The treatment of helicobacter pylori infection and its sequelae with emphasis on nitroimidazole resistance
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Rapid increase in the prevalence of metronidazole resistant *Helicobacter pylori* in the Netherlands.

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Abstract.

Objective: To study the prevalence of primary metronidazole resistance of *Helicobacter pylori* (*H. pylori*) in 1993 and 1996 in three Dutch hospitals and the 1994/1995 prevalence in one of these hospitals.

Methods: All cultures of antral biopsy specimens yielding *H. pylori* in the study period were considered for evaluation. Strains from patients who previously received anti-*H. pylori* treatment were excluded. Metronidazole resistance was determined by disk diffusion in 1993 and by E-test in 1994 - 1996.

Results: 1037 strains, all from different patients were included. Metronidazole resistance increased from 18/245 (7%) in 1993 to 161/509 (32%) in 1996 (p < 0.0001). This increase was observed both in males and in females. In one of the three hospitals endoscopic diagnosis, age and ethnic background of the 1993/1996 patients were known. Mean age of the patients was 55 ± 14 in 1993 and 54 ± 16 in 1996. In 1996 more non ulcer dyspeptics (NUD) (83% vs 38% in 1993) and non-Western European patients (12% vs 3% in 1993) were evaluated. However, comparable increase in metronidazole resistance was observed in NUD and peptic ulcer patients. Excluding the non-Western European patients prevalence of metronidazole resistance still increased from 5% in 1993 to 28% in 1996.

Conclusion: Primary metronidazole resistance is rapidly increasing in the Netherlands, independently of gender, endoscopic diagnosis and ethnic background.
Introduction.
Since the first description (1) of *Helicobacter pylori* (*H. pylori*) and the acceptance of its role in the pathogenesis of peptic ulcer disease (PUD) (2), different regimens to eradicate this microorganism have been used in clinical practice (3). Metronidazole is a frequently used drug in these treatment regimens. Resistance to metronidazole has been associated with treatment failure (4-6). Recently an increase of metronidazole resistance has been reported from different parts of the world (7-12). This retrospective study describes the prevalence of primary metronidazole resistance in 1993 and 1996 in three regional hospitals in the northern part of the Netherlands and the 1994 / 1995 prevalence in one of these hospitals.

Materials and methods.
All cultures of antral biopsy specimens yielding growth of *H. pylori* in the study period were considered for evaluation. Possible previous anti-*H. pylori* treatment was the only reason for exclusion. All *H. pylori* strains were isolated from different patients.
Endoscopes and biopsy equipment were thoroughly cleaned with a detergent and disinfected with 2% glutaraldehyde in an automatic washing machine between all endoscopic procedures. Biopsy specimens for culture were taken within 3 cm of the pylorus. Culture was performed as described elsewhere (13). Susceptibility to metronidazole was determined by disk diffusion in 1993 and the E-test in 1994 - 1996. For these tests plates were inoculated with a suspension adjusted to a turbidity approximating that of a McFarland no. 3 standard (14). For disk diffusion a 5 µg disk (Mast Laboratories, Liverpool, UK) was used and read after at least 3 days of incubation. Strains with an inhibition zone of 10 millimeter or more were regarded as susceptible (15). The E-test (AB Biodisk, Solna, Sweden) (16) was performed according to the instructions of the manufacturer and read after at least 3 days. The strains were considered to be metronidazole resistant when the minimum inhibitory concentration was above 8 µg per ml.
In one of these hospitals (hospital C) data were available on endoscopic diagnosis, age and ethnic background of the patients from whom the strains were isolated in 1993 and 1996. Therefore, in this hospital it was possible to compare the prevalence of metronidazole resistance of PUD patients with the prevalence in patients suffering from non ulcer dyspepsia (NUD) and to look at resistance rates in patients of different ethnic background. Statistical analysis was performed using Fisher's exact test on binomial
data and Student's t-test on continuous data. Differences were considered significant when \( P < 0.05 \).

