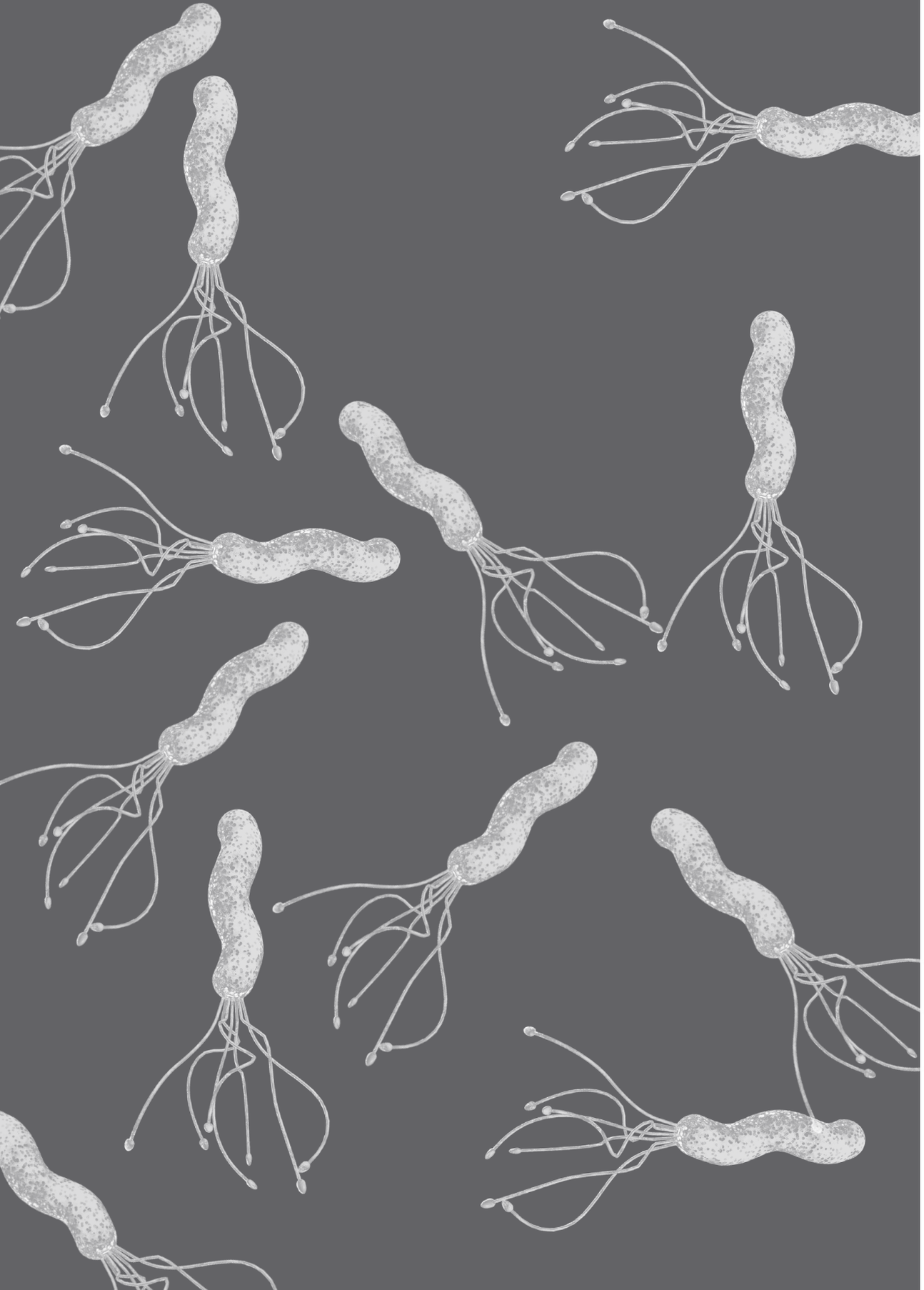
