Increase in resistance to new fluoroquinolones from 1998 to 2001 in the Bacteroides fragilis group

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Susceptibility to anti-anaerobic agents in the Bacteroides fragilis group varies according to the geographical region studied. In recent years there has been a reduction in the susceptibility of such isolates, especially to certain antibiotics such as clindamycin and cefoxitin. The antimicrobial susceptibilities of 200 strains of the B. fragilis group isolated in 1998 (100) and 2001 (100) from faecal samples of healthy people to anti-anaerobic agents were determined. Meropenem, metronidazole and chloramphenicol showed excellent activity against all isolates. The efficacy of cefoxitin was low, with only 43% of strains isolated in 2001 showing susceptibility. A high prevalence of resistance to clindamycin, reaching 56% of recent isolates, was observed. A significant increase in resistance to new fluoroquinolones, from 0% in 1998 to 12% in 2001, was detected.

Keywords: fluoroquinolone resistance, Bacteroides

Introduction

Members of the Bacteroides fragilis group are the most important anaerobic Gram-negative human pathogens, being the most common anaerobic organisms isolated from blood and intra-abdominal infections.1

Historically, antimicrobial regimens for the treatment of B. fragilis group infections have been limited to selected β-lactams, clindamycin, chloramphenicol or metronidazole. In recent years there has been a reduction in the susceptibility of such isolates, especially to certain antibiotics such as clindamycin and cefoxitin. In a study carried out in our hospital with strains isolated in 1998 we found that only 49% and 46% of strains were susceptible to clindamycin and to cefoxitin, respectively.2 The data obtained in that study led to a modification in the antibiotic prophylaxis protocol in abdominal surgery in our centre, with the substitution of clindamycin by other anti-anaerobic agents, principally metronidazole.

Recently developed fluoroquinolones have been demonstrated to have activity in vitro against B. fragilis group and other anaerobic bacteria.3,4 Their intrinsic antibacterial action against other common intra-abdominal pathogens make selected fluoroquinolones attractive as single-agent therapies for polymicrobial intra-abdominal infections.5

We studied the susceptibility to anti-anaerobic agents of 100 strains of the B. fragilis group isolated in 2001 in comparison with 100 strains isolated in 1998.

Material and methods

We studied 100 non-duplicate isolates of the B. fragilis group isolated from stool samples taken from the healthy population of an administrative health area of Madrid, Spain, in 2001, including males and females between 3 and 80 years old. The results were compared with the results obtained previously in 100 strains isolated in 1998 under the same conditions. The individuals providing the stool samples were not the same for both studies and they had not taken any antimicrobial agent during the 30 days prior to donating their stool.

A selective medium, Bacteroides bile–aesculin (BBE) agar, was used to culture B. fragilis group from faecal specimens. Strains were identified using standard methods, and fluoroquinolone-resistant strains were identified to species

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level using the Rapid ID 32A system (bioMérieux, Marcy l’Étoile, France).

MICs of the following antibiotics were determined: co-amoxiclav (SmithKline Beecham, Toledo, Spain), meropenem (Zenea, Pontevedra, Spain), cefoxitin (Sigma, St Louis, MO, USA), clindamycin (Upjohn, Crawley, UK), metronidazole (Sigma), chloramphenicol (Zyma Farmaceutica, Barcelona, Spain), trovafloxacin (Pfizer, Groton, CT, USA) and moxifloxacin (Bayer, Barcelona, Spain). The same antibiotics were tested against the strains isolated in 1998 and in 2001, with the exceptions of the introduction of chloramphenicol and the substitution of trovafloxacin, now withdrawn from the market, by moxifloxacin in the group of strains isolated in 2001. However, in all strains resistant to moxifloxacin, trovafloxacin was also tested in order to confirm cross-resistance.

MICs were determined by agar dilution following NCCLS recommendations on Wilkins–Chalgren agar (Difco Laboratories, Detroit, MI, USA). Final concentrations per plate were 0.5–256 mg/L for clindamycin, 0.12–8 mg/L for meropenem, 4–64 mg/L for cefoxitin, 0.25–8 mg/L for metronidazole, 1/0.5–16/8 mg/L for co-amoxiclav, 2–8 mg/L for chloramphenicol and 0.06–8 mg/L for trovafloxacin and moxifloxacin. Isolates were grown in supplemented brain–heart infusion broth to logarithmic phase, and their turbidity adjusted to a 0.5 McFarland standard. The inocula were delivered to the surface of the agar with a Steers replicator (~10^5 cfu/spot). The plates were incubated in an anaerobic atmosphere at 35°C for 48 h. MICs were read as the lowest concentration of antibiotic that resulted in no visible growth. MICs were determined by agar dilution following NCCLS criteria. Susceptibility data for moxifloxacin were calculated using the breakpoints established for trovafloxacin (S, ≤2 mg/L; R, ≥8 mg/L) as the NCCLS has not yet established breakpoints for moxifloxacin.

Results and discussion

The MICs for the two control strains were always within recommended limits. The resistance trend from 1998 to 2001 is summarized in Table 1, showing range, \( \text{MIC}_{50} \) and \( \text{MIC}_{90} \) as well as percentages of susceptible, intermediate and resistant strains for each of the antibiotics tested.

