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ORIGINAL ARTICLE

Antibiotic resistance of *Helicobacter pylori*: a cross-sectional study in consecutive patients, and relation to ethnicity

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Objectives To assess primary antibiotic resistance in a given population and relate the results to ethnicity.

Materials and methods Consecutive cultures were tested for antibiotic susceptibility with the Etest. Three populations were studied separately: ethnic Dutch people, patients of Turkish descent, and patients originating from Africa and the Middle East.

Results Over a period of 5.5 years, 976 (32%) biopsy specimens from 3010 patients were positive for *Helicobacter pylori*. Metronidazole and clarithromycin resistance were present in 25.8% and 4.8% of the strains, respectively. The number of metronidazole-resistant strains showed a gradual decrease, while clarithromycin resistance showed a slight increase during the study period. Antimicrobial resistance in patients of Turkish descent and in those originating from Africa or the Middle East was significantly higher than in ethnic Dutch people, 35% and 9.1% versus 21% and 2.9%, respectively ($P = 0.003$ and $P = 0.002$).

Conclusion It is important to take ethnicity into account when studying antibiotic resistance. The numbers of metronidazole- and clarithromycin-resistant strains can vary considerably between people of different ethnic origin living in the same region.

Keywords *H. pylori*, antibiotic resistance, metronidazole, clarithromycin

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INTRODUCTION

The description of *Helicobacter pylori* and its recognition in human pathology has been the most important breakthrough in gastroenterology in recent years [1]. The infection is treated with a combination of acid-suppressive drugs and antibiotics. Triple or quadruple therapy with a combination of proton pump inhibition and amoxicillin, tetracycline, nitroimadozole derivatives or clarithromycin is usually applied [2].

Successful eradication of *H. pylori* significantly decreases if resistance to these antibiotics exists. Resistance against metronidazole and clarithromycin is well known. Analysis of pooled data has shown that the cure rate of infection is between 0% and 50% when the strain is resistant to macrolides. The substantial difference between in vitro and in vivo resistance is demonstrated by the observation that such a dramatic impact is not found in cases of metronidazole resistance. The eradication rate decreases by only 20% [3]. Prior treatment with antibiotics for reasons other than *H. pylori* eradication and poor patient compliance is a risk factor for inducing antibiotic resistance. People also may have been infected with a resistant strain in a population with a high prevalence of resistant strains. The occurrence of antibiotic resistance is reported as varying between people of different ethnicity [4,5].

However, not many data exist on the yearly incidence of resistance. In addition, results of antibiotic sensitivity testing are seldom viewed in

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relation to people of different ethnic origin living in the same region. A cross-sectional study was done in order to assess the primary resistance of metronidazole and clarithromycin in a given population and relate the results to ethnicity.

MATERIALS AND METHODS

All patients undergoing upper gastrointestinal endoscopy at the De Heel Zaans Medisch Centrum, a large community hospital for the Zaanstreek region, in whom histologic and microbiological examination of the antrum was deemed appropriate, were studied. Biopsy specimens are almost always routinely taken for histologic examination as well as culture. Included in the present study were all patients, irrespective of clinical or endoscopic diagnosis, from whom biopsy specimens were taken for culture and for testing of antibiotic susceptibility.

Culture was introduced as a routine diagnostic procedure in May 1995. Testing for antibiotic susceptibility was introduced by the end of 1995. All consecutive cultures in a 5.5-year period (May 1995 to December 2000) were included in the present study.

A large group of people (almost 10% of the total number of inhabitants) of Turkish descent (born in Turkey as well as The Netherlands) lives in the Zaanstreek region. In addition, there is a small group of people originating from Africa and the Middle East (mostly refugees). These groups of patients were studied separately.

During upper gastrointestinal endoscopy using Olympus EVIS 100 videoendoscopes, antral biopsy specimens were taken for standard hematoxylin and eosin stain, Gram stain, and culture.

The biopsy specimens were inoculated on Columbia agar enriched with 7% hemolysed horse blood and *Helicobacter*-specific supplement (Oxoid, Basingstoke, UK) containing vancomycin, trimethoprim lactate, cefsoludin, and amphotericin B. The plates were incubated at 37 °C in a humid micro-aerophilic environment. Growth was determined at three to four and seven to eight days. Suspected colonies were Gram stained and evaluated by testing catalase, oxidase and urea production [6].

The antibiotic susceptibility of *H. pylori* was determined by Etest on IsoSensitest (Oxoid) agar enriched with 10% horse blood [7]. Etest strains were inoculated in McFarland 3 turbidity. Incubation was done under micro-aerophilic conditions at 35 °C for three to four days to conform with Etest guidelines. For interpretation of susceptibility we used NCCLS breakpoints for metronidazole at MIC 8.0 mg/L and clarithromycin at MIC 2.0 mg/L.

Statistical analysis was done with chi-square test for contingency tables, and *t*-test if indicated. A *P*-value less than 0.05 was regarded as statistically significant.