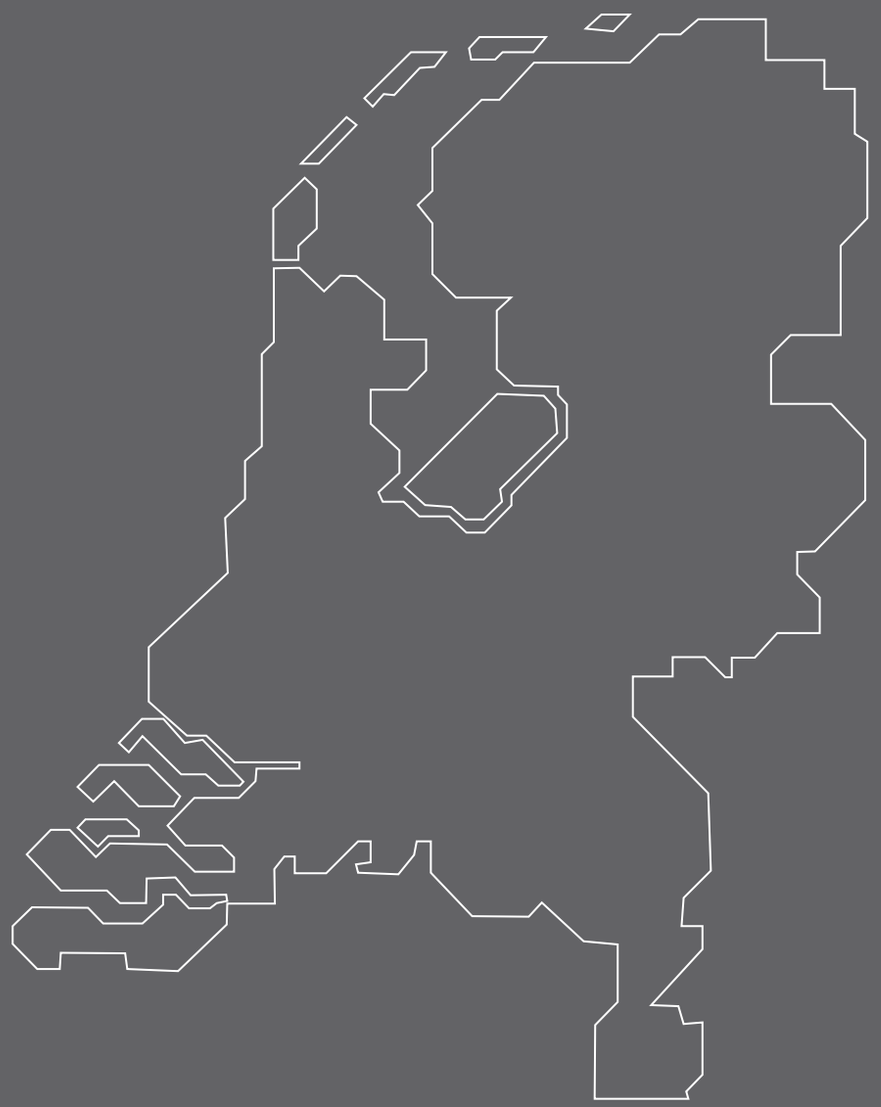


