Clinical aspects in Helicobacter pylori infections

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CHAPTER I

Introduction, aims and outline of this thesis
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General introduction and historical perspective

Peptic ulcer disease, caused by an infection with Helicobacter pylori is one of the most common chronic infectious diseases in the world. The prevalence of H. pylori ranges from approximately 25% in developed countries to more than 80% in the developing world.1, 2 These microorganisms can establish a long-term colonization of the gastric mucosa.3

Spiral organisms in the stomach were first described in dogs and, around the turn of the century, also in humans, from post-mortem studies.4,5 The possible involvement of these bacteria in peptic ulcer disease was suggested but soon forgotten. Until H. pylori were cultured in 1982, physicians and microbiologists believed that the stomach was sterile, due to its acid environment. In 1979, Warren and Marshall observed curved bacteria in gastric biopsy specimens obtained via endoscopic procedures from patients with upper gastrointestinal complaints. They also noted that these bacteria were present only in inflamed tissue and they were the first to culture this microorganism, then termed Campylobacter pyloridis.4,8 Subsequently Koch's postulates were fulfilled for Campylobacter pyloridis and gastritis. Marshall himself proved this by ingesting a culture of the organism and showing it to be responsible for an acute dyspeptic illness associated with histological gastritis.9 In 1989, Goodwin et al10 demonstrated that this bacterium did not belong to the genus Campylobacter and renamed it Helicobacter pylori, because of its helical appearance and its most common site of isolation, the pylorus of the stomach.

The isolation of this bacterium opened a new era in the understanding of gastroduodenal pathology, mainly of gastritis and peptic ulcer. The publication of the complete genome sequence of H. pylori in 1997, only the seventh bacterium for which this was achieved and only 15 years after H. pylori was first discovered, demonstrates the intense interest in the role of this organism as a human pathogen.11 This interest in H. pylori is also reflected in the more than 10,000 references that can be found in the medical database Medline when keywords ‘Helicobacter pylori’ or ‘campylobacter pyloridis’ are entered. We now know that H. pylori is the main cause of peptic ulcer disease and the discovery of this bacterium changed peptic ulcer disease from a chronic, relapsing disease of unknown cause to a specific and curable disease. We now also know that H. pylori plays a role in other diseases, e.g., in gastric mucosa-associated lymphoid tissue (MALT) lymphoma and in gastric cancer.12-14

Diagnosis of infection

Several invasive and non-invasive tests have been developed to diagnose H. pylori. Each test has unique features that offer additional information over other tests, but all have their shortcomings.15 Therefore, for clinical trials a combination of tests is mandatory. Most tests show good sensitivity and specificity; however, this is dependent on local preference and expertise. For example, pathologists vary in their ability, technique and experience in
detecting the organism, which is an important determinant for local results. The success of culturing H. pylori also varies significantly between laboratories, with the best results being derived from laboratories with a particular interest in culturing H. pylori. Similarly, the accuracy of rapid urease tests varies with several commercial as well as 'home-made' kits. Urea breath tests can also be performed via different protocols, with different detection methods, and with different cut-off values. Also, the conditions under which the tests are performed, e.g., the use of antisecretory drugs, vary. Therefore, published results of diagnostic tests may not always reflect the sensitivity and specificity of those tests in individual centers. Even in published trials, an exact description of the performed diagnostic methods is not always stated, which may lead to otherwise unexplainable differences in outcome.

Histological examination of gastric biopsy specimens can show H. pylori on the gastric mucosa, as well as the degree of mucosal damage. After a meeting in Sydney, pathologists began to standardize their histological description. The sensitivity and specificity is generally good; however, interobserver variability exists among pathologists. Accuracy is also linked to the quality of the material provided. H. pylori may be missed on histologic evaluation because of a patchy distribution, the presence of intestinal metaplasia, and the recent use of antibiotics, bismuth, or proton pump inhibitors. Special stains, including Genta, giemsa and Warthin-Starry, may be helpful in detecting H. pylori. Immunohistochemistry, using purified polyclonal H. pylori antiserum, has been advised because of its high specificity and low interobserver variability.

Culture is 100% specific for an H. pylori infection; however, sensitivity varies widely among centers. Culturing H. pylori requires local expertise and strict transport conditions. Culturing also provides information on antimicrobial susceptibility and allows for typing of strains via different methods. A disadvantage of culture is the delay in obtaining the results. Growth of H. pylori occurs at best after 3 days, and susceptibility tests take another 3 days. If no growth occurs, the plates must be kept for 12 days before recording a negative result.

Biopsy-based urease tests are simple, rapid, inexpensive, and quite specific. This test is based on H. pylori's usually potent urease. Urease hydrolyses urea to release ammonia and carbon dioxide. The free ammonia increases the pH in the medium, and the pH indicator changes color. Optimal results are obtained when the test result is measured at 24 hours. Sensitivity drops when early reading is performed. Sensitivity was also reported lower in the post-treatment setting.

