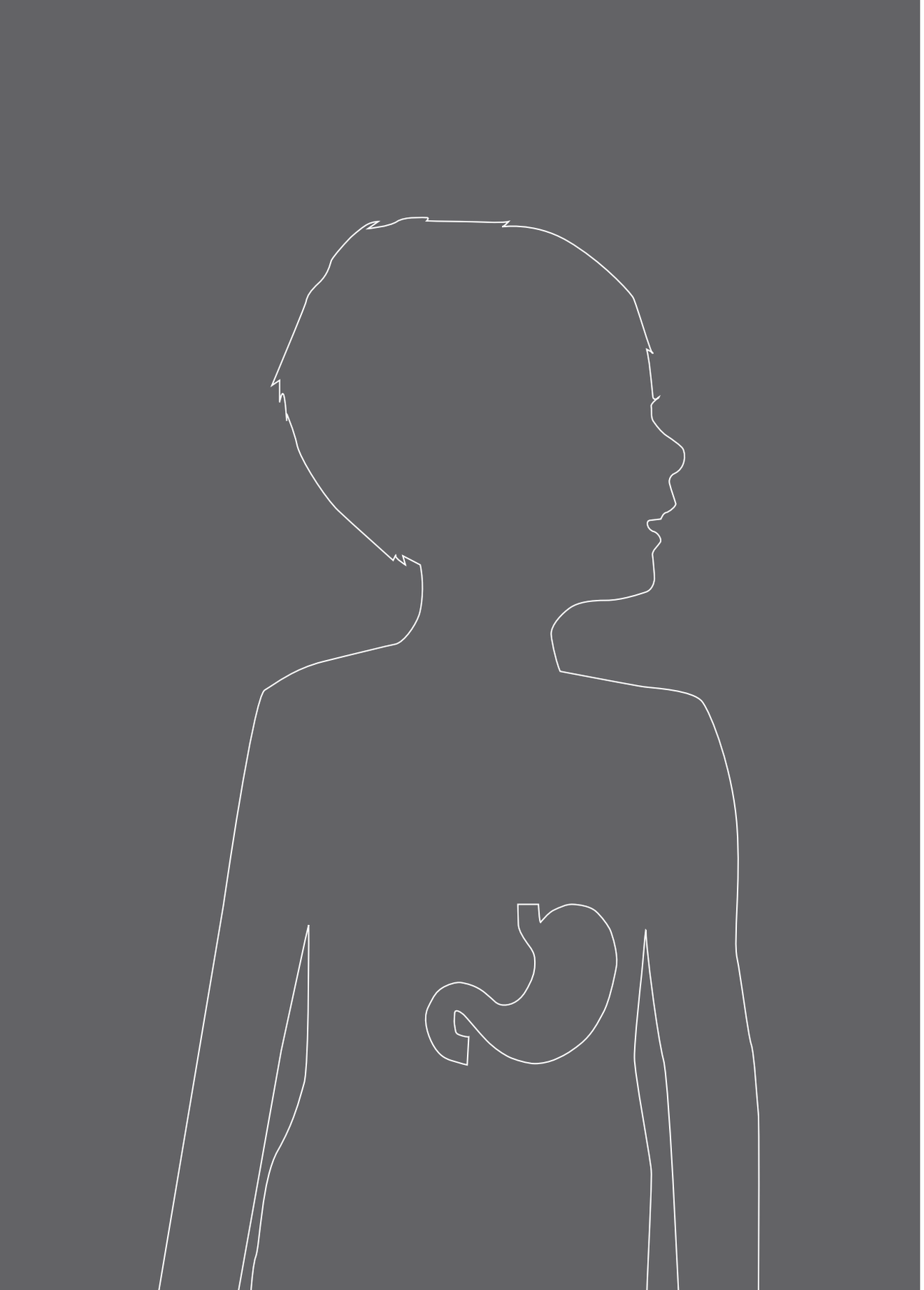

CHAPTER 1
Introduction

1.1 DEFINITION AND HISTORY

Helicobacter pylori (*Hp*) has cohabitated with humans for over 50.000 years, thereby affecting directly more than half of the world's human population. The Gram-negative spiral bacterium with its up to six unipolar sheathed flagella, has the unique ability to colonize the human stomach. Infection with *Hp* has been widespread and therefore geographic variations in the type of *Hp* have successfully been linked to the pathways of early human migrations. Direct evidence of early infections has been found in the form of *Hp*-DNA fragments in a Pre-Columbian mummy dating back to 1350 AD in current Mexico[1].

From 1875 onward, researchers have tried to reveal a positive association between a human infection with microorganisms and the development of peptic ulcers. In line with their hypothesis they already attempted treating ulcers with anti-microbial bismuth-containing compounds. In the 1950s Lykoudis found evidence that peptic ulcer disease and gastritis had in fact an infectious origin and he therefore decided to prescribe antibiotics to thousands of his patients[2].
More than 30 years later two Australian physicians, Barry Marshall (gastroenterologist) and Robin Warren (pathologist), were the first to describe the presence of a Campylobacter-like organism in the stomach of patients suffering from gastritis and peptic ulcers[3,4]. In 1984 Marshall started an attempt to assess Koch's postulates for these diseases in piglets, but the experiment turned out unsuccessful. Eventually, in a bold approach to prove his hypothesis he infected his own stomach with a colony of the bacterium and fell ill himself. He underwent endoscopy and it was revealed that spiral bacteria were present in his antral biopsies and had caused his gastritis. This time, his experiment had succeeded and ever since, *Hp* has been recognized as a human pathogen. Marshall recovered from his gastritis by treatment with amoxicillin and a proton pump inhibitor, and he proved the causal relationship between his disease and *Hp* by fulfilling all four Koch's postulates[5,6].
In recognition of their new discovery, Warren and Marshall were awarded with the Nobel Prize in Physiology or Medicine in 2005, particularly for their role in the observation of "the bacterium *Helicobacter pylori* and its role in gastritis and peptic ulcer disease" as well as "further identification of *Helicobacter pylori*". Initially, they named the bacterium "Campylobacter-like organism (CLO)", because of its similarities with the Campylobacter species. Later the name was changed to Campylobacter pylori-dis, Campylobacter pylori and, finally, it became known as *Helicobacter pylori* by 1989.
Nowadays, *Hp* is recognized as one of the most important pathogens for a wide range of gastrointestinal diseases in the human species. Colonization commonly leads to gastritis and in 10-15% of the cases it progresses to peptic ulcer of the du-

