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# *Helicobacter pylori* antibiotic resistance in a Dutch region: trends over time

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## ABSTRACT

**Aim:** Most patients treated for *H. pylori* infection receive empirical therapy based on epidemiological data of antibiotic resistance. However, previous European studies indicate that resistance patterns may be changing. Therefore, the aim of this study was to investigate the prevalence of primary clarithromycin and/or metronidazole-resistant *H. pylori* strains over a six-year period (1997-2002) in a regional hospital.

**Methods:** All patients visiting Slingeland Hospital in Doetinchem, the Netherlands between 1997 and 2002 with a positive *H. pylori* culture were included in this study. Susceptibility to metronidazole and clarithromycin was determined by disk diffusion.

**Results:** Of the 1355 patients with an *H. pylori* positive culture, 1127 did not have a history of *H. pylori* eradication, 58 did, and for 170 this information was not available. Mean rates of primary resistance to metronidazole and clarithromycin were 14.4% (162/1125) and 1.0% (11/1123), respectively. Primary metronidazole resistance was stable throughout the study period and primary clarithromycin resistance showed a decreasing trend. Patients of foreign descent and from secondary care had a higher chance of harbouring primary metronidazole-resistant *H. pylori* (adjusted OR (95% CI) 1.75 (1.1 to 2.8), and 1.60 (1.1 to 2.2), respectively). Patients with failed *H. pylori* eradication had a higher chance of harbouring metronidazole-resistant *H. pylori* (43 vs 14%,  $p < 0.0001$ ) and clarithromycin-resistant *H. pylori* (5.3 vs 1.0%,  $p = 0.004$ ) than untreated patients.

**Conclusion:** Primary metronidazole resistance is stable at a low level, while primary clarithromycin resistance is virtually absent in the eastern part of the Netherlands. Therefore, triple therapy with a proton pump inhibitor, clarithromycin and amoxicillin can remain the empirical treatment of choice in the Netherlands.

## KEYWORDS

Antibiotic resistance, clarithromycin, *Helicobacter pylori*, metronidazole

## INTRODUCTION

During the past decade it has been established that not only patients with peptic ulcer disease but also a subgroup of patients with functional dyspepsia benefit from *Helicobacter pylori* eradication.<sup>1,2</sup> Therefore, *H. pylori* test-and-eradication has been incorporated in most guidelines for treatment of primary care patients with dyspeptic symptoms.<sup>3,5</sup> As a result, many patients now receive therapy for *H. pylori* infection.

Several therapy regimens are effective for treatment of this infection, but the current European guidelines as well as the Dutch guidelines recommend triple therapy based on a proton pump inhibitor or ranitidine bismuth citrate, combined with two antibiotics (clarithromycin and amoxicillin or metronidazole) as first-line treatment.<sup>3,5</sup> These regimens reach high cure rates in clinical trials.<sup>6</sup> However, cure rates are substantially lower in case of resistance to the antibiotics used.<sup>7-9</sup>

*H. pylori* can be (or become) resistant to clarithromycin and metronidazole and ideally therapy should be based on culture results. However, with the new noninvasive management strategies, fewer patients have upper gastrointestinal endoscopy.<sup>10</sup> Even if endoscopy is performed, taking biopsies for culture is often omitted because of the high cost. Therefore culture-based antimicrobial susceptibility data are not generally available in routine clinical practice. Thus, the choice of therapy is usually based on epidemiological data of the local prevalence of antibiotic-resistant *H. pylori* strains.

