Prevalence of *Helicobacter pylori* resistance to metronidazole, clarithromycin, amoxycillin, tetracycline and trovafloxacin in The Netherlands

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Successful treatment of *Helicobacter pylori* infection is becoming compromised by emerging resistance. We report the prevalence rates of *H. pylori* resistance to metronidazole, clarithromycin, amoxycillin, tetracycline and trovafloxacin in The Netherlands. A total of 231 *H. pylori* clinical isolates were collected throughout the country over a period of 6 months during 1997–1998. The MICs of the above-mentioned antibiotics were determined in a single laboratory. The overall percentage of resistance for clarithromycin and metronidazole was 1.7% and 21.2%, respectively. None of the strains was resistant to amoxycillin or tetracycline. The primary resistance rate of trovafloxacin was as high as 4.7%. Since trovafloxacin has not yet been introduced on to the Dutch market, the resistance is probably induced by the use of other quinolones. Our data indicate that treatment outcome would benefit from susceptibility testing before starting therapy, especially when prescribing metronidazole.

**Materials and methods**

**Strains and growth conditions**

A total of 231 clinical isolates of *H. pylori* were collected from 21 participating laboratories in The Netherlands. Each participating laboratory collected 10–15 consecutive clinical isolates. All strains were cultured from gastric biopsies of patients with peptic ulcer disease who underwent a diagnostic endoscopy. These strains were collected between September 1997 and February 1998. Isolates were frozen in 20 vol% glycerol in Brain Heart Infusion broth.

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(Difco Laboratories, Detroit, MI, USA) transferred to our laboratory and subcultured on to Columbia agar (Becton Dickinson, Cockeysville, MD, USA) supplemented with 10% lysed horse blood (Bio Trading, Mijdrecht, The Netherlands), and H. pylori selective supplement (Oxoid, Basingstoke, UK), referred to as Dent plates. Plates were incubated for 72 h at 37°C in an atmosphere of O2/CO2/N2 (5:10:85). Identification was performed by means of Gram's stain, catalase, oxidase, and urea hydrolysis.

Determination of MIC

Inocula were prepared from a H. pylori culture grown on Dent plates. MICs were determined by Etest (AB Biodisk, Solna, Sweden) essentially as described by Glupczynski et al., on Columbia agar supplemented with 10% lysed horse blood plates inoculated with approximately $2 \times 10^8$ cfu in 20 μL of 0.9% NaCl.

Consumption of macrolide antibiotics and metronidazole

Data on the consumption of macrolide antibiotics and metronidazole were obtained from the ‘Stichting Farmaceutische K engetallen The Hague, The Netherlands’.

Interregional differences in antibiotic consumption and resistance rates

To obtain an insight into potential interregional differences in antibiotic consumption and resistance rates, we divided The Netherlands into three regions, namely West, North-East and South regions. Patients whose strains were studied were classified into one of these three regions based on their place of residence.

![Figure 1](image-url)

**Figure 1.** (■), West region; (□), North-East region; (△), South region. Distribution of MIC among the tested 231 H. pylori isolates for metronidazole (a), tetracycline (b), trovafloxacin (c), and clarithromycin (d). The numbers within the bars represent the percentage of strains per region with the particular MIC.
Results

The overall percentages of resistance are summarized in the Table. Figure 1 gives an overview of the distribution of MICs of clarithromycin, metronidazole, trovafloxacin and tetracycline. For all strains the MICs of amoxycillin were <0.064 mg/L (data not shown). The two most active antibiotics against H. pylori in vitro were amoxycillin and clarithromycin (both with MIC< 0.016 mg/L). For clarithromycin the distribution of MICs is in line with the recent finding that resistance is due to a single point mutation. In contrast with clarithromycin the distribution of MICs of metronidazole showed a gradual progression, showing a cluster of strains with MICs in the range 0.125–8 mg/L (cut-off level), with peaks at 1.5 and 3 mg/L, whereas most of the resistant strains had high MICs (>265 mg/L). Trovafloxacin was found to be less active than clarithromycin and amoxycillin (MIC< 0.23 mg/L).

The antibiotic consumption of macrolides and metronidazole in The Netherlands from 1993 to 1997 is shown in Figure 2. It is interesting to note that the consumption of clarithromycin and azithromycin increased markedly, whereas the consumption of roxithromycin remained the same. In 1997 there were no major differences in consumption for clarithromycin and metronidazole between the three regions (data not shown).