The medical ethics committee of De Heel Zaans Medisch Centrum approved the study.

RESULTS

Over a period of 5.5 years, biopsy specimens from 3010 patients were sent to the Department of Microbiology for culture. Of these specimens, 976 (32%) were positive for *H. pylori*. Positive cultures from 21 of these patients were excluded from analysis, because these cultures were done as a result of follow-up after anti-*H. pylori* therapy, which proved to be unsuccessful.

Table 1 shows the endoscopic diagnosis in the culture-positive patients.

Because the Etest was introduced towards the end of 1995, very few strains were tested, and the results from 1995 were excluded from further analysis. In the following years, 14–20% of the cultured strains did not grow on the Etest plates used for determination of antibiotic susceptibility.

Of the 727 strains available for testing of metronidazole susceptibility, 188 (25.8%) were resistant. Of the 724 strains available for testing of clarithromycin susceptibility, 35 (4.8%) were resistant. In

Table 1 Endoscopic diagnosis in patients with positive culture for *H. pylori*. More than one diagnosis is possible in a patient

Diagnosis	Number
No macroscopic abnormalities	242
Endoscopic signs of gastritis	278
Bulbitis	58
Duodenal ulcer	62
Gastric ulcer	33
Ulcer scar	25
Hiatus hernia	110
Reflux esophagitis	80
Duodenitis	4
Gastric cancer	3
Gastric lymphoma	1
Barrett's esophagus	12
Billroth resection	9

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Table 2 The individual combination of antibiotic susceptibility of different *H. pylori* strains tested

Metronidazole	Clarithromycin	Number
?	?	211
R	S	168
R	R	15
S	S	504
S	R	20
?	S	17
?	R	0
R	?	5
S	?	15

?, not known; R, resistant; S, sensitive.

Table 2, data on the susceptibility combinations of individual strains are shown.

The number of metronidazole-resistant strains gradually decreased over the time period studied (Figure 1). Clarithromycin resistance, on the other hand, showed a slight increase during the study period.

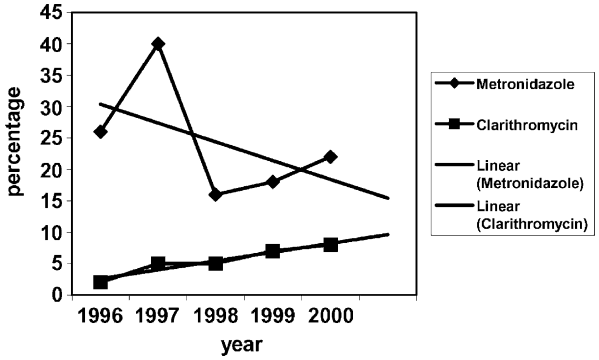

Figure 1 Yearly percentages of metronidazole- and clarithromycin-resistant strains over a period of 5 years. The bold lines represent the trend curve.

In the studied population, 269 people were of Turkish descent (group I) (144 men, 125 women, mean age 38 years, range 15–71 years). Fifty-two patients originated from the Middle East or Africa (group II) (27 men, 25 women, mean age 40 years, range 16–74 years), and the remaining 634 patients (group III) (279 men, 262 women, mean age 54 years, range 18–89 years) were ethnically Dutch. There was no statistically significant difference in gender between the groups. However, the native Dutch were significantly older ($P < 0.001$).

Table 3 shows the antibiotic susceptibilities of *H. pylori* in the three different populations. Resistance to metronidazole and clarithromycin in patients from groups I and II was 35% and 9.1%, respectively, while it was 21% and 2.9% in cultures from patients of group III. Resistance to metronidazole and clarithromycin was significantly more often present in patients from group I and II; $P = 0.003$ and $P = 0.002$, respectively. If all cases without of susceptibility testing were considered either susceptible or resistant, these results did not change. There was no difference in antibiotic resistance between men and women for either of the populations.

Figures 2 and 3 show the trend of resistant strains in the three groups of patients in consecutive years.

DISCUSSION

There are differences in outcome of antimicrobial sensitivity testing, depending on the test method used, Etest or agar dilution [8]. The present paper represents a single-center study of consecutive cultures in previously untreated patients using

Table 3 Antibiotic susceptibility in the three studied populations

Antibiotic and number of isolates studied	Number of *H. pylori* isolates susceptible (%)					
	1996	1997	1998	1990	2000	Total
Metronidazole						
Group I (189)	38 (68%)	28 (61%)	21 (78%)	26 (90%)	19 (63%)	122 (65%)
Group II (43)	5 (39%)	7 (78%)	6 (66%)	4 (100%)	5 (72%)	28 (65%)
Group III (495)	107 (80%)	62 (58%)	75 (87%)	65 (77%)	67 (84%)	389 (79%)
$P = 0.003$						
Clarithromycin						
Group I (189)	53 (96%)	42 (88%)	23 (88%)	28 (96%)	25 (83%)	172 (91.1%)
Group II (43)	12 (100%)	10 (91%)	7 (88%)	3 (75%)	7 (100%)	39 (90.7%)
Group III (492)	125 (98%)	110 (99%)	83 (98%)	78 (93%)	77 (95%)	478 (97.1%)
$P = 0.002$						

Group I, patients of Turkish descent; group II, patients originating from Africa and the Middle East; group III, ethnic Dutch patients.