However, the prevalence of antibiotic-resistant *H. pylori* strains may be changing. Van der Wouden *et al.*<sup>11</sup> reported a rapid increase in metronidazole resistance in the northern part of the Netherlands. And several studies in other countries also showed increasing rates of both metronidazole and clarithromycin resistance.<sup>12-15</sup> Therefore, in order to be able to decide which combination of antibiotics should be used for the treatment of *H. pylori* infection recent data on the local antibiotic resistance patterns are needed. Unfortunately there is only one recent Dutch study on this subject. This study by Loffeld *et al.*<sup>16</sup> showed fairly stable rates of antibiotic resistance. But more research is necessary to confirm this for other parts of the Netherlands. Therefore, the aim of this study was to evaluate the prevalence of both primary and secondary clarithromycin and/or metronidazole resistant *H. pylori* strains in the eastern part of the Netherlands and to monitor changes over a six-year period (1997-2002).

## METHODS

### Study population

All patients who underwent diagnostic upper gastrointestinal endoscopy in Slingeland Hospital in Doetinchem, the Netherlands, between 1 January 1997 and 31 December 2002, and who had a culture positive for *H. pylori* were included in this study. Data regarding antibiotic susceptibility, gender, age, country of origin, referring physician (primary or secondary care), and previous (failed) *H. pylori* eradication were entered into a database.

### *H. pylori* culture and antibiotic susceptibility testing

*H. pylori* was cultured from one gastric biopsy specimen (antrum or corpus) on chocolate agar and a Skirrow plate (Regional Laboratory Arnhem, the Netherlands).

Plates were incubated in a micro-aerobic atmosphere at 37°C and examined after three, seven and ten days of incubation. *H. pylori* was identified by colony appearance, Gram staining and positive biochemical tests (catalase, oxidase and urease).

Susceptibility to metronidazole and clarithromycin was determined by disk diffusion: a 16 µg metronidazole disk and a 30 µg clarithromycin disk were placed on separate chocolate agar plates with three to five suspected colonies of *H. pylori*. Plates were incubated in a microaerophilic atmosphere at 37°C for 72 hours. Antibiotic susceptibility was determined by measuring the growth inhibition zone around the disk. Strains were considered resistant to clarithromycin when the growth inhibition zone was <19 mm and to metronidazole when it was <23 mm.<sup>17</sup>

### Data analysis

Primary outcome was the prevalence of resistance to metronidazole or clarithromycin. Baseline characteristics of patients harbouring antibiotic resistant and susceptible strains were compared using the  $\chi^2$  test. Baseline characteristics and study year were related to the presence of antibiotic resistance using unadjusted and adjusted logistic regression analyses. Data were analysed using SAS software (SAS Institute Inc., USA). Statistical significance was defined as a p value <0.05. Missing values were excluded from analyses.

## RESULTS

### Population

During the study period 1355 patients had a culture positive for *H. pylori*. Fifty-eight of these patients had had a previously failed attempt to eradicate *H. pylori*, for 170 there were no data available regarding prior *H. pylori*

**Table 1.** Baseline characteristics in relation to primary metronidazole resistance and primary clarithromycin resistance

	Metronidazole		Clarithromycin	
	Susceptible % (n) n=963	Resistant % (n) n=162	Susceptible % (n) n=1112	Resistant % (n) n=11
Gender				
- Male	54 (520)	49 (79)	53 (594)	36 (4)
- Female	46 (442)	51 (83)	47 (517)	64 (7)
Age in years: mean (SD)	55 (16)	56 (17)	55 (16)	60 (20)
Descent				
- Dutch	86 (822)	80 (130)	85 (939)	91 (10)
- Foreign	14 (135)	20 (32)	15 (167)	9 (1)
Referring physician*				
Primary	64 (602)	52 (82)	62 (676)	50 (5)
Secondary	36 (345)	48 (76)	38 (417)	50 (5)

\*P<0.05 for the difference between metronidazole susceptible and resistant strains.

eradication. The remaining 1127 patients who did not have a history of *H. pylori* eradication were studied for primary metronidazole and clarithromycin resistance. Table 1 shows the baseline characteristics of these patients related to the presence of primary metronidazole and clarithromycin resistance.