Discussion

The introduction of the new macrolide clarithromycin and the azalide azithromycin did not result in the anticipated decreased prescription rate for the older macrolide erythromycin. The increased consumption of macrolides is expected to influence the prevalence of H. pylori resistance to clarithromycin. The number of strains included in this study was too small to draw any solid conclusions on inter-regional differences in macrolide resistance. The observed
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Table. Percentages of H. pylori resistance to metronidazole, clarithromycin, and trovafloxacin in The Netherlands

<table>
<thead>
<tr>
<th>Region of The Netherlands</th>
<th>No. resistant strains (total)</th>
<th>%</th>
<th>95% CI (^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metronidazole R (MIC &gt;8 mg/L)</td>
<td>West</td>
<td>30 (123)</td>
<td>24.3</td>
</tr>
<tr>
<td></td>
<td>North-East</td>
<td>14 (65)</td>
<td>21.5</td>
</tr>
<tr>
<td></td>
<td>South</td>
<td>5 (43)</td>
<td>11.6</td>
</tr>
<tr>
<td></td>
<td>Overall</td>
<td>49 (231)</td>
<td>21.2</td>
</tr>
<tr>
<td>Clarithromycin R (MIC &gt;2 mg/L)</td>
<td>West</td>
<td>2 (123)</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>North-East</td>
<td>1 (65)</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>South</td>
<td>1 (43)</td>
<td>2.3</td>
</tr>
<tr>
<td></td>
<td>Overall</td>
<td>4 (231)(^b)</td>
<td>1.7</td>
</tr>
<tr>
<td>Trovafloxacin R (MIC &gt;1 mg/L)</td>
<td>West</td>
<td>3 (123)</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>North-East</td>
<td>6 (65)</td>
<td>9.2</td>
</tr>
<tr>
<td></td>
<td>South</td>
<td>2 (43)</td>
<td>4.6</td>
</tr>
<tr>
<td></td>
<td>Overall</td>
<td>11 (231)(^c)</td>
<td>4.7</td>
</tr>
</tbody>
</table>

\(^a\) CI, confidence interval.
\(^b\) Two strains also metronidazole R.
\(^c\) Four strains also metronidazole R.

Overall percentage of resistance to clarithromycin is 1.7%; this is only marginally higher than the <1% resistance reported in 1994/1995.\(^7\) Based on these findings, to start empirical treatment with clarithromycin is appropriate; however, development of resistance is the main reason for treatment failure with clarithromycin monotherapy. Since resistance is due to a single point mutation in the 23S rRNA gene of H. pylori,\(^,33\) we strongly advise that clarithromycin should always be combined with a second anti-H. pylori drug.

Two out of a total of four clarithromycin-resistant strains in this study were also resistant to metronidazole. This number is high, since in the total bacterial population tested only one out of five (20%) strains was metronidazole resistant. High prevalence rates for double resistance may be the result of combination therapy as indicated by the findings of Buckley et al.\(^14\) If the infecting strain is already resistant to metronidazole, the patient can be treated effectively with clarithromycin monotherapy.

Metronidazole use in The Netherlands has increased steadily over the last 4 years. We found an overall metronidazole resistance rate of 21.2%. This is 3% higher than in 1994/1995.\(^7\) With a desired cure rate of >90%, the observed metronidazole resistance may preclude the use of this drug for empirical treatment. Our metronidazole resistance rates compare with the 32% metronidazole resistance reported by van der Wouden et al.\(^8\) In the present study five out of 11 strains originating from the same area as those studied by van der Wouden et al. were resistant to metronidazole, and although the numbers are small this could indicate that there are regional variations in the prevalence of metronidazole resistance in The Netherlands. Metronidazole consumption in this area was similar to elsewhere in the country and so the increased incidence of resistance may be due to poor patient compliance, inadequate dosing regimens, or clonal distribution of a metronidazole-resistant strain.

A further interesting finding of our study was that in spite of the fact that trovafloxacin has not yet been introduced on
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to the Dutch market the resistance to trovafloxacin is as high as 4.7%. Most likely the observed resistance results from induction by related quinolones. The role of trovafloxacin in the treatment of H. pylori infection needs further investigation.

In conclusion, although resistance to amoxicillin and tetracycline was not observed in this study, the resistance rates for clarithromycin and metronidazole in H. pylori in The Netherlands have increased over the last 3 years. We recommend susceptibility testing before starting therapy with metronidazole.

Acknowledgements

We would like to thank all participants of the 21 medical microbiology laboratories for collecting the strains. For financial support we thank Abbott, The Netherlands and The Netherlands Digestive Disease Foundation.

References

Received 28 July 1998; returned 12 October 1998; revised 28 October 1998; accepted 18 November 1998