<td>0.5</td>
<td>0.125</td>
</tr>
<tr>
<td>RMA 5081</td>
<td>2</td>
<td>0.25</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>WAL 13054</td>
<td>2</td>
<td>0.25</td>
<td>&gt;256</td>
<td>1</td>
</tr>
<tr>
<td>RMA 6600</td>
<td>2</td>
<td>&gt;32</td>
<td>0.5</td>
<td>32</td>
</tr>
<tr>
<td>WAL 13267</td>
<td>4</td>
<td>0.125</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>RMA 0309</td>
<td>4</td>
<td>&gt;32</td>
<td>0.5</td>
<td>&gt;256</td>
</tr>
<tr>
<td>RMA 5798</td>
<td>8</td>
<td>0.125</td>
<td>0.5</td>
<td>4</td>
</tr>
<tr>
<td>RMA 5691</td>
<td>8</td>
<td>0.25</td>
<td>1</td>
<td>16</td>
</tr>
<tr>
<td>RMA 5138</td>
<td>&gt;256</td>
<td>0.5</td>
<td>0.5</td>
<td>2</td>
</tr>
<tr>
<td>RMA 6791</td>
<td>&gt;256</td>
<td>0.5</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

*a* s, susceptible; *r*, resistant.
bactam. However, no statistical differences in killing rates could be found even between 0.5 × C_{max} and 4 × C_{max}.

Comparing the prior established MICs by Etest with the assessed kill kinetics, a good correlation could be found when the organisms were susceptible to the respective antibiotic agent. Furthermore, in the present study imipenem showed a slightly better effect than did piperacillin-tazobactam. In contrast, clinical trials comparing piperacillin-tazobactam with imipenem/cilastatin in patients with intra-abdominal infections revealed equal efficacy (5, 18) or slight advantages for piperacillin-tazobactam (4).

Against resistant strains, clindamycin showed no effect on those strains while metronidazole could show a rather good effect. Thus, metronidazole appeared to be the most effective investigated substance but still needs to be combined with another antibiotic to cover infections with aerobic bacteria in mixed infections.

In summary, the kill kinetics over time could provide additional information on local resistance patterns, which is of utmost importance for an adequate prophylaxis and treatment in anaerobic or mixed infections. Kill kinetics assays should be performed in studies after establishing MIC values and also choosing resistant strains.

REFERENCES