odenum or the stomach, in less than 1% it leads to to mucosa-associated lymphoid tissue (MALT) lymphomas and in 1% to gastric carcinoma[7,8]. However, colonization of the human gastric mucosa does not necessarily result in the development of symptoms and typically more than 70% of infected people remain asymptomatic[7].
The first report on *Hp* in the Netherlands, a letter to the *Lancet* by Langenberg *et al* in 1984, described gastric antral biopsies of 50 outpatients with upper abdominal complaints who were referred for upper gastrointestinal endoscopy[9]. The study unambiguously confirmed a positive association between colonization with *Hp* and gastritis and furthermore noted that *Hp*-associated gastritis may be present in apparently healthy individuals, thereby raising doubts about the clinical significance of *Hp* infections.
In 1986, the first report on the isolation of *Hp* from the stomach of children was published by Hill *et al* [10]. Cadranel and colleagues followed with the first European paper on *Hp* and children. They performed a small prospective study on twenty-five children, eight of whom were found *Hp*-positive. The latter study confirmed the presence of *Hp* in children and established a positive correlation with epigastric pain and chronic gastric inflammation[11].
Currently more than twenty-five *Helicobacter* species have been identified in the gastrointestinal tract of animals and humans, whose infection correlates positively with the occurrence of gastrointestinal and systemic diseases[12].
In figure 1 the rapid development of the scientific field of *Hp*-research, taking off at pioneering work of Marshall and Warren, is illustrated, as well as the relatively small fraction of that work that includes research on children.

1.2 CLINICAL FEATURES OF *HELICOBACTER PYLORI* INFECTION

1.2.1 Transmission and virulence factors

Generally *Hp*-infection is acquired during childhood and if not treated, the infection remains life-long. Spontaneous eradication in childhood appears to occur, but may be attributed to the unreported use of antibiotics to cure respiratory or parasitic diseases.
The principal transmission route of *Hp* has not clearly been defined, nor has its source, but two models prevail: firstly, the vertical route in which parents infect their children, probably by gastro-oral, fecal-oral or oral-oral routes and secondly the horizontal transmission route in which intensive direct contact between infants and children or via for example dentists, endoscopists, or through environmental contamination of food and drinking water[13-21]. For the latter only indirect evidence exists in the presence of *Hp*-DNA in food and water.
Coccoid morphological states of *Hp* have been detected in surface water, but so far

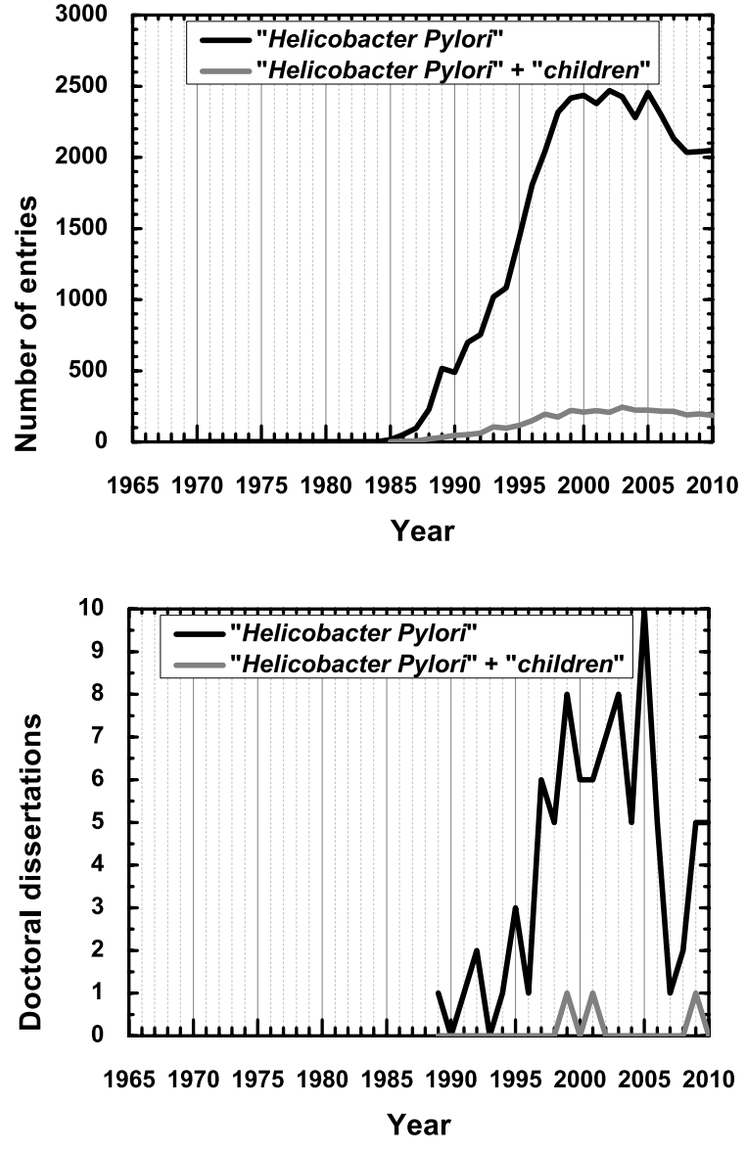

Fig 1: Panel (a) The number of entries in the scientific publications search engine Scopus (www.scopus.com) for the search strings "*Helicobacter pylori*" (black curve) and "*Helicobacter Pylori* children" (grey curve) as a function of the year of publication. Panel (b) The number of doctoral dissertations filed in PubMed (http://www.ncbi.nlm.nih.gov/pmc/) for the search strings "*Helicobacter pylori*" (black data) and "*Helicobacter pylori* AND children" (grey data) as a function of the year of publication.

could not be cultured *in vitro*, raising doubts about their potential for the transmission of the infection. Recognized risk factors for the infection onset are thought to be:
- low socio-economic status and educational level
- living in a family with a high number of siblings (crowding)
- being born to an infected mother
- the use of river or municipal water instead of spring water.

