Susceptibility of Clinical Isolates of *Campylobacter pylori* to Twenty-One Antimicrobial Agents

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The MICs of 21 antimicrobial agents were determined for 97 clinical isolates of *Campylobacter pylori*. The beta-lactams (penicillin, ampicillin, cefoxitin and cephalaxin), macrolides (erythromycin and azithromycin), quinolones (ciprofloxacin and ofloxacin), nitrofurans, gentamicin and tetracycline all had MIC90 values of < 0.5 mg/l. Aztreonam, flucloxacillin, amifloxacin and rifampicin had moderate activity. All isolates were resistant to vancomycin, cefsulodin and amphotericin B. Five percent of the strains were inhibited by 8 mg/l of polymyxin. Of the oral agents tested, the nitrofurans and ampicillin are probably the most appropriate antimicrobial agents. Azithromycin and the oral form of cefuroxime are promising alternatives. Cefsulodin, vancomycin and amphotericin B would be suitable constituents of selective media for isolation of *Campylobacter pylori*.

The treatment of *Campylobacter pylori* infection is difficult as in vitro activity does not always correlate with in vivo success (1, 2). Relapse has followed apparent clearance of the organisms with bismuth salts and amoxicillin (3). Acquired resistance has occurred with the quinolones (2). Since it has become apparent that long-term eradication of *Campylobacter pylori* will be difficult, a search for alternative antimicrobial agents is needed. The determination of in vitro efficacy of antimicrobial agents against *Campylobacter pylori* is the first step in this search. We report the activity of 21 agents against *Campylobacter pylori*, including the new macrolide azithromycin (CP 62993), and the newer quinolones amifloxacin and ofloxacin. Also tested were five agents (penicillin, erythromycin, cefoxitin, gentamicin and ciprofloxacin) whose activity had been previously determined against a smaller number of isolates (4).

Materials and Methods

Organisms. Strains were cultured from gastric antral biopsy specimens taken at routine endoscopy during investigation of upper gastrointestinal symptoms. Patients had not received antimicrobial agents for *Campylobacter pylori* infection in the six weeks prior to the collection of gastric biopsy specimens. The organisms were identified as *Campylobacter pylori* using previously described methods (1): colonial morphology, Gram stain appearance and positive rapid urease test. Organisms were stored in liquid nitrogen until subcultured for this study.

Antimicrobial Agents. The agents tested were penicillin, flucloxacillin, ampicillin (Boehem, UK), cefoxitin (Merck, Sharpe and Dohme, UK), cephalexin (Eli Lilly, UK), cefuroxime (Glaxo, UK), cefsulodin and rifampicin (CIBA, UK), aztreonam (Squibb, UK), erythromycin (Abbott, UK), azithromycin, (Pfizer, UK), tetracycline (Revlin, UK), ciprofloxacin (Bayer, UK), ofloxacin (Hoechst, UK), amifloxacin (Sterling Research, UK), nitrofurantoin (Norwich Eaton, UK), furazolidone (Norwich Eaton, USA), gentamicin (Roussel, UK), vancomycin (Eli Lilly, UK), polymyxin (Wellcome, UK), and amphotericin B (Squibb, UK). A measured aliquot of each antibiotic was taken from stock solutions of 1000 mg/l or 100 mg/l and added to 20 ml of agar at about 50°C to make each of the final concentrations tested.

Susceptibility Testing. MICs were determined as previously described using an agar dilution technique with *Staphylococcus aureus* NCTC 6571, *Escherichia coli* NCTC 10418 and *Pseudomonas aeruginosa* NCTC 10662 were included on each plate. Plates were incubated for 72 h at 37°C in a microaerobic atmosphere of 6 % O2 and 10 % CO2 (partial evacuation and replacement technique). The MIC of an antimicrobial agent was defined as the concentration (in mg/l of agar) at which there was a reduction (by counting) to ten or fewer colonies.

Results

The susceptibility of the isolates to the antimicrobial agents, expressed in terms of inhibitory range, and MICs for 50% and 90% of the strains tested (MIC50

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Table 1: MIC values of 21 antimicrobial agents for isolates of *Campylobacter pylori*.