SECTION C

DIAGNOSIS



CHAPTER 7

Antibiotic resistance of *Helicobacter pylori* in the Netherlands

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ABSTRACT

Guidelines on treatment of Helicobacter pylori (*Hp*) infections in adults and children recommend triple therapy (amoxicillin, clarithromycin or metronidazole and a proton pump inhibitor). The increasing antimicrobial resistance of *Hp* is one of the main reasons for eradication failure. Failure of first eradication treatment has shown to diminish future eradication success and therefore it is clinically relevant to get information on local resistance of *Hp*. In the Netherlands data on the resistance of *Hp* to clarithromycin and metronidazole are available up to 2003. No recent data on *Hp* resistance are known from adults or from children. We investigated the resistance prevalence of *Hp* to clarithromycin and metronidazole in 1080 Dutch adults and 72 children from 2000 to 2009. *Hp* resistance to clarithromycin was 8.5-9.4% and 6.5-7.2% in adults and children, respectively, while resistance to metronidazole was detected in 20.7-22.9% and 10.4-11.7%, respectively. Resistance to both clarithromycin and metronidazole was found in 2.8% of the adults and it was absent in children. Resistance rates were low compared to previous data of adults in the Netherlands, while resistance rates in children were low compared to other European countries. We conclude that a test-and-treat regimen is justified for adults as well as for children in the Netherlands.

Keywords: Helicobacter pylori, resistance, clarithromycin, metronidazole, children, adults

INTRODUCTION

Hp is acquired mainly in childhood and is one of the most important pathogens for a wide spectrum of human gastrointestinal diseases, including acute and chronic gastritis, peptic ulcer disease, gastric mucosa-associated lymphoid tissue lymphoma (MALT) and gastric malignancy[1]. After detection, the bacterium should be eradicated, as spontaneous clearance of the infection is rare.

Consensus guidelines on treatment of *Hp* in adults as well as in children recommend 7-10 day triple therapy, i.e. amoxicillin, clarithromycin or metronidazole and a proton pump inhibitor (PPI) in areas with clarithromycin resistance prevalences less than 15-20% and other regimens if the resistance rate is higher [2,3]. With this regimen eradication rates vary from 60-90%. However, the success rate of standard triple therapy for *Hp* eradication is decreasing worldwide and is a point of concern. Reasons for therapeutic failure include lack of compliance to therapy and an increasing antimicrobial resistance of *Hp* to clarithromycin and/or metronidazole [4-12].

Resistance of *Hp* to clarithromycin mainly results from point mutations occurring in the 23S rRNA gene, and resistance to metronidazole is associated with alterations of the nitroreductase-encoding genes rdxA and frxA as well as an increase of the TolC effluxpump [13,14]. *Hp* resistance to clarithromycin and metronidazole is thought to be caused by the extending use of clarithromycin for respiratory infections (especially in children) and the use of metronidazole for parasitic infections. Prescription of alternative regimens containing tetracyclines or bismuth is not allowed in children in many countries. Currently sequential therapy is a topic of research in adults as well as in children to improve the eradication rate [15-18].

The gold standard for assessment of *Hp* infection is upper endoscopy plus mucosal biopsies of the antrum and corpus of the stomach, hereby allowing getting material for the urease test, histology and culture to determine the in vitro susceptibility of the bacteria before any treatment. Determining the in vitro susceptibility before starting treatment will increase the eradication rate after first treatment and seems to be cost effective for clarithromycin-resistant *Hp* [19]. The additional advantage of endoscopy is that it can detect complications of *Hp* infection such as ulcer and carcinoma and that it is able to rule out other upper gastrointestinal disorders such as celiac disease, esophagitis and Crohn's disease. However, endoscopy is an invasive and expensive procedure and requires the use of sedation or anesthesia in children.

Dutch guidelines for adults recommend test-and-treat, which is safe and as effective as prompt endoscopy in absence of alarm symptoms in persons less than 45 years

of age [20]. In the Netherlands there is also a tendency to test-and-treat *Hp* infection in children, even if the current recommendation is to perform a culture of stomach biopsies taken during endoscopy. According to this approach, non-invasive tests include antibody-based stool tests (with a sensitivity of 80-98%), the urea breath test and serology. However, serology does not distinguish between an active and a past infection [21].
In the Netherlands, data on resistance of *Hp* isolated from adults are only known between 1993 and 2003. Resistance to clarithromycin varied from 1 to 5%, while resistance to metronidazole was 7-31% [9,22-28]. Since 2006 no further data have been published and resistance of *Hp* in children has never been investigated. The aim of this study was to analyze the prevalence of *Hp* resistance in adults and children in comparison to reported data on European individuals, in order to estimate whether the test-and-treat approach is justified in the Netherlands.

