Since the first description (1) of Helicobacter 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 has frequently been used as a component in these treatment regimens. H. pylori resistance to metronidazole has been associated with treatment failure (4-7). Recently, an increase of metronidazole resistance has been reported from different parts of the world (8-13). This retrospective study describes the prevalence of primary metronidazole resistance occurring in H. pylori strains in 1993 and 1996 in three regional hospitals in the northern part of the Netherlands and in 1994-95 in one of these hospitals.

Sampling
All cultures of antral biopsy specimens yielding growth of H. pylori in the study period were considered for evaluation. Previous anti-H. pylori treatment was the only reason for exclusion. All 1,037 H. pylori strains evaluated were isolated from different patients. Biopsy specimens for culture were taken within 3 cm of the pylorus. Endoscopes and biopsy equipment were thoroughly cleaned with a detergent and disinfected with 2% glutaraldehyde in an automatic washing machine between procedures. Culture was performed as described elsewhere (14).

Susceptibility Testing
Susceptibility to metronidazole was determined by disk diffusion in 1993 and the Epilipsometer-test (E-test) in 1994-1996. For these tests, plates were injected with a suspension adjusted to a turbidity approximating that of a McFarland No. 3 standard (15). For disk diffusion, a 5-µg disk (Mast Laboratories, Liverpool, United Kingdom) was used and read after at least 3 days of incubation. Strains with an inhibition zone of 10 mm or more were regarded as susceptible (16). The E-test (AB Biodisk, Solna, Sweden)(17) was performed according to the instructions of the manufacturer and read after at least 3 days. The strains were considered metronidazole resistant when the minimum inhibitory concentration was above 8 g/ml (18).

To test the equivalence of the two methods of susceptibility testing, a prospective study compared the E-test and disk diffusion. In 124 different H. pylori strains, results were concurrent in all but six.

Prevalence of Metronidazole-Resistant H. pylori
In one of the 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. In this hospital, it was possible to compare the prevalence
of metronidazole resistance in PUD patients with that in nonulcer dyspepsia (NUD) patients and to look at resistance rates in patients of different ethnic backgrounds. Statistical analysis was performed by Fisher's exact test on binomial data and Student's t test on continuous data. Differences were considered significant when \( p < 0.05 \).

The number of \( H. \) pylori strains isolated in the three hospitals was 245 in 1993 and 509 in 1996. In Hospital C, an additional 137 strains from 1994 and 146 strains from 1995 were studied. Looking at the sex of the study population, we found a male-to-female ratio in 1993 of 1.75:1, in 1994 of 1.36:1, in 1995 of 1:1, and in 1996 of 1.18:1 (the total male-to-female ratio was 1.28:1). The proportion of women in the population examined was higher in 1996 (46%) than in 1993 (36%) \( (p = 0.02) \). The prevalence of metronidazole resistance did not differ, however, between men and women. The prevalence of metronidazole resistance increased significantly from 1993 to 1996 in the total sample group (Figure 1) and also among men (from 7% to 30%, \( p < 0.0001 \)) and women (from 8% to 33%, \( p < 0.0001 \)).

In Hospital C, the number of strains isolated from PUD patients decreased from 83% to 38%. No significant difference, however, was noted in the prevalence of resistance between NUD patients and PUD patients in 1993 or in 1996 (Figure 2, \( p < 0.0001 \)). In Hospital C more strains from non-Western European patients were included in 1996 than in 1993 \( (p = 0.04) \), and the prevalence of metronidazole resistance was higher in this group than in the total population. Exclusion of this patient group still resulted in an increase in the prevalence among the Western Europeans \( (p < 0.0001) \). The mean age of the patients from whom \( H. \) pylori strains were isolated in Hospital C was the same in 1993 and 1996 \( (55 \pm 14 \text{ [mean \pm standard deviation]} \text{ and } 54 \pm 16 \text{ years, respectively}) \).

Our study shows a rapidly increasing prevalence of metronidazole resistance in \( H. \) pylori in the Netherlands. This increase was observed in all three hospitals included in the study. Our results are consistent with the findings of some investigators \( (8-13) \) but not with those of others \( (19,20) \). It confirms our own previous experience of increasing resistance in this part of the Netherlands \( (21,22) \).

We explored the possibility that the observed rise in metronidazole resistance was due to some known confounding factor such as age, sex, endoscopic diagnosis, or ethnicity. More \( H. \) pylori strains were isolated from NUD patients in 1996 than in 1993. In 1996, the proportions of women and foreigners in the examined populations were also higher. In contrast with the results of Ching et al. \( (23) \), however, we found that the prevalence of metronidazole resistance in NUD patients and in PUD patients was the same. Furthermore, the prevalence of metronidazole resistance was comparable among men and women in both 1993 and 1996. Exclusion of the non-Western European patients from the analysis still showed a rapid increase in metronidazole resistance. Several authors have suggested that the prevalence of metronidazole resistance is higher in the young and middle-aged \( (19,20,24-26) \). In our study population, however, the mean age was the same in 1993 and 1996. The use of different techniques to measure metronidazole susceptibility could confound the validity.
of our results (26). However, our prospective study comparing the E-test and disk diffusion, as well as other studies (27,28), show a very high intertest agreement when using the above-stated criteria for metronidazole resistance. We cannot exclude the possibility that other methodologic factors are involved. However, because procedures were standardized and the increase was observed in three different hospitals, each with its own laboratory, we consider this unlikely. Therefore, the observed rapid increase seems real and is relevant for clinical practice (4-7).

Possible Causes of Resistance
Several authors have suggested that the use of imidazoles for other indications, such as gynecologic infections, could account for the resistance increase (6,22-24,28,29). This would also explain the higher prevalence of metronidazole resistance in women that has been observed in several studies (6,19,21,25). Our study, however, did not show a significant difference between men and women or a more apparent increase in women. Moreover, out-of-hospital prescription of metronidazole in the Netherlands increased only slightly from 1989 until 1995 (Figure 3). Some authors have suggested that imidazole-containing regimens themselves could be the cause (25,29,30). However, we consider this unlikely. First, we excluded all strains that were isolated after known anti- H. pylori treatment. We cannot completely exclude the possibility that some of the patients had been treated by their general practitioner without our knowledge. We are, however, confident that this is a rare occurrence because in our region most physicians prescribe their treatment on the basis of endoscopic findings and culture of the biopsy specimens, and we purposely excluded all patients from whom H. pylori was previously isolated. In our region, breath testing is not available for general practitioners, and serologic tests are rarely used. Moreover, imidazole-containing anti-H. pylori regimens are highly effective (3,7), and metronidazole resistance could be induced only in the few H. pylori strains escaping eradication. Finally, as infection is rare during adulthood, it is unlikely that strains rendered resistant in that way spread in the population (31,32). Therefore, although general practitioners may have been treating H. pylori infections more frequently in recent years, it seems unlikely that this could have caused the observed fourfold increase in the prevalence of resistance.

The cause of the rapid increase in metronidazole resistance in H. pylori that we observed can 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. Our study suggests that the prevalence of
metronidazole resistance in H. pylori is rapidly increasing in the Netherlands. The cause of this increase, however, is still elusive.

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