The presence of bacterial virulence factors is one of the possible pathogenic mechanisms of *Hp*-induced gastroduodenal disease; putative virulence factors, especially CagA, VacA, bab A, hom B and OipA, are associated with the gene jph05632, that encodes for the cell envelope protein glycosyltransferase which appears important for the progression to peptic ulcer disease[22-25].

1.2.2 *Helicobacter pylori* and gastrointestinal symptoms in children

Bacterial virulence, host factors and potentially environmental exposures are considered to be key factors in the type of gastroduodenal inflammation and disease outcome that is associated with *Hp*-infection. It remains unclear as to: (l) why *Hp* causes symptomatic disease in only 20-30% of infected people; (2) how many of the organisms are needed to establish a persistent infection; and (3) who is prone for which disease phenotype. In children, antral predominant gastritis usually develops after colonization. Children may have transient impaired gastric acid secretion (hypochlorhydria), which, as is the case for adults, is thought to add to an increased susceptibility for enteric infections[26,27].

Although dyspepsia in adults is generally regarded to be caused by *Hp*, a specific clinical scenario of *Hp* infection in children is not known. The relation between recurrent abdominal pain (RAP) and *Hp* as described by Apley in 1985[28] has been assessed in many studies, but nevertheless the role of *Hp* in causing such symptoms has remained controversial[29-32]. A literature review over the years 1983-1998 found no specific correlation between RAP and *Hp* infection in children[33]. More recently, a systematic review confirmed the absence of such a correlation. Evidence was found for an association between epigastric pain and *Hp* infection in children except for children in primary care. Other gastro-intestinal symptoms as nausea, vomiting, regurgitation, diarrhea, flatulence or dyspepsia were not associated with *Hp* infection, but randomized control studies on this topic are needed[34].

1.2.3 Peptic ulcer

Duodenal and gastric ulcer disease in childhood are much less common in childhood than for adults and children develop gastric ulcers much less often than duodenal ulcers. Remarkably, the prevalence of non-*Hp*-associated ulcer disease seems to increase lately[35,36]. Longstanding *Hp* infections have been associated with gastric

atrophy and intestinal metaplasia with development of gastric adenocarcinoma in adults. Gastric atrophy and metaplasia have also been described in children[37]. Few cases of gastric MALT-lymphoma in children have been reported, treated only with eradication medication for the *Hp* infection even in immune-compromised pediatric patients. Reports of gastric adenocarcinoma in children are, however, rare[38-44].

1.2.4 *Helicobacter pylori* and extra-intestinal symptoms in children

Idiopathic thrombocytopenic purpura (ITP)

Several reports on recovery of thrombocytopenia after *Hp* eradication in adults and children with chronic ITP have been published[45-48].

In 47 Dutch children with chronic ITP the prevalence of *Hp* infection was 6.4% and all *Hp* positive patients achieved partial or complete remission of their ITP, however the follow-up time was rather short (six months) and unlike in adults the disease is characterized by spontaneous remissions in one-third of the children, particularly in the first year after the diagnosis.

Gastroesophageal Reflux Disease (GERD)

In adults, *Hp* infection might have a protective role against GERD. The role of *Hp* in GERD in children remains controversial and currently insufficient data have been published [49-51].

Short stature

There is insufficient evidence that *Hp* infection is causally related to short stature[52]. Many studies on this item have been conducted in countries where confounders with negative effects on growth in children might play a role: low socio-economic status, helminthic infections, iron deficiency anemia and malnutrition. In those studies height and weight were usually not expressed as standard deviation scores, local reference curves were not available (usually WHO reference curves were used), and parental heights were unknown.

With respect to the possible pathophysiology, laboratory examinations have so far focused on Ghrelin, a strong growth hormone secretagogue, that is predominantly produced by entero-endocrine cells in the stomach. However, so far, most studies have been reported on total Ghrelin concentration, rather than the acylated form or the ratio between acylated and total Ghrelin. It is now generally believed that acylated Ghrelin is the active form of the hormone[27,53-56].

Iron deficiency anemia

There are some plausible explanations for links between iron deficiency anemia

and *Hp* infection. One can imagine that the *Hp* infection can produce gastritis and/or erosions and ulcers with chronic blood loss. Moreover, *Hp* gastritis may have reducing effects on gastric acid secretion and iron absorption. Another mechanism could be utilization of iron by *Hp* for its growth.
Confounders in studies on *Hp* and iron deficiency anemia are low socio-economic state, poor hygienic standards and malnutrition. Only one study described an increasing blood hemoglobin value in *Hp*-positive children after eradication therapy. More well-designed studies in developed as well as developing countries would be useful [57-59].
Recent guidelines recommend that in children with refractory iron-deficiency anemia, testing for *Hp* infection may be regarded after other causes have been ruled out [52].

1.3 DIAGNOSIS

So far, guidelines for the management of *Hp* infection in children recommend endoscopy to exclude other pathological causes for the child's symptoms[52]. The reasoning behind this recommendation is that no specific complex of clinical symptoms and signs has been established for children. Reflux-esophagitis, eosinophilic esophagitis, celiac disease or Crohn's disease could cause symptoms similar to those caused by gastritis or ulcer and can only be ruled out by endoscopy. The current gold standard for the detection of an *Hp* infection is endoscopy with gastric biopsies for histology, a rapid urease test and a culture with susceptibility testing. Cultures of gastric tissues have a specificity of 100%, but a relatively low sensitivity of 38-80%. PCR testing in gastric tissue can detect genes associated with virulence factors and antibiotic resistance. As endoscopy is both invasive and expensive and particularly in children usually requires deep sedation or anesthesia, non-invasive tests have been developed. The ^{13}C-urea breath test (UBT) and the monoclonal stool test have been validated well in children older than 6 years for the detection as well as the eradication control of *Hp*. Unfortunately these non-invasive tests have not sufficiently been validated in younger children, below the age of 6 years[60-62].