DNA techniques, especially polymerase chain reaction (PCR), can also be used to detect H. pylori in gastric biopsies. This is a quick technique that does not require strict transport conditions. PCR techniques can also be used for molecular typing. Several commercially available tests, which detect IgG antibodies against H. pylori using an enzyme-linked immunosorbent assay (ELISA) technique, are available. The main difference among these kits is the antigen: some are semi-purified and others are a mixture of purified antigens from several strains. These tests evaluate the whole stomach, are easily tolerated, simple to use, and provide quick results. An advantage of serology is that it is not influenced by the use of antimicrobials or antisecretory drugs. Sensitivity and specificity of the different serological tests
vary and should be locally validated. Serological tests reflect not only current but also previous exposure to *H. pylori*: antibody titers fall after eradication therapy only after several months and the rate of fall is variable. Nevertheless, monitoring of *H. pylori* eradication after antimicrobial treatment has been reported to produce reliable results when the test is performed no sooner than 6 months after the end of treatment and is compared to the pre-treatment sample.\(^{31,32}\) Tests have also been developed to detect IgG antibodies against *H. pylori* saliva and urine; however, these tests are still under research.

Urea breath tests are also used to diagnose *H. pylori* infections. The principle of the urea breath test is based on the identification of urease activity utilizing \(^{13}\)C- or \(^{14}\)C-labeled urea. The urea is metabolized by urease, which is produced by *H. pylori*, yielding labeled CO2, which is absorbed across the gastric mucosa and expired in the breath where it can be measured. Two carbon isotopes can be used: \(^{14}\)C, which is radioactive and \(^{13}\)C, which is a non-radioactive isotope. Labeled carbon can be measured using a mass spectrometer, infrared, or laser spectrometer\(^{34,35}\). The urea breath tests vary with respect to the detection method for the isotope, dose and formulation of the isotope, use of test meals or liquids, use of baseline samples, timing of the sampling, method and volume of breath collection, and cut-off value. Furthermore, breath testing during therapy with antisecretory drugs is less reliable. When a locally validated protocol is used, the \(^{13}/^{14}\)C-urea breath test is a reliable way to detect the presence or absence of an *H. pylori* infection in adults as well as in children.\(^{36-40}\) However, relatively few data are available regarding the value of breath testing after antibiotic therapy. The advantage of breath testing is clearly its non-invasiveness, cost effectiveness, and simplicity. Furthermore, the absence of a sampling error favors the use of breath tests.

The latest development in diagnostic tools to detect *H. pylori* is an antigen test for stools (Premier Platinum HpSA, for which very promising results were reported.\(^{31,42}\)

**Post-eradication**

All of the above mentioned tests can also be applied to test for cure after an antimicrobial regimen has been given; however, the sensitivity and specificity of these tests may be lower than in the pre-treatment setting.\(^{37,43-46}\)

After antimicrobial therapy, when the microorganism may be suppressed but not eradicated, diagnostic tests are more likely to give false-negative results. The number of false-negative biopsy-based tests may be reduced if multiple specimens are taken and more than one test is used. Biopsies should be taken from both antrum and corpus of the stomach, since the organism may be patchily distributed and exhibit a lower density after a failed eradication regimen. Also, proximal migration of *H. pylori* within the stomach after the use of proton pump inhibitors has been documented.\(^{34,47}\) However, especially after therapy, when a diagnosis has already been made, non-invasive tests are preferred because of lower cost and greater convenience for the patients.

The accuracy of a test is reflected in a combination of both sensitivity and specificity. In routine practice, the clinician wishes to know that if a test result is positive or negative, it presents a true reflection of whether the patient has or does not have the disease. This information is best
conveyed by the positive and negative predictive values (PPV and NPV). Unlike sensitivity and specificity, PPV and NPV depend on the prevalence of the disease in the population tested. For example, assuming the sensitivity and specificity of a given test is 95%, the NPV is 99.7% at a prevalence of 5%, 95% at a prevalence of 50%, and only 50% when the prevalence is 95%. The performance of the test, therefore, will depend on the pre-test likelihood that the infection is present. After treatment, the clinician wants to know whether the treatment has been successful, that is, that a negative result is indeed a true reflection of *H. pylori* status.