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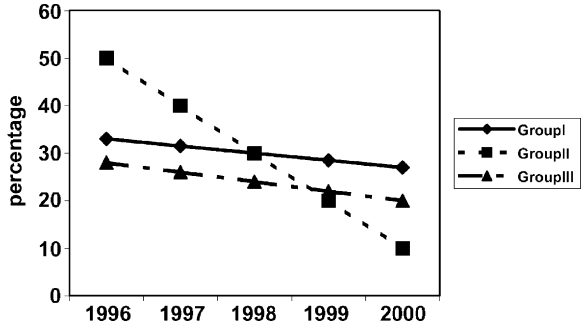

Figure 2 Percentages of metronidazole-resistant strains in the three groups of patients in consecutive years. The bold lines represent the trend curves of the three groups.

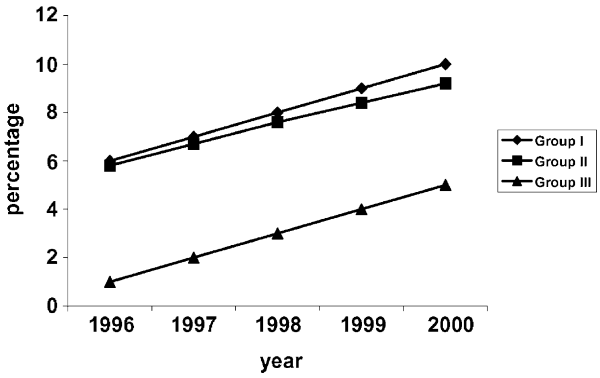

Figure 3 Percentages of clarithromycin-resistant strains in the three groups of patients in consecutive years. The bold lines represent the trend curves of the three groups.

the Etest. The number of isolates resistant to metronidazole or clarithromycin is in accordance with studies reported in the literature [9,10].

Patients originating from countries other than The Netherlands, mostly from Turkey but also from Africa and the Middle East, harbored metronidazole-resistant strains significantly more often. These patients were younger than patients harboring susceptible strains [11]. In developing countries, the prevalence of metronidazole resistance is high, probably as a consequence of the frequent use of nitroimidazole derivatives for infection with protozoa [12]. The level of metronidazole resistance varies markedly between countries, and was found to be higher in subjects of African descent [9]. One large US study did not find regional differences in antimicrobial resistance; however, the results were not corrected for ethnicity [2]. Metronidazole resistance is invariably reported to be higher in women [9,10,14], possibly due to the use of these drugs for gynecologic infections. In contrast, the present study, in keeping with other studies [14], did not show any difference between men and women.

The number of metronidazole-resistant strains increased sharply from 5% to 28% over a period of three years in another Dutch study [14]. The reported rise in antibiotic resistance can be explained by three phenomena: the use of different test methodologies, the inclusion of cultures from patients previously treated unsuccessfully with anti-*H. pylori* treatment, and a real rise in primary resistance. The present study, however, shows a gradual overall decrease in the yearly occurrence of metronidazole resistance. However, the numbers of cultures available from patients of group II is small, so the results in this group should be interpreted cautiously. Other studies did not correct for ethnicity [14]. The reason for this gradual decrease in metronidazole resistance is not clear. Resistance to antibiotics is especially high in populations in which there is a high level of use of certain antibiotics. Antimicrobial resistance can result from the acquisition of a resistant strain, as can be expected in populations where the risk of person-to-person transmission is high, or from selection of a resistant strain in a patient due to the use of antibiotics. It can be assumed that few patients receive pretreatment with metronidazole for reasons other than *H. pylori* eradication. However, the study period is too short to permit firm conclusions to be drawn, and this observation needs to be confirmed.

By the late 1990s, significantly higher levels of resistance against clarithromycin had been described from many Western countries: 8–15%, with higher and increasing levels reported in some countries [15–17]. The prevalence of clarithromycin resistance in the USA increased from 4% in 1993–94 to 12% in 1995–96, due in part to an increase in the number of patients who failed therapy [18]. We report a slight increase in clarithromycin resistance. Clarithromycin resistance was higher in ethnic Turkish patients and in those originating from Africa and the Middle East. Since clarithromycin is an expensive drug, we can tentatively assume that patients originating from other countries would have received other less expensive macrolides and that cross-resistance with clarithromycin is present. In summary, it is important to take ethnicity into account when studying antibiotic resistance. The number of primary metronidazole-resistant strains shows a

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gradual decrease in the Zaanstreek region, while the number of clarithromycin-resistant strains has increased slightly. These data are important, because resistance is a major determinant in assessing the effect of anti-*H. pylori* treatment.

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