MATERIAL AND METHODS

Design of the study

We conducted a single center, retrospective database study at Leiden University Medical Center (LUMC), the Netherlands, from January 2000 to December 2009 to analyze the resistance to clarithromycin and metronidazole of *Hp* positive cultures of biopsies from the gastric antrum and/or corpus of adults and children.

Patients

The endoscopy unit of the LUMC is a reference center for family doctors as well as medical specialists, with a regional and national function. All consecutive patients that were referred for upper gastrointestinal endoscopy and had positive biopsies for *Hp*, were included. The data of all *Hp*-isolates were divided into two groups: 0-17.99 years of age (children) and ≥18 years of age (adults). If later biopsies and positive cultures from a patient were obtained after an interval of ≥3 months, the results were analyzed separately, and used to estimate the development of resistance under appropriate treatment. In adults, information about the medical history was limited due to the fact that most of them had been sent for endoscopy by the family doctor with an incomplete history of abdominal complaints. For most patients it was unknown whether or not they had undergone non-invasive testing before, and whether or not they had been treated before.
The children attended the outpatient department of our hospital or a regional hospital before endoscopy. None of the children had been treated for *Hp* before the first endoscopy. Children with a migrational background (at least one non-Dutch parent, or adopted from abroad) were analyzed separately.

Culture and susceptibility testing

Cultures for *Hp* were carried out at the laboratory of Medical Microbiology of the LUMC. Biopsies from gastric antrum and corpus were sent to the laboratory as soon as possible in NaCl 0.9% and inoculated on a blood plate (BioMérieux, France) and on a specific plate for *Hp*, Pyloria agar (PYL-plate, Bio-Mérieux, France). Plates were checked after 3-5 and 7 days of incubation under microaerophilic circumstances. *Hp* positivity was determined with a Gram stain and a positive oxidase, katalase and urease test. Minimal inhibitory concentrations (MIC's) were determined by the epsilometer test (E-test) (AB Biodisk, Solna, Sweden). Strains were considered clarithromycin sensitive if MIC ≤0.25 and resistant if MIC > 0.25 mg/L and metronidazole sensitive if MIC ≤ 8mg/L, intermediate if >8 and ≤ 16 mg/L and resistant if > 16 mg/L, according to the Eucast-criteria (www.eucast.org).

Table 1A. Demographic data and results

Parameters	Adults (N=1080)	Children (N=72)
Male (N) Mean age, yr (range)	510 55.8 (18.7-90.3)	42 11.5 (2.9-17.8)
Migrational background (%)	unknown	69
Number of Hp isolates (N)	1137	77
Resistance to Cla % #		
R S NT*	8.5 81.2 10.4	6.5 △ 83.1 10.4
Resistance to Cla, % of tested strains	9.4	7.2
Resistance to MNZ, % #		
R I S NT*	20.7 0.4 69.2 9.7	10.4 △ △ 3.9 74.0 11.7
Resistance to MNZ, % of tested strains	22.9	11.7
Double Resistance** %	2.8	0

R = resistant; S = sensitive; I = intermediate. Cla = Clarithromycin. MNZ = Metronidazole.
NT* = Not tested due to viability problems of the strains
**Double resistance to Clarithromycin and Metronidazole
percentage of isolates
△ non-Dutch N= 3, △ △ non-Dutch N= 6

Table 1B. Changing resistances in second and third occasion biopsies

	Adults N	Children N
Patients with two cultures Cla S→ Cla R MNZ S→ MNZ R No change	41 4 4 33	2 0 0 2
Patients with three cultures Cla S→Cla R MNZ S→MNZ R No change	6 1 2 3	2 0 0 2
Patients with four cultures No change	1 1	0