### Prevalence of antibiotic resistance

Metronidazole susceptibility was successfully tested in 1125 patients and resistance was found in 162 (14.4%, 95% CI 12.3 to 16.5%) of these patients. Clarithromycin susceptibility was successfully tested in 1123 patients and resistance was found in 11 (1.0%, 95% CI 0.4 to 1.6%) of these patients.

Figure 1 shows that the prevalence of metronidazole resistance was fairly stable during the study period (odds ratio for study year 0.96 (95% CI 0.9 to 1.1)). Furthermore, figure 1 shows that the prevalence of clarithromycin resistance decreased during the study period (odds ratio for

study year 0.58 (95% CI 0.40 to 0.9)), although this result is difficult to interpret due to the low number of patients with clarithromycin-resistant strains.

### Factors associated with primary antibiotic resistance

Table 2 shows that patients of foreign descent and patients referred by a secondary care physician were more likely to harbour metronidazole-resistant strains. It was not feasible to perform these analyses for clarithromycin resistance due to the very low number of clarithromycin-resistant *H. pylori* strains.

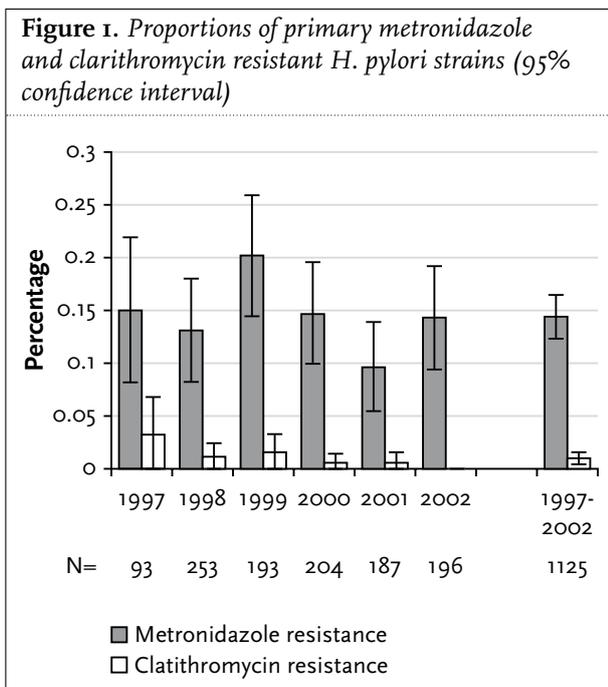
### Secondary antibiotic resistance

Prevalence of both metronidazole and clarithromycin resistance was higher in the 58 patients with a previous (failed) attempt to eradicate *H. pylori* than in previously untreated patients (metronidazole: 43 vs 14%  $p < 0.0001$  and clarithromycin: 5.3 vs 1.0%  $p = 0.004$ , respectively).

## DISCUSSION

Because most patients are treated for *H. pylori* without prior susceptibility testing it is important to gather epidemiological data on the current prevalence of antibiotic resistance to guide empirical therapy, which was the aim of this study. The present study shows that primary metronidazole resistance was stable throughout the study period (1997 to 2002) with a mean prevalence of 14%. Furthermore, the results show that the prevalence of primary clarithromycin resistance was very low (mean prevalence 1%) and showed a decreasing trend.

Our figures are somewhat lower than those recently reported by Loffeld *et al.*<sup>16</sup> for 976 *H. pylori* positive cultures from another Dutch region (26% primary metronidazole resistance and 5% primary clarithromycin resistance). This can partly be explained by the higher proportion of patients of Mediterranean descent (who have a higher prevalence of antibiotic resistance) in the study by Loffeld *et al.* However, our results are comparable with data from other Dutch regions, published as abstracts only.



