
Ewen A. B. Cameron,† Katharine U. Powell, Lynette Baldwin, Philip Jones, G. Duncan Bell‡ and Simon G. J. Williams

Department of Gastroenterology and Public Health Laboratory Service, Ipswich Hospital, Heath Road, Ipswich IP4 5PD, UK

**Correspondence**

Ewen A. B. Cameron
camerone99@aol.com

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**INTRODUCTION**

*Helicobacter pylori* is involved in the pathogenesis of a number of gastrointestinal diseases including acute and chronic gastritis, peptic ulceration, gastric carcinoma and gastric lymphoma (Calam, 1995). Diagnosis of infection can be achieved by using a number of direct and indirect methods such as direct gastric urease tests, histology, direct culture of gastric biopsies, urea breath testing and serological testing.

The treatment of *H. pylori* infection usually relies on the simultaneous administration of various combinations of drugs. Most commonly, an acid suppressor (usually a proton pump inhibitor) is prescribed in combination with two antibiotics (usually from amoxycillin, metronidazole and clarithromycin). The success of eradication depends on a number of factors, including antibiotic resistance (Jenks, 2002).

The level of antibiotic resistance amongst *H. pylori* strains varies markedly even within Western Europe. Rates of metronidazole resistance range from 15·9 % (France) to 41·9 % (Norway), whilst clarithromycin resistance is seen in between 1 % (Norway) and 5 % of isolates (UK and Ireland) (Megraud et al., 1999). As a result, local sensitivity data aid the choice of eradication regime.

Ipswich Hospital is a district general hospital in Suffolk (East Anglian region of England) that has been involved in the study of *H. pylori* infection since shortly after its discovery, including the development of the C14 urea breath test (Bell et al., 1987) as a means of monitoring the success of various candidate eradication regimes and monitoring reinfection/recrudescence rates, as well as the development of histological stains for identification of the organism (Trowell et al., 1987). We have prospectively collected data on antibiotic sensitivity of *H. pylori* isolates since 1991. Our local dyspepsia guidelines recommend first-line treatment of infection, in the absence of antibiotic sensitivities, with a 1 week course of omeprazole 40 mg o.d., amoxycillin 500 mg t.d.s. and metronidazole 400 mg t.d.s. We have examined the changing...
pattern of resistance to metronidazole since 1991 and to clarithromycin since 1995 and its variation with age and sex. We have also examined the effect of time, age and sex on the eradication rate with triple therapy regimes consisting of a proton pump inhibitor, amoxycillin and metronidazole (PPI/A/M) or clarithromycin (PPI/A/C) since 1994.

METHODS

Antibiotic resistance in *H. pylori* isolates. Between 1991 and the end of 2001, all consecutive isolates of *H. pylori* from gastric biopsies were studied to allow assessment of trends in antibiotic resistance. Initially, only sensitivity testing for metronidazole was performed, with the addition of testing for clarithromycin from 1995 (although not all isolates were routinely tested until 1998). Sensitivity to metronidazole and/or clarithromycin was prospectively evaluated for all isolates. Sensitivity testing was performed as described previously (Bell et al., 1995) using a disc diffusion method on Isosensitest agar containing 5% lysed horse blood at 35–37 °C under microaerophilic conditions. Discs contained either 5 μg metronidazole or 15 μg clarithromycin. Isolates were defined as resistant if no zone of inhibition was seen around the disc. Patient demographic data were collected regarding age and sex for all patients with positive isolates.

Efficacy of *H. pylori* eradication. Data were prospectively collected for all consecutive serology positive patients referred for urea breath testing to confirm successful eradication of *H. pylori* after treatment with either PPI/A/M or PPI/A/C, including demographic data and the result of urea breath testing.

Statistical analysis was performed using the SPSS version 9.0 statistics package. Comparisons between groups were performed using Pearson’s chi-squared test and, when appropriate, Fisher’s exact test. Statistical significance was set at the 5% level. Where more than one factor was independently associated with resistance, multiple logistic regression with forward stepwise selection was performed to ensure the independence of factors associated with antibiotic resistance.