1.4 EPIDEMIOLOGY AND PREVALENCE

The prevalence of *Hp* infection varies greatly between developing and developed countries, respectively 90% versus 40% at the age of 40 years, and is declining worldwide over the last decades[63]. The prevalence of *Hp* in children in the Netherlands is 1.2% at the age of 2-4 years and 9% at the age of 7-9 years and most of the infected children are offspring of originally non-Dutch parents[64,65]. In

developed countries where the prevalence of *Hp* infection in children is low, the prevalence is increasing with age because of a cohort effect [63,65,66]. In institutions of intellectually disabled persons a relatively high prevalence has been found, including in the Netherlands[15,66-68].
Therefore, despite the decreasing prevalence of *Hp* infection in developed countries such as the Netherlands, *Hp* infections deserve attention and focus should be on those particular population groups, where the prevalence of *Hp* is relatively high.

1.5 RE-INFECTION AND SPONTANEOUS CLEARANCE

Studies in adults in industrialized countries have shown that the rate of *Helicobacter* re-infection following an initial satisfactory eradication response is relatively low (3.4%), while the highest rates of recurrence (8.7%) have been reported in geographical regions with a lower development index and a very high prevalence[69].
Most cases of recurrence take place within twelve months after treatment. Studies using molecular fingerprinting techniques (e.g. polymerase chain reaction) favor the hypothesis that reappearance of a *Hp* strain identical to the pretreatment strain is defined as recrudescence of the *Hp* present in that patient prior to treatment and usually not as a true re-infection and describe lower recurrence rates [69-71]. Niv *et al* described a literature search of 17 studies on *Helicobacter pylori* recurrence in adults in developed countries and in developing countries and found annual recurrence rates of 2.67% and 13.0%, respectively [72]. Studies in children indicate a re-infection rate of 2.3-12.8% depending on the *Hp* prevalence in the country and the follow-up time (see table 1).

Table 1 Re-infection rates in children

Year	Country	*Hp* reinfection (%)	Follow-up Months
1992 [73]	Italy (Turin)	9 0 11	6 12 18
1998 [74]	Japan	2.4	22
1999 [75]	Ireland/Dublin	11.5 (4.3 if >5 years of age)	24+/- 14
2002 [76]	Germany	2.3	15.5
2004 [77]	N-Ireland/ Belfast	2.4	
2004 [78]	Estonia*	6.7	84
2005 [79]	Italy (South)	12.8	18-43
2006 [80]	France	5.4	148 patient years

1.6 GUIDELINES FOR DIAGNOSIS AND TREATMENT

1.6.1 Adults

Updated guidelines on behalf of the European *Helicobacter* Study Group (Maastricht11 Consensus Report) for management of *Helicobacter pylori* infection in adults have been published in 2007[81]. One small paragraph in that report provides directives on *Hp* testing in children with recurrent abdominal pain and iron deficiency anemia. In the fourth edition of the Maastricht consensus report (2012) no recommendations on *Hp* in children have been included [82]. The Dutch standard for general practitioners (NHG-standard) lacks recommendations for treating children with *Hp* infection[81,83].

1.6.2 Children

Dutch evidence-based guidelines for *Hp* infection in children do not currently exist. Up to now, directives from Ireland and Canada have been used by the Dutch Society of Pediatrics. Recently NASPGHAN (North American Society Pediatric Gastroenterology, Hepatology and Nutrition) and ESPGHAN (European Society Pediatric Gastroenterology, Hepatology and Nutrition) published joint evidence-based clinical guidelines for the diagnosis and treatment of *Hp* infection in children in North-America and Europe [52,84-87]. In these guidelines it is recommended that the initial diagnosis of *Hp* infection should be based on either histopathology plus a rapid urease test on the biopsy or a positive culture. In contrast, the recommendations for adults suggest a test-and-treat regimen, based on one positive non-invasive diagnostic test under strict conditions. "Test-and-treat" is definitely not recommended in children. First-line eradication therapy usually comprises triple therapy (protonpump-inhibitor + amoxicillin+ clarithromycin (or metronidazole) or bismuth salts + amoxicillin+ metronidazole during 7-10 days, or sequential therapy. The ^{13}C-urea breath test and the monoclonal stool test are reliable non-invasive tests to determine whether *Hp* has been eradicated 4-8 weeks after completion of therapy.
In the Netherlands the publication of evidence based guidelines is scheduled for 2012.

1.7 RESISTANCE TO ANTIBIOTICS

Antibiotic resistance of *Hp* is one of the main reasons for eradication failure in adults as well as in children and there is evidence that the prevalence of resistance is increasing. Generally the prevalence of resistance to macrolides is higher in isolates from children than those from adults as opposed to results for metronidazole[88]. In a French study on 27 treatment regimens a global failure of 25.8% was found and resistance to clarithromycin almost perfectly predicted failure[89]. The resistance rate

to amoxicillin is very low in Europe. More recently alternative therapeutic regimens have been introduced including sequential therapy and quadruple therapy[90-92], to overcome the problems of resistance. So far, promising results have been obtained, although the knowledge on local resistance rates remains key to effective therapy. In most Northern and Western European countries antibiotic resistance does not play a major role, but in Southern-Europe, Africa and Asia high resistance percentages have been found for clarithromycin and metronidazole. The relatively low prevalence of resistant strains in our country (1-5 and 7-33% to clarithromycin and metronidazole, respectively) has led to guidelines on test-and-treat-policy for adults below the age of 45 years (see table 2). However resistance rates are increasing in Dutch adults, and therefore surveillance will remain necessary in order to maintain this regimen for the future.