**Results.**

In the three hospitals 245 \( H. \text{pylori} \) strains isolated in 1993, and 509 strains isolated in 1996 were evaluated. In hospital C another 137 strains from 1994 and 146 strains from 1995 were studied. The strains were obtained from 89 females (36\%) and 156 males (64\%) in 1993, 58 females (42\%) and 79 males (58\%) in 1994, 73 females (50\%) and 73 males (50\%) in 1995, and 234 females (46\%) and 275 (54\%) males in 1996. The proportion of females in the population examined was higher in 1996 as compared to 1993 \( (p = 0.02) \). However, the prevalence of metronidazole resistance in males did not differ from the prevalence in females. Both in males, females and the total group the prevalence of metronidazole resistance increased significantly from 1993 to 1996 (table 1).

**Table 1.** Prevalence of metronidazole resistance of \( H. \text{pylori} \) from 1993 to 1996 in 3 different hospitals.

<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>All strains.</td>
<td>18/245 (7%)</td>
<td></td>
<td>161/509 (32%)</td>
<td>( p &lt; 0.0001 )</td>
</tr>
<tr>
<td>Hospital A.</td>
<td>5/75 (7%)</td>
<td></td>
<td>46/170 (27%)</td>
<td>( p &lt; 0.0008 )</td>
</tr>
<tr>
<td>Hospital B.</td>
<td>7/82 (9%)</td>
<td></td>
<td>55/137 (40%)</td>
<td>( p &lt; 0.0001 )</td>
</tr>
<tr>
<td>Hospital C.</td>
<td>6/88 (7%)</td>
<td>13/137 (9%)</td>
<td>21/146 (14%)</td>
<td>60/202 (30%)</td>
</tr>
<tr>
<td>Males.</td>
<td>11/156 (7%)</td>
<td>4/79 (5%)</td>
<td>9/73 (12%)</td>
<td>83/275 (30%)</td>
</tr>
<tr>
<td>Females.</td>
<td>7/89 (8%)</td>
<td>9/58 (16%)</td>
<td>12/73 (16%)</td>
<td>78/234 (33%)</td>
</tr>
</tbody>
</table>

Data are given as number of metronidazole resistant \( H. \text{pylori} \) strains / total number of \( H. \text{pylori} \) strains (percentage of metronidazole resistant \( H. \text{pylori} \) strains). \( p \) values are given as 1993 compared to 1996 (Fisher's exact test).
In hospital C 83% and 38% of the strains were isolated from PUD patients in 1993 and 1996 respectively (table 2, p < 0.0001). However, there was no significant difference in the prevalence of resistance between NUD patients or PUD patients in 1993 nor in 1996. In this hospital more strains from non-Western-European patients were included in 1996 then in 1993 (p = 0.04) and the prevalence of metronidazole resistance was higher in this group as compared to the total population. However, when this patient group was excluded the observed increase could still be detected in the traditional local population (p < 0.0001). The mean age of the patients from whom the \textit{H. pylori} strains were isolated in hospital C was the same in 1993 and 1996 (55 ± 14 (mean ± SD) and 54 ± 16 years, respectively.)

Table 2.

Distribution of metronidazole resistance of \textit{H. pylori} in 1993 and 1996 in hospital C.

<table>
<thead>
<tr>
<th></th>
<th>1993</th>
<th>1996</th>
</tr>
</thead>
<tbody>
<tr>
<td>All strains</td>
<td>6/88 (7%)</td>
<td>60/202 (30%)</td>
</tr>
<tr>
<td>Non ulcer</td>
<td>1/15 (7%)</td>
<td>36/124 (29%)</td>
</tr>
<tr>
<td>Peptic ulcer</td>
<td>5/73 (7%)</td>
<td>23/76 (30%)</td>
</tr>
<tr>
<td>Gastric malignancy</td>
<td>-</td>
<td>1/2 (50%)</td>
</tr>
<tr>
<td>Western-European</td>
<td>4/85 (5%)</td>
<td>50/178 (28%)</td>
</tr>
<tr>
<td>Eastern-European, African and Asian</td>
<td>2/3 (67%)</td>
<td>10/24 (42%)</td>
</tr>
</tbody>
</table>

Data are given as: number of metronidazole resistant strains / total number of \textit{H. pylori} strains (percentage of metronidazole resistant \textit{H. pylori} strains).
Discussion.

In agreement with the results of some investigators (7-12) but in contrast with those of others (17,18), our study shows a rapidly increasing prevalence of metronidazole resistance in *H. pylori*. The increase did occur in all three hospitals. It confirms our previous experience of increasing resistance in this part of the Netherlands (19,20).