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>No. of isolates tested</th>
<th>MIC (mg/l)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MIC50</td>
<td>MIC90</td>
</tr>
<tr>
<td>Penicillin</td>
<td>70</td>
<td>0.015</td>
<td>0.03</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>97</td>
<td>0.015</td>
<td>0.03</td>
</tr>
<tr>
<td>Flucloxacinillin</td>
<td>50</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>97</td>
<td>≤ 0.12</td>
<td>≤ 0.12</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>70</td>
<td>0.12</td>
<td>0.12</td>
</tr>
<tr>
<td>Cephalixin</td>
<td>97</td>
<td>≤ 0.25</td>
<td>0.5</td>
</tr>
<tr>
<td>Cefadolin</td>
<td>97</td>
<td>16</td>
<td>&gt; 16</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>97</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>97</td>
<td>0.12</td>
<td>0.25</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>97</td>
<td>0.12</td>
<td>0.25</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>97</td>
<td>0.25</td>
<td>0.5</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>21</td>
<td>0.12</td>
<td>0.25</td>
</tr>
<tr>
<td>Amoxifloxacin</td>
<td>97</td>
<td>0.5</td>
<td>4</td>
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<tr>
<td>Nitrofurantoin</td>
<td>96</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Furazolidone</td>
<td>96</td>
<td>0.12</td>
<td>0.25</td>
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<tr>
<td>Gentamicin</td>
<td>70</td>
<td>0.12</td>
<td>0.25</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>70</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>50</td>
<td>≤ 1</td>
<td>≤ 1</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>21</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>Vancomycin</td>
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<td>&gt; 64</td>
<td>&gt; 64</td>
</tr>
<tr>
<td>Polymyxin</td>
<td>97</td>
<td>&gt; 128</td>
<td>&gt; 128</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>50</td>
<td>&gt; 32</td>
<td>&gt; 32</td>
</tr>
</tbody>
</table>

and MIC90 respectively), are shown in Table 1. The MICs of *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were within one dilution step of those previously reported (5).

Penicillin and ampicillin had the greatest activity of the antimicrobials tested, with an MIC50 of 0.015 mg/l and MIC90 of 0.03 mg/l. Flucloxacinillin was less active, with an MIC90 of 2 mg/l, but the inhibitory range went as high as 8 mg/l. Of the cephalosporins cefuroxime (MIC90 ≤ 0.12 mg/l) was the most active, with an MIC50 and MIC90 of less than 0.12 mg/l. Cefoxitin was also very active, with an MIC90 of 0.12 mg/l and range up to 0.5 mg/l; cephalexin was slightly less active (MIC90 0.5 mg/l), with an inhibitory range up to 2 mg/l. For all isolates MICs of cefadolin were high, with an inhibitory range of 8 — > 16 mg/l. The monobactam aztreonam had moderate activity, with an MIC90 of 2 mg/l and inhibitory range of 0.06—8 mg/l. The two macrolides, erythromycin and azithromycin, were equipotent (MIC50 0.012 mg/l, MIC90 0.25 mg/l) but were ten times less active than penicillin. The two quinolones, ciprofloxacin and ofloxacin, had similar activity to the macrolides; the third quinolone, amoxifloxacin, was ten times less active with an MIC90 of 4 mg/l and inhibitory range of 1—8 mg/l. The two nitrofurans had activity similar to that of the macrolides and quinolones; furazolidone (MIC90 0.25 mg/l) was slightly more active than nitrofurantoin (MIC90 0.5 mg/l). Tetracycline (MIC90 0.25 mg/l) and rifampicin (MIC90 1 mg/l) both had good activity. Vancomycin and amphotericin B had little or no activity against *Campylobacter pylori*. The majority of strains were resistant to polymyxin (MIC90 > 128 mg/l), though five strains were inhibited by less than 8 mg/l.

**Discussion**

In order to detect small numbers of resistant strains it is important to study a large number of isolates. Other groups (6, 7, 8) have tested the activity of a range of antimicrobial agents against *Campylobacter pylori* using ten, 20 and 50 isolates respectively. In this study a larger number of isolates and several additional antimicrobials including quinolones, nitrofurans, aztreonam, cefuroxime and azithromycin were used. No patient from whom a strain of *Campylobacter pylori* was isolated had received antibiotics in the preceding six weeks, which is important because acquired resistance to quinolones and imidazoles has occurred after short courses of treatment. Reproducibility of MIC results is greatest when inocula are prepared from liquid cultures (9). Other authors have prepared inocula from growth on solid media suspended in either saline or broth; this method will produce degenerate nonviable bacteria. Armstrong et al. (10) suggest degenerate forms will be decreased by two transfer cultures in liquid medium. We prepared our inocula from 48 h liquid media cultures. Caution should therefore be used when comparisons are made between results from different centres.