### RESULTS AND DISCUSSION

Between 1991 and 2001, *H. pylori* was isolated from 1263 endoscopically collected gastric biopsies. Metronidazole susceptibility was successfully tested in 1257 of these and resistance was found in 31.7% (95% confidence interval 29.1–34.2%) of isolates. A significant increase in metronidazole resistance was found between 1991–1994 (29.1%, 25.2–33.1%) and 1999–2001 (37.0%, 31.4–42.5%) (*P* = 0.022). Metronidazole resistance was commoner in women than in men (*P* < 0.001) and in patients ≤40 years old than in patients >40 (*P* < 0.001) (Table 1). Multiple logistic regression with forward stepwise selection confirmed both youth (odds ratio 2.02; 95% confidence interval 1.37–2.92; *P* = 0.0004) and the female sex (odds ratio 1.70; 95% confidence interval 1.33–2.16; *P* < 0.0001) to be independently associated with the risk of metronidazole resistance.

Susceptibility to clarithromycin was tested in 388 isolates between 1995 and 2001, 5.4% (3.1–7.5%) of which were resistant. Clarithromycin resistance fell significantly between 1995–1998 (9.3%, 3.8–14.9%) and 1999–2001 (3.8%, 1.6–6.0%) (*P* = 0.014). There was no significant association between the presence of clarithromycin resistance and either age or sex (Table 1).

Although available in our hospital since 1987, a urea breath test service was only made generally available to all local general practitioners in 1994 to allow confirmation of eradication in patients found to be positive for *H. pylori* on serological testing. Testing utilized urea labelled with either C13 or C14 as described previously (Bell & Weil, 1992; Logan, 1992). Between 1994 and 2001, 706 patients were referred for urea breath testing by their general practitioners following a course of eradication therapy with a PPI/A/M triple therapy regime. The overall eradication rate was 89.9% (87.7–91.4%)

### Table 1. Metronidazole (1991–2001) and clarithromycin (1995–2001) resistance in *H. pylori* isolates divided according to sex and age

<table>
<thead>
<tr>
<th>Result</th>
<th>Sex</th>
<th></th>
<th>Age (years)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Men</td>
<td>Women</td>
<td>&gt;40</td>
<td>≤40</td>
</tr>
<tr>
<td>Metronidazole†</td>
<td>755</td>
<td>502</td>
<td>1140</td>
<td>117</td>
</tr>
<tr>
<td>Isolates</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resistant</td>
<td>206</td>
<td>192</td>
<td>345</td>
<td>53</td>
</tr>
<tr>
<td>Percentage (95% confidence intervals)</td>
<td>27.3 (24.1–30.5)</td>
<td>38.2 (34.0–42.5)</td>
<td>30.3 (27.6–32.9)</td>
<td>54.3 (36.3–54.3)</td>
</tr>
<tr>
<td>P-value</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Clarithromycin‡</td>
<td>221</td>
<td>167</td>
<td>348</td>
<td>40</td>
</tr>
<tr>
<td>Isolates</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resistant</td>
<td>12</td>
<td>9</td>
<td>17</td>
<td>4</td>
</tr>
<tr>
<td>Percentage (95% confidence intervals)</td>
<td>5.4 (2.4–8.4)</td>
<td>5.4 (2.0–8.8)</td>
<td>4.9 (0.7–19.3)</td>
<td>10 (2.6–7.1)</td>
</tr>
<tr>
<td>P-value</td>
<td>0.986</td>
<td>0.255</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

†Comparisons made using Pearson’s chi-squared test.
‡Comparisons made using Fisher’s exact test.
92.2%), with no statistically significant change in eradication rate with time. Whilst the eradication rate was significantly lower in patients ≤40 years old than in those older (P < 0.001), there was no difference between men and women (Table 2). During the same time period, 63 patients were referred following a course of eradication therapy with PPI/A/C. The eradication rate in these patients was 92.1% (85.4–98.7%) with no statistically significant difference in eradication rates compared with patients treated with PPI/A/M (P = 0.589).

This study provides important information with regards to changing patterns of antibiotic resistance in H. pylori infection in Suffolk. There has been a slow but steady rise in metronidazole resistance without an accompanying rise in clarithromycin resistance. Recent data from Nottingham and Dublin suggest rates of resistance of 26–6% to metronidazole and 5% to clarithromycin (Megraud et al., 1999). Numbers from these two centres were, however, small (60 patients overall) and limited to patients with duodenal ulcer disease. The large number of our own isolates (1263) from all disease processes and the duration of our study provide valuable information that can be applied to all patients with H. pylori infection.