R = resistant
S = sensitive
Cla = Clarithromycin.
MNZ = Metronidazole

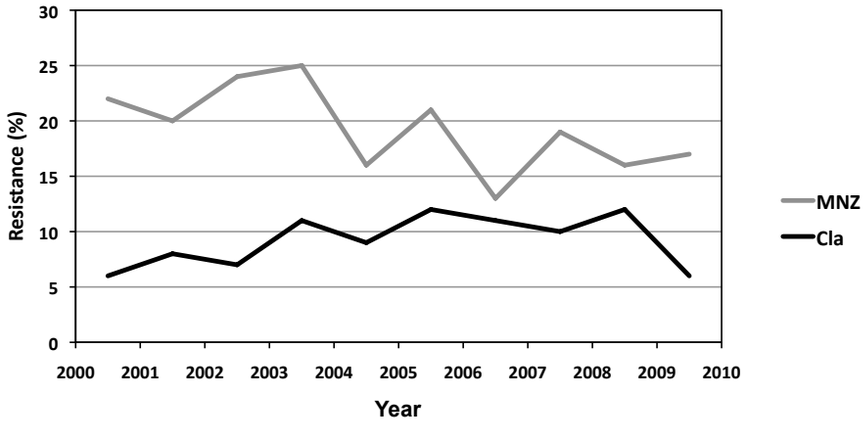

Fig 1. *Hp* resistance to Clarithromycin and Metronidazole in 2000-2009 in the Netherlands

RESULTS

From 2000 to 2009 all *Hp*-positive patients who underwent endoscopy, were included: 72 children and 1080 adults. Of these 1152 patients, 1214 cultures were positive for *Hp*. Susceptibility of clarithromycin and metronidazole could be determined in 1088 (90%) and 1095 (90%) cultures, respectively.

Demographic data and results of resistance to antibiotics are summarized in Table 1A and Table 1B. *Hp*-resistance rate against clarithromycin was 8.5-9.4% in adults and 6.5-7.2% in children. Resistance to metronidazole was observed in 20.7-22.9% of adults and 10.4-11.7% of children. Resistance to both clarithromycin and metronidazole in adults was 2.8%, equally divided among genders, and there was no time trend (2.9% in 2000-2004, 2.7% in 2005-2009). Double resistance to clarithromycin and metronidazole was absent in children. The *Hp* resistance rate against any antibiotic in the whole decade was 29.2% in adults, and 16.9% in children.

Sixty-nine percent of the children with *Hp* positive cultures in the study had a migrational background. Three out of 5 clarithromycin resistant strains of the children and 6 of 8 metronidazole resistant strains were detected in offspring of a non-Dutch mother. All clarithromycin resistant strains in children were detected in the period after 2004, while metronidazole resistant strains were divided equally over the whole period. The development of resistance to clarithromycin and metronidazole over time reveals that the resistance to clarithromycin slightly increased in the Netherlands (with the exception of the year 2009) and that resistance to metronidazole slightly decreased (figure 1).