We explored the possibility that the observed rise in the rate of resistance was the result of a change in the population examined. In fact, in 1996 more *H. pylori* strains were isolated from NUD patients, reflecting the gradually changed habit of the endoscopists to take routine biopsy samples for culture at each gastroscopy. Furthermore, in 1996 larger proportions of the examined populations were females and foreigners. However, in contrast with the results of Ching et al. (21), we found that the prevalences of metronidazole resistance in NUD patients and of PUD patients were the same. Furthermore, the prevalences of metronidazole resistance were comparable among males and females in both 1993 and 1996. If non-Western-European patients were excluded, the rapid increase was still observed. Several authors have suggested that the prevalence of metronidazole resistance is higher in the young and middle aged (17,18,22-24). In our study population however, mean age was the same in 1993 and 1996.

The fact that we used different techniques to measure metronidazole susceptibility is an important issue (24). However, in a prospective study comparing the E-test and disk diffusion in 124 different *H. pylori* strains we found concurrent results in all but six cases (unpublished). These results, as well as other studies (25,26) show a very high inter-test agreement between the two tests when using the above stated criteria for metronidazole resistance.

We cannot exclude the possibility that other methodological factors are involved. However, as during the study period procedures were standardized and the increase was observed in three different hospitals with their own laboratory, we consider this unlikely. Therefore, the observed rapid increase seems real and is relevant for clinical practice (4-6).

What possible explanations for the apparent increase in metronidazole resistance can be postulated? Several authors have suggested that the use of nitroimidazoles for other reasons, for instance gastrointestinal parasitic (21-22,27) or gynaecological infections (6,21,24,27), could be implicated. The latter would be an explanation for the higher prevalence of metronidazole resistance in women that was observed in several studies.
Our study, however, did not show a significant difference between males and females, nor was the increase more apparent in women. Moreover, as is shown in table 3 out of hospital prescription of metronidazole in the Netherlands only increased slightly from 1989 up till 1995. Some authors have suggested that the treatment with nitroimidazole containing regimens of \textit{H. pylori} infection itself could be the cause (23,27,28). However, we consider this unlikely. Firstly, we excluded all strains that were isolated after known anti-\textit{H. pylori} treatment. Admittedly, we cannot exclude completely that some of the patients were previously treated by their general practitioner, without our knowledge. We are, however, confident that this is a rare occurrence as in our region most physicians prescribe their treatment based on endoscopic findings and culture of the biopsy specimens and we excluded all patients from whom \textit{H. pylori} was isolated at a previous occasion. In our region breath testing is not available for general practitioners and so far serology is used only rarely. Moreover, nitroimidazole containing anti-\textit{H. pylori} regimens are highly effective (3,29) and metronidazole resistance could only be induced in the few \textit{H. pylori} strains escaping eradication. Epidemiological data make it unlikely that strains rendered resistant in that way spread in the population (30,31). Therefore, although it is possible that general practitioners have been treating \textit{H. pylori} infections more frequently in recent years, it seems unlikely that this could have caused the observed four times increase in prevalence of resistance.

\textbf{Table 3.} The out of hospital use of metronidazole in the Netherlands in the years 1989 to 1995.

<table>
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<tbody>
<tr>
<td>use of metronidazole</td>
<td>10,5</td>
<td>11,2</td>
<td>11,7</td>
<td>12,4</td>
<td>12,6</td>
<td>12,8</td>
<td>13,3</td>
</tr>
</tbody>
</table>

Data are given as number of prescriptions / 1000 insured.
Data derived from the Health Insurance Council (Ziekenfondsraad), Drug Information Project, Amstelveen, the Netherlands.
The actual explanation for the rapid increase in metronidazole resistance in *H. pylori* that we observed can therefore only be a matter of speculation. Apparently metronidazole resistant *H. pylori* strains somehow have a survival advantage and the increase in metronidazole resistance may be the result of some as yet unknown environmental pressure.

In summary, our study suggests that the prevalence of metronidazole resistance in *H. pylori* is rapidly increasing in the Netherlands. The explanation for this increase, however, is still elusive and can only be speculated about.

References.