Table 2 *Hp* resistance to Clarithromycin and Metronidazole in Dutch adults (1993-2003)

Year of publication and reference	Period	Region/city* of the Netherlands	Clarithromycin % (N)	Metronidazole % (N)
1997[93]	1993 1996	Not known	Not tested	7 (245) 32 (509)
1996[94]	1994-1995	North South Amsterdam*	 1 (780)	18 (200) 16 (430) 19 (150)
1999[95]	1997-1998	West North-East South	1.6 (123) 1.5 (65) 2.3 (43)	24.3 (123) 21.5 (65) 11.6 (43)
2001[96]	1998	Amsterdam* Hoogeveen*	5 (100) 1.3 (77)	33 (100) 23.4 (77)
2001[97]	1995-2000	North-East	2.1 (5946)	18.8 (5946)
2003[98]	1995-2000	Zaandam*	4.8 (724)	25.8 (727)
2005[99]	1998-2003	Den Bosch*	3 (959)	14 (960)
2006[100]	1997-2002	Doetinchem*	1 (1123)	14.4 (1125)

1.8 AIM, RESEARCH QUESTIONS AND OUTLINE OF THE THESIS

Introduction of non-invasive diagnostic tests and test-and-treat policy have led to a decreasing prevalence of the infection and its main complications. However, the interest of the scientific society working on *Hp* has mainly highlighted work on adults, as can be seen from Figure 1. There are important reasons for pediatric gastroenterologists to focus on *Hp*:

- the continuous immigration of children from relatively high-prevalence countries (particularly Turkey, Morocco, Somalia and China), as well as the adoption of children from abroad. As the infection is usually acquired early in life, these children may already have been infected in their respective country of origin.
- the increasing resistance rates of the bacterium, which diminishes the eradication rate and which could be the reason for recrudescence of the infection after a negative eradication control.
- the awareness that the first stage of the infection in humans occurs usually during childhood. The discovery of this should have given an impulse on the study of *Hp* in children.

The aim of this dissertation is to present scientific data on the prevalence and treatment of *Hp* infection in children.
The thesis is divided in four parts:
Section A (*chapters 2 and 3*) provides an overview of the literature on *Hp* in pediatrics published between 2005-2006 and 2009-2010 respectively.
Section B (*chapters 4-6*) focuses on the prevalence of *Hp* infection in children and discusses its complications. Chapter 4 contains data on the prevalence of *Hp* infection in young Dutch children within the general population. Chapter 5 describes aspects of one of the severe complications of *Hp* infection in children, ulcer disease, in a European-wide multicenter study. In chapter 6 results of a prevalence study of *Hp* infection in a developing country (Bandung, Indonesia) are described. Here a new monoclonal stool antigen test was used for a group of 150 young Indonesian children.
Section C (*chapter 7*) discusses the main reason for eradication failure, the antibiotic resistance of *Hp*, for both adults and children in the Netherlands.
The results of this thesis are discussed in **Section D**, *chapter 8* and in this chapter also considerations about future scientific research on *Hp* are addressed.

REFERENCES

1. Castillo-Rojas G, Cerbon MA, Lopez-Vidal Y. Presence of Helicobacter pylori in a Mexican pre-Columbian mummy. *Bmc Microbiology* 2008;8.
2. Rigas B, Feretis C, Papavassiliou ED. John Lykoudis: an unappreciated discoverer of the cause and treatment of peptic ulcer disease. *Lancet* 1999;354(9190):1634-1635.
3. Marshall B. Unidentified Curved Bacilli on Gastric Epithelium in Active Chronic Gastritis. *Lancet* 1983;1(8336):1273-1275.
4. Warren JR. Unidentified Curved Bacilli on Gastric Epithelium in Active Chronic Gastritis. *Lancet* 1983;1(8336):1273.
5. Marshall BJ, Armstrong JA, McGechie DB, Glancy RJ. Attempt to fulfil Koch's postulates for pyloric Campylobacter. *Med J Aust* 1985;142(8):436-439.
6. Marshall B. Helicobacter connections. *Chemmedchem* 2006;1(8):783-802.
7. Correa P, Piazuelo MB. Natural history of Helicobacter pylori infection. *Digestive and Liver Disease* 2008;40(7):490-496.
8. Schistosomes, liver flukes and Helicobacter pylori. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Lyon, 7-14 June 1994. *IARC Monogr Eval Carcinog Risks Hum* 1994;61:1-241.
9. Langenberg ML, Tytgat GNJ, Schipper MEI, Rietra PJGM, Zanen HC. Campylobacter-Like Organisms in the Stomach of Patients and Healthy-Individuals. *Lancet* 1984;1(8390):1348.
10. Hill R, Pearman J, Worthy P, Caruso V, Goodwin S, Blincow E. Campylobacter Pyloridis and Gastritis in Children. *Lancet* 1986;1(8477):387.
11. Cadranel S, Goossens H, Deboeck M, Malengreau A, Rodesch P, Butzler JP. Campylobacter Pyloridis in Children. *Lancet* 1986;1(8483):735-736.
12. Fox JG. The non-H pylori helicobacters: their expanding role in gastrointestinal and systemic diseases. *Gut* 2002;50(2):273-283.
13. Hulten K, Han SW, Enroth H et al. Helicobacter pylori in the drinking water in Peru. *Gastroenterology* 1996;110(4):1031-1035.
14. Goodman KJ, Correa P, Aux HJT et al. Helicobacter pylori infection in the Colombian Andes: A population-based study of transmission pathways. *American Journal of Epidemiology* 1996;144(3):290-299.
15. Vincent P, Gottrand F, Pernes P et al. High Prevalence of Helicobacter-Pylori Infection in Cohabiting Children - Epidemiology of A Cluster, with Special Emphasis on Molecular Typing. *Gut* 1994;35(3):313-316.
16. Ng BL, Ng HC, Goh KT, Ho B. Helicobacter pylori in familial clusters based on antibody profile. *FEMS Immunol Med Microbiol* 2001;30(2):139-142.
17. Rothenbacher D, Winkler M, Gonser T, Adler G, Brenner H. Role of infected parents in transmission of Helicobacter pylori to their children. *Pediatric Infectious Disease Journal* 2002;21(7):674-679.