DISCUSSION

We have shown that the *Hp* resistance to clarithromycin in the Netherlands has increased from less than 5% [22,24,26-28] to 8.5-9.4% in adults. This rate is comparable to the resistance rates reported in the European multicenter study in 1998 [9] and in studies from Finland, UK and Belgium in the same period [29-31]. However, this resistance rate is low compared to increasing resistance rates to clarithromycin in adults in other European countries [32-36], where prevalences have been reported of 17-26% (supporting information Table 2). In case of secondary resistance rate, the resistance rate can be up to 68%, as was shown in a study from France [34]. We speculate that the most likely explanation of the low resistance rates of *Hp* to clarithromycin in the Netherlands is the relative reluctance of prescribing antibiotics by physicians in the Netherlands [37]. The prescription of clarithromycin, the most commonly used macrolide in primary care, has stabilized since 2003 (www.swab/nethmap.nl).
Hp resistance to metronidazole in this study was 20.7-22.9%, while in the period before 2003 rates in Dutch adults varied from 7 to 33%. This, again, is comparable to data from Sweden (16.2%) [38] and much lower than the resistance rates in other European countries (27-61%) [29-36,39] and the resistance rates reported in the European multicentre study in adults (33.1%) [9] (Supporting information Table 2). Metronidazole resistance of *Hp* varies geographically, being higher in developing countries, where this class of antibiotics is frequently used to treat parasitic infections.
Double resistance to clarithromycin and metronidazole was detected in only 2.8% of the strains of adults and remained stable over time. The low resistance rates to clarithromycin and metronidazole as well as the stable and low double resistance rate are remarkable, because since implementation of the stool antigen test in 2000, the test-and-treat regimen has been introduced gradually in the Netherlands without determining the susceptibility of *Hp* before treatment. With such approach, one would have expected higher secondary resistance rates, since the endoscopic samples were probably more often from patients who failed first line therapy. Resistance of *Hp* to amoxicillin in Europe and USA is very low and stable, and clinically negligible. In 1998, Glupczynski reported 0% primary resistance to amoxicillin all over Europe with an exception for Italy (8.2%) and Copenhagen (4%) [9]. We did not determine the susceptibility to amoxicillin in all *Hp* positive strains systematically during the last decade.

This study is the first to report data on the antimicrobial resistance of *Hp* in children living in the Netherlands. The prevalence of *Hp* in young children is low (1.2%-9%) and most infected children are offspring of at least one non-Dutch parent [40,41]. In our study 69% of the *Hp*-positive children had a migrational background. We determined

resistance rates of 6.5-7.2% to clarithromycin and 10.4-11.7% to metronidazole. Seventy-five percent of the metronidazole resistant strains and sixty percent of the clarithromycin resistant strains were detected in offspring of a non-Dutch mother.
The resistance rates of *Hp* strains of children in our study are much lower than the resistance rates of 24% and 25% to clarithromycin and metronidazole, respectively, that were detected in an European study [42], and also lower than rates reported from various European countries [36,43-50](Supporting information Table 3). In the European study, 41% of the children with resistant strains were offspring of non-European mothers, while double resistance to both clarithromycin and metronidazole was 6.9%. Several studies have suggested that resistance to clarithromycin is generally higher in children than in adults, probably due to the previous use of macrolides in respiratory infections [9,30,35]. However, we detected lower clarithromycin resistance rates in children (6.5%) compared to adults (8.5%).

A limitation of the study is the single center design, due to unavailability of data on resistance rates in the past ten years and differences in testing methods and standardization in other centres. Therefore, geographical variation in resistance rates could not be analyzed. However, previous publications on Dutch adults have reported only a slightly different resistance pattern in diverse areas of our country [9,22,24], although the resistance rate may be higher in an area of the country with a higher percentage of immigration. Another limitation is the retrospective nature of our study, conducted on the basis of medical files. Since general practitioners in the Netherlands directly refer adult patients for endoscopy, primary or secondary resistance could not be distinguished. In general, secondary resistance is far higher than primary resistance, and is one of the main reasons for eradication failures [33,35]. Although it is likely that some of the *Hp*-positive patients in our study have used antibiotics before, and in spite of the gradual introduction of the test-and-treat-regimen in adults in 2007 [2], the resistance rates have remained low.

The resistance rate for clarithromycin in Dutch adults below 15-20% supports the recommendation [2] to routinely perform a test-and-treat regimen. However, we believe that also in the coming years surveillance of regional *Hp* resistance is needed, and that this should be well communicated to the clinicians. Previous studies have shown that most physicians are insufficiently acquainted with regional resistance data [51-53]. Up-to-date information on resistance rates is needed to timely modify treatment regimens. Hopefully, future development of non-invasive susceptibility tests for clarithromycin in stool samples will facilitate this.
Our data on resistance rates in children suggest that also in this age group a test-and-treat approach can be used. However, we suggest that the clinician should

inquire if the child has received clarithromycin in the previous 3 months, and if so, exchange this by metronidazole. In case of eradication failure after the first clarithromycin-based triple therapy, a second triple therapy with metronidazole instead of clarithromycin could be prescribed. A second eradication failure should lead to referral of the patient for endoscopy with biopsies for susceptibility testing.