As previously documented, we found higher rates of metronidazole resistance amongst women (Weil et al., 1990; Wolle et al., 2002; Osato et al., 2001; Meyer et al., 2002), a phenomenon thought to occur as a result of metronidazole use in the treatment of gynaecological infections. Previous reports have also suggested higher rates of metronidazole resistance in younger patients (Mollison et al., 2000), but this is far from being a consistent finding (Osato et al., 2001). When deciding on the choice of eradication regime in patients without the use of sensitivity data, it is important to be able to identify subgroups of patients at high risk of antibiotic resistance. Continued use of metronidazole in eradication regimes in the absence of sensitivity data might seem counter-intuitive; however, increasing rates of clarithromycin resistance are being seen around the world (Cabrita et al., 2000; Megraud, 1998). The avoidance of widespread use of clarithromycin in the treatment of H. pylori infection may help to prevent this rise in resistant organisms.

Recently, a consensus has been reached on the methods that are to be used in the testing for antibiotic susceptibility in H. pylori (McNulty et al., 2002). We recognize the limitations of the disc diffusion method we have used. However, whilst some cases of low-level resistance may have been missed, this would not have affected the overall trend observed. In addition, it is important that we have used the same method throughout the study period, allowing direct comparison across time periods.

Despite increasing levels of metronidazole resistance, no effect was seen on eradication rates amongst patients treated with PPI/A/M. Importantly, these cases were not treated as part of a clinical trial and, as such, are representative of the real situation in clinical practice. The patient group studied was selected as it represents a sample of the local population in whom identification of H. pylori infection was made without the use of culture and sensitivity testing. This ensured that eradication rates were not falsely elevated by selection of patients with metronidazole-susceptible isolates. In fact, as one would expect urea breath testing to be performed more often in patients with ongoing symptoms after therapy than in those with symptom resolution, this group probably contained a slight excess of treatment failures and, as a result, metronidazole resistance.

Whilst it is possible that some of the cases of H. pylori infection identified with serological testing might have been false positives, the rate of successful eradication was similar to that found locally in clinical trials (Bell et al., 1995) and no significant difference was seen with PPI/A/C regimes. False positives would have been expected in similar proportions of both groups. The much smaller number of patients treated with PPI/A/C reflected the local recommendation to use omeprazole, amoxycillin and metronidazole as a first-line regime.

It is not clear why increasing rates of metronidazole resistance had little impact on eradication rates. High eradication rates with metronidazole-containing regimes have been seen despite in vitro metronidazole resistance in H. pylori isolates (Megraud et al., 1999; Graham et al., 1996), and this may occur due to differences between in vitro and in vivo susceptibility (Jenks, 2002). The reason for the fall in rates of clarithromycin resistance over the study period is also not obvious. The small number of isolates tested between 1995 and 1998 and the fact that not all isolates were routinely...
tested over this time period may have contributed to this finding. Between 1995 and 1998, clarithromycin resistance was utilized as a research tool on isolates from one consultant’s endoscopy lists. Whilst this led to smaller numbers of isolates being tested during this time period, the cases on these lists were not specially selected and, as a result, should have been representative of all isolates.

The population in Suffolk may not be entirely representative of other populations within the UK. The ethnic minority population in Suffolk is considerably smaller than the national mean, with 97.2% of the local population being white (http://www.statistics.gov.uk/census2001/), potentially affecting the generalizability of the results to areas with different ethnic characteristics.

Clarithromycin-containing regimes are currently recommended in the UK by the Primary Care Society for Gastroenterology (http://www.pcsg.org.uk/) and the British Society of Gastroenterology (http://www.bsg.org.uk/clinical_prac/guidelines/dyspepsia.htm) in the first-line treatment of H. pylori infection. However, whilst overall eradication rates with PPI/A/M remain approximately 90% [a figure comparable to that previously reported in open and double-blind studies in Suffolk (Bell et al., 1995)], the use of amoxycillin and metronidazole as first-line eradication therapy in combination with a proton pump inhibitor seems reasonable for the majority of patients. This approach may help to reduce the spread of clarithromycin resistance and is considerably cheaper. The current cost of a 1 week course of clarithromycin 500 mg b.d. is £24.74, compared with £3.50 for metronidazole 500 mg t.d.s. (http://www.bnf.org). Avoidance of metronidazole-containing regimes in subgroups of patients with high rates of metronidazole resistance (such as young women and certain ethnic groups) in whom sensitivity testing has not been performed is, however, probably indicated. Whilst most centres utilise direct urease tests for the identification of H. pylori in gastric biopsies, we have continued to culture the organism in Ipswich directly. This continued surveillance of antibiotic sensitivity will be vital in directing future eradication therapy.

REFERENCES