18. Konno M, Fujii N, Yokota S et al. Five-year follow-up study of mother-to-child transmission of Helicobacter pylori infection detected by a random amplified polymorphic DNA fingerprinting method. *J Clin Microbiol* 2005;43(5):2246-2250.
19. Schwarz S, Morelli G, Kusecek B et al. Horizontal versus familial transmission of Helicobacter pylori. *PLoS Pathog* 2008;4(10):e1000180.
20. Roma E, Panayiotou J, Pachoula J et al. Intrafamilial Spread of Helicobacter pylori Infection in Greece. *Journal of Clinical Gastroenterology* 2009;43(8):711-715.
21. Vale FF, Vitor JMB. Transmission pathway of Helicobacter pylori: Does food play a role in rural and urban areas? *International Journal of Food Microbiology* 2010;138(1-2):1-12.
22. Talarico S, Gold BD, Fero J et al. Pediatric Helicobacter pylori Isolates Display Distinct Gene Coding Capacities and Virulence Gene Marker Profiles. *Journal of Clinical Microbiology* 2009;47(6):1680-1688.
23. Rick JR, Goldman M, Semino-Mora C et al. In Situ Expression of cagA and Risk of Gastroduodenal Disease in Helicobacter pylori-infected Children. *Journal of Pediatric Gastroenterology and Nutrition* 2010;50(2):167-172.
24. Yamaoka Y, Reddy R, Graham DY. Helicobacter pylori Virulence Factor Genotypes in Children in the United States: Clues about Genotype and Outcome Relationships. *Journal of Clinical Microbiology* 2010;48(7):2550-2551.
25. Oleastro M, Santos A, Cordeiro R, Nunes B, Megraud F, Menard A. Clinical relevance and diversity of two homologous genes encoding glycosyltransferases in Helicobacter pylori. *J Clin Microbiol* 2010;48(8):2885-2891.
26. Wu MS, Chen CJ, Lin JT. Host-environment interactions: their impact on progression from gastric inflammation to carcinogenesis and on development of new approaches to prevent and treat gastric cancer. *Cancer Epidemiol Biomarkers Prev* 2005;14(8):1878-1882.
27. Windle HJ, Kelleher D, Crabtree JE. Childhood Helicobacter pylori infection and growth impairment in developing countries: a vicious cycle? *Pediatrics* 2007;119(3):e754-e759.
28. Apley J, Naish N. Recurrent Abdominal Pains - A Field Survey of 1,000 School Children. *Archives of Disease in Childhood* 1958;33(168):165-170.
29. van der Meer SB, Forget PP, Loffeld RJ, Stobberingh E, Kuijten RH, Arends JW. The prevalence of Helicobacter pylori serum antibodies in children with recurrent abdominal pain. *Eur J Pediatr* 1992;151(11):799-801.
30. Chong SKF, Leu QY, Asnicar MA et al. Helicobacter-Pylori Infection in Recurrent Abdominal-Pain in Childhood - Comparison of Diagnostic-Tests and Therapy. *Pediatrics* 1995;96(2):211-215.
31. Macarthur C, Saunders N, Feldman W. Helicobacter-Pylori, Gastroduodenal Disease, and Recurrent Abdominal-Pain in Children. *Jama-Journal of the American Medical Association* 1995;273(9):729-734.
32. Malaty HM, Abudayyeh S, Graham DY, Gilger MA, Rabeneck L, O'Malley K. A prospective study for the association of Helicobacter pylori infection to a multidimensional measure for recurrent abdominal pain in children. *Helicobacter* 2006;11(4):250-257.

33. Macarthur C. Helicobacter pylori infection and childhood recurrent abdominal pain: lack of evidence for a cause and effect relationship. *Can J Gastroenterol* 1999;13(7):607-610.
34. Spee LAA, Madderom MB, Pijpers M, van Leeuwen Y, Berger MY. Association Between Helicobacter pylori and Gastrointestinal Symptoms in Children. *Pediatrics* 2010;125(3):E651-E669.
35. Kalach N, Bontems P, Koletzko S et al. Frequency and risk factors of gastric and duodenal ulcers or erosions in children: a prospective 1-month European multicenter study. *Eur J Gastroenterol Hepatol* 2010;22(10):1174-1181.
36. Huang SC, Sheu BS, Lee SC, Yang HB, Yang YJ. Etiology and treatment of childhood peptic ulcer disease in Taiwan: a single center 9-year experience. *J Formos Med Assoc* 2010;109(1):75-81.
37. Guarner J, Bartlett J, Whistler T et al. Can pre-neoplastic lesions be detected in gastric biopsies of children with Helicobacter pylori infection? *J Pediatr Gastroenterol Nutr* 2003;37(3):309-314.
38. Conley ME, Ziegler MM, Borden S, Huff DS, Boyle JT. Multifocal Adenocarcinoma of the Stomach in A Child with Common Variable Immunodeficiency. *Journal of Pediatric Gastroenterology and Nutrition* 1988;7(3):456-460.
39. Munck A, Bellaiche M, Ferkadji L et al. Carcinoma of the Stomach in A Child. *Journal of Pediatric Gastroenterology and Nutrition* 1993;16(3):334-336.
40. Ashorn P, Lahde PL, Ruuska T, Makipernaa A. Gastric lymphoma in an 11-year-old boy: a case report. *Med Pediatr Oncol* 1994;22(1):66-67.
41. Blecker U, McKeithan TW, Hart J, Kirschner BS. Resolution of Helicobacter pylori-associated gastric lymphoproliferative disease in a child. *Gastroenterology* 1995;109(3):973-977.
42. Blecker U. Helicobacter pylori - Associated gastroduodenal disease in childhood. *Southern Medical Journal* 1997;90(6):570-576.
43. Ohno Y, Kosaka T, Muraoka I et al. Remission of primary low-grade gastric lymphomas of the mucosa-associated lymphoid tissue type in immunocompromised pediatric patients. *World J Gastroenterol* 2006;12(16):2625-2628.
44. Slotta JE, Heine S, Kauffels A et al. Gastrectomy with isoperistaltic jejunal parallel pouch in a 15-year-old adolescent boy with gastric adenocarcinoma and autosomal recessive agammaglobulinemia. *J Pediatr Surg* 2011;46(9):e21-e24.
45. Neefjes VME, Heijboer H, Tamminga RYJ. H-pylori infection in childhood chronic immune thrombocytopenic purpura. *Haematologica-the Hematology Journal* 2007;92(4):576.
46. Kuhne T, Michaels LA. Helicobacter pylori in children with chronic idiopathic thrombocytopenic purpura: Are the obstacles in the way typical in pediatric hematology? *Journal of Pediatric Hematology Oncology* 2008;30(1):2-3.
47. Treepongkaruna S, Sirachainan N, Kanjanapongkul S et al. Absence of Platelet Recovery Following Helicobacter pylori Eradication in Childhood Chronic Idiopathic Thrombocytopenic Purpura: A Multi-center Randomized Controlled Trial. *Pediatric Blood & Cancer* 2009;53(1):72-77.