**Table 2.** Factors associated with primary metronidazole resistance

Factor	Unadjusted analysis odds ratio (95% CI)	Adjusted analysis odds ratio (95% CI)
Age group (old vs young)	1.11 (0.8-1.6)	1.23 (0.8-1.8)
Gender (female vs male)	1.24 (0.9-1.7)	1.22 (0.9-1.7)
Descent (foreign vs Dutch)	1.50 (1.0-2.2)	1.75 (1.1-2.8)*
Referring physician (secondary vs primary care)	1.62 (1.2-2.3)*	1.60 (1.1-2.2)*
Year of the study period	0.96 (0.9-1.1)	0.93 (0.8-1.1)

\* $P < 0.05$ .

Arents *et al.*<sup>18</sup> studied 6648 *H. pylori* positive cultures in the north of the Netherlands and found that primary metronidazole resistance had decreased from 28% in 1996 to 13% in 2001, and that clarithromycin resistance ranged from 1 to 3% without evident trends. Moreover, Janssen *et al.*<sup>19</sup> found a 14% metronidazole resistance and a 3% clarithromycin resistance when studying 961 *H. pylori* positive cultures in the south of the Netherlands.

Our results do not confirm the rapid increase in metronidazole resistance (from 7% in 1993 to 32% in 1996) as reported by Van der Wouden *et al.*<sup>11</sup> for 1037 isolates from the north of the Netherlands. However, more recent data from that area did not confirm this increase; in fact they showed that this increase turned into a decrease after 1996.<sup>18</sup>

Compared with other European countries both primary metronidazole resistance and primary clarithromycin resistance are low in the Netherlands.<sup>12-14,20,21</sup> These differences in primary antibiotic resistance may be related to the use of antibiotics for other indications. In the Netherlands sales of antibiotics are lower than in any other country of the European Union. In fact, in France, Spain, Italy and Greece sales of macrolide antibiotics are about four times higher than in the Netherlands and this may explain the higher prevalence of clarithromycin resistance in these countries.<sup>22-24</sup>

Our results showed that patients originating from foreign countries (nearly all from Turkey) were more likely to harbour strains resistant to metronidazole than patients of Dutch descent. This has been confirmed by other research<sup>16</sup> and it probably reflects the higher frequency of metronidazole use for other indications in these countries. Furthermore, both metronidazole and clarithromycin resistance were about four times higher in patients with a history of failed *H. pylori* eradication than in untreated patients. This reflects the induction of secondary resistance to metronidazole and/or clarithromycin depending on the antibiotics used in the failed *H. pylori* eradication. Therefore, it is important to take a thorough medical history regarding previous failed *H. pylori* treatments in order to determine which antibiotics can be used for a subsequent attempt to eradicate *H. pylori*.

In this study *H. pylori* susceptibility was tested using disk diffusion. Although agar dilution is considered the gold standard, this method is too demanding for everyday practice and it can be replaced by E-test or disk diffusion. Initially E-test was considered superior to disk diffusion, but several studies show that both methods produce comparable results. In comparison with agar dilution both methods are hampered with some discordant results for metronidazole susceptibility, especially in the intermediate susceptibility range.<sup>25-27</sup>

Based on our results it should be advised to use clarithromycin-based triple therapy rather than metronidazole-based triple therapy for empirical first-line treatment of *H. pylori* infections in our region. Triple therapy with clarithromycin and amoxicillin is the treatment of choice because it is not hampered by metronidazole resistance<sup>28</sup> and because this regimen cannot induce double antibiotic resistance (to both clarithromycin and metronidazole).<sup>29,30</sup> Therefore, failure of this therapy still leaves the option of empirical second-line therapy based on metronidazole, preferably quadruple therapy since this therapy may overcome metronidazole resistance.<sup>9,30</sup> Regarding the low prevalence of primary antibiotic resistance, culture and susceptibility testing are not necessary for this combination of first-line and second-line therapy.

In conclusion, in the eastern part of the Netherlands, primary metronidazole resistance was stable at a low level, while primary clarithromycin resistance was virtually absent. Therefore, triple therapy with clarithromycin and amoxicillin can remain the empirical treatment of choice in this area.

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