CONCLUSION

The resistance of *Hp* to clarithromycin, although still low compared to resistance in other European countries, is slowly increasing in Dutch adults, while the resistance to metronidazole has been stable. The resistance of *Hp* to clarithromycin and metronidazole in Dutch children is low compared to European data and lower than in adults. Double resistance to both clarithromycin and metronidazole is low in adults and absent in children. Both for adults and children, a test-and-treat approach can be used, but continuing surveillance of antibiotic resistance remains necessary.

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Tables 2 and 3
Supporting information

Table 2 Reported prevalence of *H.pylori* resistance to clarithromycin (CLA) and metronidazole (MNZ) in adults in Europe

Year and reference	Country	Period	No of ptts	CLA res %	MNZ res %	AMX res or CLA + MNZ
2004[1]	Finland	2000-2002	292	2	38	Not done
2011 [2]		2000-2008	505	8	40	AMX 0
2006 [3]	Sweden		333	1.5	16.2	0
2007 [4]	Italy	2004-2006	255	16.9	29.4	
2007 [5]	UK (Gwynned)	2000-2005	664	8.3	28.6	Cla +MNZ 4.4
	UK (Essex)	2000-2005	646	12.7	36.3	Cla + MNZ 8.4
2009 [6]	Poland	1997-1998 2007-2008	66 76	9.1 18.4	36.4 44.7	Amoxy 0
2010 [7]	France	2004-2007	530	26 P* 19 S** 68	61.1	Amoxy 0
2010 [8]	Ireland	2007-2008	222	13.2 P* 9.3 S* 32.4	31.5	Cla+MNZ 8.6
2008 [9]	Bulgaria	1996-1999	120	9.8	27.5	
2010 [10]		2007-2009	428	18	27.3	
2005 [11]	Belgium	2002	436	3	31	Amoxy 0
2011 [12]		1990-2009	7903	P* 5.2 S** 8,5	P 26.1 S 49	Amoxy 0
2001 [13]	European multicenter study	1998	1274	9,9	33.1	0.8

P*: primary resistance S**: secondary resistance

Table 3 Reported antibiotic resistance of *H.pylori* in children in Europe (last decade)

Year of publication and reference	Year (period)	No of patients	Country	Resistance		
				CLA %	MNZ %	AMX %
2001 [14]	1989-1995 1995-2000	551	Belgium	6-16 16.6	18 18	0 0
2001 [15]	1998-2000	98	Poland	23.5	unknown	unknown
2002 [16] 2008 [9] 2010 [10]	2000-2001 2005-2007 2007-2009	115 75 73	Bulgaria	12.4 Naive 18.7 Naive 27.4 Naive	15.8 Naive 16 Naive 16.4 Naive	0
2000 [17] 2005 [18] 2011 [19]	1998-1999 1999-2003 2000-2009	58 109 1115	Portugal	44.8 39.4 34.7	19.0 16.5 13.9	0 0 0
2001 [20] 2009 [21]	1991-1995 2002-2006	246 101	Spain	3.5 54.6	19.9 35.7	0 0
2003 [22] 2010 [23] 2011 [24]	1997-2000 2002-2008 2002-2009	117 153 74	Austria Vienna Vienna Graz	20.3 34 22	16 23 22	0 0
2006 [25]	2000-2003	58	Germany	9	16	unknown
2008 [26]	2002-2006	157	Italy	42	12	unknown
2007 [27]	1994-2005	377	France	22.8	36.7	0
2006 [28]	1999-2002	1233	Europe* (Multicenter)	24	25	0.6

* no Dutch children included

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