48. Ferrara M, Capozzi L, Russo R. Effect of Helicobacter pylori eradication on platelet count in children with chronic idiopathic thrombocytopenic purpura. *Hematology* 2009;14(5):282-285.
49. Moon A, Solomon A, Beneck D, Cunningham-Rundles S. Positive association between Helicobacter pylori and gastroesophageal reflux disease in children. *J Pediatr Gastroenterol Nutr* 2009;49(3):283-288.
50. Emiroglu HH, Sokucu S, Suoglu OD, Gulluoglu M, Gokce S. Is there a relationship between Helicobacter pylori infection and erosive reflux disease in children? *Acta Paediatr* 2010;99(1):121-125.
51. Abdollahi A, Morteza A, Khalilzadeh O, Zandieh A, Asgarshirazi M. The role of Helicobacter pylori infection in gastro-oesophageal reflux in Iranian children. *Ann Trop Paediatr* 2011;31(1):53-57.
52. Koletzko S, Jones NL, Goodman KJ et al. Evidence-based Guidelines From ESPGHAN and NASPGHAN for Helicobacter pylori Infection in Children. *J Pediatr Gastroenterol Nutr* 2011;53(2):230-243.
53. Sykora J, Rowland M. Helicobacter pylori in Pediatrics. *Helicobacter* 2011;16 Suppl 1:59-64.
54. Chimonas MA, Baggett HC, Parkinson AJ, Muth PT, Dunaway E, Gessner BD. Asymptomatic Helicobacter pylori infection and iron deficiency are not associated with decreased growth among Alaska Native children aged 7-11 years. *Helicobacter* 2006;11(3):159-167.
55. Cherian S, Forbes D, Sanfilippo F, Cook A, Burgner D. Helicobacter pylori, helminth infections and growth: a cross-sectional study in a high prevalence population. *Acta Paediatr* 2009;98(5):860-864.
56. Plonka M, Bielanski W, Konturek SJ et al. Helicobacter pylori infection and serum gastrin, ghrelin and leptin in children of Polish shepherds. *Dig Liver Dis* 2006;38(2):91-97.
57. Choe YH, Kim SK, Son BK, Lee DH, Hong YC, Pai SH. Randomized placebo-controlled trial of Helicobacter pylori eradication for iron-deficiency anemia in preadolescent children and adolescents. *Helicobacter* 1999;4(2):135-139.
58. Pacifico L, Anania C, Osborn JF, Ferraro F, Chiesa C. Consequences of Helicobacter pylori infection in children. *World J Gastroenterol* 2010;16(41):5181-5194.
59. Annibale B, Capurso G, Lahner E et al. Concomitant alterations in intragastric pH and ascorbic acid concentration in patients with Helicobacter pylori gastritis and associated iron deficiency anaemia. *Gut* 2003;52(4):496-501.
60. Koletzko S, Feydt-Schmidt A. Infants differ from teenagers: use of non-invasive tests for detection of Helicobacter pylori infection in children. *European Journal of Gastroenterology & Hepatology* 2001;13(9):1047-1052.
61. Megraud F. Comparison of non-invasive tests to detect Helicobacter pylori infection in children and adolescents: results of a multicenter European study. *J Pediatr* 2005;146(2):198-203.
62. Guarner J, Kalach N, Elitsur Y, Koletzko S. Helicobacter pylori diagnostic tests in children: review of the literature from 1999 to 2009. *European Journal of Pediatrics* 2010;169(1):15-25.

63. Pounder RE, Ng D. The prevalence of Helicobacter pylori infection in different countries. *Aliment Pharmacol Ther* 1995;9 Suppl 2:33-39.
64. Mourad-Baars P, Chong S. Helicobacter pylori infection in pediatrics. *Helicobacter* 2006;11 Suppl 1:40-45.
65. den Hoed CM, Vila AJ, Holster IL et al. Helicobacter pylori and the birth cohort effect: evidence for stabilized colonization rates in childhood. *Helicobacter* 2011;16(5):405-409.
66. Roosendaal R, Kuipers EJ, Buitenwerf J et al. Helicobacter pylori and the birth cohort effect: evidence of a continuous decrease of infection rates in childhood. *Am J Gastroenterol* 1997;92(9):1480-1482.
67. Bohmer CJ, Klinkenberg-Knol EC, Kuipers EJ et al. The prevalence of Helicobacter pylori infection among inhabitants and healthy employees of institutes for the intellectually disabled. *Am J Gastroenterol* 1997;92(6):1000-1004.
68. Lewindon PJ, Lau D, Chan A, Tse P, Sullivan PB. Helicobacter pylori in an institution for disabled children in Hong Kong. *Dev Med Child Neurol* 1997;39(10):682-685.
69. Gisbert JP. The recurrence of Helicobacter pylori infection: Incidence and variables influencing it. A critical review. *American Journal of Gastroenterology* 2005;100(9):2083-2099.
70. Van Der Hulst RW, Rauws EA, Koycu B et al. Helicobacter pylori reinfection is virtually absent after successful eradication. *J Infect Dis* 1997;176(1):196-200.
71. vanderEnde A, vanderHulst RWM, Dankert J, Tytgat GNJ. Reinfection versus recrudescence in Helicobacter pylori infection. *Alimentary Pharmacology & Therapeutics* 1997;11:55-61.
72. Niv Y, Hazazi R. Helicobacter pylori recurrence in developed and developing countries: meta-analysis of 13C-urea breath test follow-up after eradication. *Helicobacter* 2008;13(1):56-61.
73. Oderda G, Vaira D, Ainley C et al. 18 Month Follow-Up of Helicobacter-Pylori Positive Children Treated with Amoxicillin and Tinidazole. *Gut* 1992;33(10):1328-1330.
74. Kato S, Abukawa D, Furuyama N, Iinuma K. Helicobacter pylori reinfection rates in children after eradication therapy. *Journal of Pediatric Gastroenterology and Nutrition* 1998;27(5):543-546.
75. Rowland M, Kumar D, Daly L, O'Connor P, Vaughan D, Drumm B. Low rates of Helicobacter pylori reinfection in children. *Gastroenterology* 1999;117(2):336-341.
76. Feydt-Schmidt A, Kindermann A, Konstantopoulos N et al. Reinfection rate in children after successful Helicobacter pylori eradication. *European Journal of Gastroenterology & Hepatology* 2002;14(10):1119-1123.
77. Farrell S, Milliken I, Doherty GM, Murphy JL, Wootton SA, McCallion WA. Total family unit Helicobacter pylori eradication and pediatric re-infection rates. *Helicobacter* 2004;9(4):285-288.
78. Oona M, Rago T, Maaroos HI. Long-term recurrence rate after treatment of Helicobacter pylori infection in children and adolescents in Estonia. *Scandinavian Journal of Gastroenterology* 2004;39(12):1186-1191.