In the past decade the literature has reported increasing resistance in \( B. \ fragilis \) group species to antimicrobial agents, as well as regional and institutional differences in resistance rates. Consequently, the results of periodic susceptibility studies are useful and necessary as a guide in the choice of agents for correct prophylaxis and empirical treatment.

In an attempt to update our data concerning the susceptibility of the \( B. \ fragilis \) group in our hospital we performed a second study with 100 strains isolated in 2001 under the same conditions as those studied in 1998. The increase in resistance to clindamycin was confirmed, although the difference was not statistically significant, rising from 49% to 56% (\( P = 0.32 \)) in spite of the marked reduction in its use in our hospital. There were no significant differences in susceptibility to coamoxiclav and cefoxitin, whilst metronidazole and meropenem retained excellent in vitro activity with 100% of strains susceptible and MICs similar to those of 3 years earlier.

Nevertheless, we have observed a significant increase in resistance to new fluoroquinolones, from 0% resistance to trovafloxacin in 1998 to 12% (12/100) resistance to moxifloxacin in 2001 (\( P < 0.001 \)), with an \( \text{MIC}_{90} \) of 0.5 mg/L in the first case and >8 mg/L in the second. In order to confirm the presence of cross-resistance between trovafloxacin and moxi-

### Table 1. Comparison of the susceptibility pattern of 100 \( B. \ fragilis \) group isolated in 1998 with 100 \( B. \ fragilis \) group isolated in 2001.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>1998 range</th>
<th>( \text{MIC}_{50} )</th>
<th>( \text{MIC}_{90} )</th>
<th>S (%)</th>
<th>I (%)</th>
<th>R (%)</th>
<th>2001 range</th>
<th>( \text{MIC}_{50} )</th>
<th>( \text{MIC}_{90} )</th>
<th>S (%)</th>
<th>I (%)</th>
<th>R (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clindamycin</td>
<td>≤0.5–&gt;256</td>
<td>4</td>
<td>256</td>
<td>49</td>
<td>2</td>
<td>49</td>
<td>≤0.5–&gt;256</td>
<td>64</td>
<td>&gt;256</td>
<td>44</td>
<td>0</td>
<td>56</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>≤4–&gt;64</td>
<td>32</td>
<td>64</td>
<td>46</td>
<td>36</td>
<td>18</td>
<td>≤4–&gt;64</td>
<td>32</td>
<td>64</td>
<td>43</td>
<td>36</td>
<td>21</td>
</tr>
<tr>
<td>Co-amoxiclav</td>
<td>≤1–16</td>
<td>≤1</td>
<td>8</td>
<td>83</td>
<td>13</td>
<td>4</td>
<td>≤1–16</td>
<td>2</td>
<td>8</td>
<td>88</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Meropenem</td>
<td>≤0.12–4</td>
<td>0.25</td>
<td>1</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>≤0.25–4</td>
<td>&lt;0.25</td>
<td>1</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>≥2–8</td>
<td>4</td>
<td>4</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Metronidazole</td>
<td>≤0.25–0.5</td>
<td>0.25</td>
<td>0.5</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>0.5–1</td>
<td>0.5</td>
<td>0.5</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Trovafloxacin</td>
<td>0.12–2</td>
<td>0.25</td>
<td>0.5</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>&lt;0.06–&gt;8</td>
<td>0.5</td>
<td>&gt;8</td>
<td>87</td>
<td>1</td>
<td>12</td>
</tr>
</tbody>
</table>

S, susceptible; I, intermediately susceptible; R, resistant.

*Expressed as mg/L.

*Using breakpoints established for trovafloxacin by the NCCLS (S, ≤2 mg/L; R, ≥8 mg/L).
Fluoroquinolone resistance in Bacteroides

trovafloxacin and to compare the two results, susceptibility to
trovafloxacin was studied in 12 moxifloxacin-resistant
strains. All (12/12), were also resistant to trovafloxacin; in
eight isolates the MIC was >8 mg/L of both antibiotics; and in
four the MICs of trovafloxacin were slightly lower than those
obtained for moxifloxacin, 8 mg/L versus >8 mg/L, without
affecting their categorization as resistant.

Isolates resistant to moxifloxacin and trovafloxacin were
grouped in species; six B. fragilis, three Bacteroides eggerthii,
one Bacteroides ovatus, one Bacteroides vulgatus and one
B. thetaiotamicron.

The new fluoroquinolones with activity against anaerobic
bacteria are considered effective against B. fragilis group and
are recommended as an excellent alternative in monotherapy
for intra-abdominal infection on account of their good activity
against the microorganisms usually involved. Various stud-
ies have demonstrated the in vitro activity of trovafloxacin
and moxifloxacin against the B. fragilis group. Recently
there have been reports of resistance to these compounds,
reaching 7% for trovafloxacin in the study of Aldridge et al.

However, in a study carried out in Spain with strains
isolated in 1997, Betriú et al. found only 1.8% resistance to
trovafloxacin, while moxifloxacin had MICs only slightly
higher.

The marked and rapid increase in resistance to the new
fluoroquinolones in B. fragilis group species in our health
area is probably due, to a certain extent, to their increasing use
in various infections, particularly those of the respiratory
tract. These data suggest the need for continuous epidemi-
ological surveillance. In the future, resistance to these com-
pounds could rule out their use as empirical therapy in
infections where B. fragilis group is suspected.

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