79. Magista AM, Ierardi T, Castellaneta S et al. Helicobacter pylori status and symptom assessment two years after eradication in pediatric patients from a high prevalence area. *Journal of Pediatric Gastroenterology and Nutrition* 2005;40(3):312-318.
80. Halitim F, Vincent P, Michaud L et al. High rate of Helicobacter pylori reinfection in children and adolescents. *Helicobacter* 2006;11(3):168-172.
81. Malfertheiner P, Megraud F, O'Morain C et al. Current concepts in the management of Helicobacter pylori infection: the Maastricht III Consensus Report. *Gut* 2007;56(6):772-781.
82. Malfertheiner P, Megraud F, O'Morain CA et al. Management of Helicobacter pylori infection--the Maastricht IV/ Florence Consensus Report. *Gut* 2012;61(5):646-664.
83. de Wit NJ, van Barneveld TA, Festen HP, Loffeld RJ, van PB, Numans ME. [Guideline "Dyspepsia"]. *Ned Tijdschr Geneeskd* 2005;149(25):1386-1392.
84. Drumm B, Koletzko S, Oderda G. Helicobacter pylori infection in children: a consensus statement. European Paediatric Task Force on Helicobacter pylori. *J Pediatr Gastroenterol Nutr* 2000;30(2):207-213.
85. Jones NL. A review of current guidelines for the management of Helicobacter pylori infection in children and adolescents. *Paediatr Child Health* 2004;9(10):709-713.
86. Bourke B, Ceponis P, Chiba N et al. Canadian Helicobacter Study Group Consensus Conference: Update on the approach to Helicobacter pylori infection in children and adolescents--an evidence-based evaluation. *Can J Gastroenterol* 2005;19(7): 399-408.
87. Rowland M, Imrie C, Bourke B, Drumm B. How should Helicobacter pylori infected children be managed? *Gut* 1999;45 Suppl 1:I36-I39.
88. Cabrita J, Oleastro M, Matos R et al. Features and trends in Helicobacter pylori antibiotic resistance in Lisbon area, Portugal (1990-1999). *J Antimicrob Chemother* 2000;46(6):1029-1031.
89. Broutet N, Tchamgoue S, Pereira E, Lamouliatte H, Salamon R, Megraud F. Risk factors for failure of Helicobacter pylori therapy--results of an individual data analysis of 2751 patients. *Aliment Pharmacol Ther* 2003;17(1):99-109.
90. Prieto-Jimenez CA, Cardenas VM, Fischbach LA et al. Double-blind randomized trial of quadruple sequential Helicobacter pylori eradication therapy in asymptomatic infected children in El Paso, Texas. *J Pediatr Gastroenterol Nutr* 2011;52(3):319-325.
91. Patrick B, Nicolas K, Giuseppina O et al. Sequential Therapy vs. Tailored Triple Therapies For Helicobacter Pylori Infection In Children: A Prospective, Open-label, Multi-center Study. *J Pediatr Gastroenterol Nutr* 2011.
92. Albrecht P, Kotowska M, Szajewska H. Sequential therapy compared with standard triple therapy for Helicobacter pylori eradication in children: a double-blind, randomized, controlled trial. *J Pediatr* 2011;159(1):45-49.
93. van der Wouden EJ, van Zwet AA, Vosmaer GD, Oom JA, de JA, Kleibeuker JH. Rapid increase in the prevalence of metronidazole-resistant Helicobacter pylori in the Netherlands. *Emerg Infect Dis* 1997;3(3):385-389.

94. van Zwet AA, de Boer WA, Schneeberger PM, Weel J, Jansz AR, Thijs JC. Prevalence of primary Helicobacter pylori resistance to metronidazole and clarithromycin in The Netherlands. *Eur J Clin Microbiol Infect Dis* 1996;15(11):861-864.
95. Debets-Ossenkopp YJ, Herscheid AJ, Pot RG, Kuipers EJ, Kusters JG, Vandenbroucke-Grauls CM. Prevalence of Helicobacter pylori resistance to metronidazole, clarithromycin, amoxycillin, tetracycline and trovafloxacin in The Netherlands. *J Antimicrob Chemother* 1999;43(4):511-515.
96. Glupczynski Y, Megraud F, Lopez-Brea M, Andersen LP. European multicentre survey of in vitro antimicrobial resistance in Helicobacter pylori. *Eur J Clin Microbiol Infect Dis* 2001;20(11):820-823.
97. Arents NL, Smeets LC, van Zwet AA et al. Implications of the simultaneous presence of metronidazole-susceptible and -resistant Helicobacter pylori colonies within a single biopsy specimen. *Eur J Clin Microbiol Infect Dis* 2001;20(6):418-420.
98. Loffeld RJ, Fijen CA. Antibiotic resistance of Helicobacter pylori: a cross-sectional study in consecutive patients, and relation to ethnicity. *Clin Microbiol Infect* 2003;9(7):600-604.
99. Janssen MJ, Schneeberger PM, de Boer WA, Laheij RJ, Jansen JB. [Low prevalence of metronidazole- and clarithomycin-resistant Helicobacter pylori in the 's-Hertogenbosch region, 1998-2003]. *Ned Tijdschr Geneeskd* 2005;149(39):2175-2177.
100. Janssen MJ, Hendrikse L, de Boer SY et al. Helicobacter pylori antibiotic resistance in a Dutch region: trends over time. *Neth J Med* 2006;64(6):191-195.