Whatever test or tests are chosen for outcome assessment, it is important that they be done no earlier than four weeks after the cessation of antibiotics or bismuth-containing compounds. Earlier testing will lead to higher false-negative rates due to suppression of the organism. Although four weeks was initially determined to be the minimum interval before biopsy testing, the accuracy of the tests may be improved by waiting longer. Proton pump inhibitors should also be avoided before follow-up testing.48

**Microbiology**

*H. pylori* is a spiral-shaped, slow-growing gram-negative micro-organism that proliferates at temperatures between 30 and 39 degrees Celsius. *H. pylori* are 2.5–5.0 μm long and 0.5–1.0 μm wide, with four to six unipolar sheathed flagella essential to bacterial motility.49-52 For its growth, it requires enriched media and a microaerophilic O2/CO2/N2 (5:10:85%) environment. Whole blood, heme, serum, charcoal, cornstarch, or egg yolk emulsions are usually added to the media as nutritional components for growth. *H. pylori* can be identified by a gram's stain and by positive oxidase, catalase, and urease reactions.53

**Antimicrobial resistance**

The goal of culturing *H. pylori* is to detect clinically relevant antimicrobial resistance. In other words, via an *in vitro* assay, one hopes to predict the likelihood of successfully treating the infection with a particular antimicrobial agent. Therefore, we use a clinical definition of antimicrobial resistance, i.e., a strain is resistant when the likelihood of eradication by a given treatment is low. To determine this, one needs to do clinical studies and compare the cure rates with the minimal inhibitory concentration (MIC) of the antibiotic *in vitro*.

There are different methods of determining antimicrobial resistance. Most often used are the agar dilution method, the epsilometer test (E-test), and the disc-diffusion method. The agar dilution method is time-consuming but is a reliable technique that is usually carried out as a reference method for evaluating the efficacy of other testing methods. However, this method is also not standardized with regard to the appropriate medium (brucella agar, Columbia agar, Wilkins Chalgren agar, etc), supplementation (5 or 10% blood), size of the inoculum (104–108 CFU/ml), incubation atmosphere, and appropriate time to read the plates. The E-test is much less laborious and easier to perform than the agar dilution method and proves a reliable technique
to obtain MIC levels of antibiotics\textsuperscript{54,55}; however, it has been stated that nitroimidazole resistance may be overestimated with the E-test.\textsuperscript{56} The disc-diffusion method is the easiest and cheapest way of testing susceptibility to antibiotics. It provides a zone of inhibition instead of a MIC level as a measure of resistance. The disc-diffusion method in the case of H. pylori has not yet been sufficiently validated. The National Committee for Clinical Laboratory Standards in the United States is currently standardizing the \textit{in vitro} susceptibility tests.

Antibiotic resistance in \textit{H. pylori} infections is increasing worldwide. The highest levels for resistance are found for metronidazole, followed by clarithromycin. Only a few reports on resistance to fluoroquinolones, rifampicin, tetracycline, and amoxicillin have been published to date.\textsuperscript{57-61}

\textbf{Nitroimidazole resistance}

The prevalence of nitroimidazole resistance varies greatly and increases over time. In developing countries, resistance rates have been reported as high as 80–90\%. In most western countries, resistance rates vary from 10–50\%.\textsuperscript{62-64} The steep increase in reported resistance rates is only partly explained by general use of nitroimidazole and by the techniques of testing resistance. It is unclear what other factors may cause this fast rising prevalence of nitroimidazole resistance.

Essentially, two nitroimidazole compounds have been used to treat \textit{H. pylori}: metronidazole and tinidazole. There is cross-resistance between these two drugs. Nitroimidazoles are weak non-ionized bases at neutral pH (in blood and tissue) that cross the gastric barrier and then become ionized due to the low pH in the stomach. This ionized nitroimidazole cannot diffuse back through the barrier and thus accumulates in the stomach. The nitro group of nitroimidazole must be reduced in order to be active. The exact mechanism of how nitroimidazoles can kill \textit{H. pylori} is not completely understood, but it seems that the reduced nitroimidazole intermediates and oxygen radicals can cause DNA damage\textsuperscript{65,67}

Cederbrant et al showed that \textit{in vitro} metronidazole resistance could disappear when the strains were incubated anaerobically for a few hours.\textsuperscript{68} Smith and Edwards showed that the rate of \textit{H. pylori} killing by metronidazole is dependent on the redox potential of the medium.\textsuperscript{65} Therefore, we should test nitroimidazole resistance at the redox potential, which is present at the site of action of the drug, i.e., at the gastric mucosa level. However, the latter probably varies from one patient to the other, and from one moment to another, and thus this is difficult to achieve \textit{in vitro}. Therefore, the results of testing nitroimidazole resistance may not always be comparable, and may not reflect true resistance \textit{in vivo}. This may also explain the apparent instability of nitroimidazole resistance.\textsuperscript{56}

The distribution of the MICs of nitroimidazoles in \textit{H. pylori} infection exhibits a continuous spectrum, without a clear break-point.\textsuperscript{69} This suggests that several pathways can be responsible for nitroimidazole resistance.\textsuperscript{70} Mutational inactivation of the \textit{rdxA} gene, which encodes an oxygen-insensitive NADPH nitroreductase, is one of the mechanisms that have recently been demonstrated to play a role in the development of nitroimidazole resistance in \textit{H. pylori} \textsuperscript{71,72}
There are many conflicting reports with regard to the impact of nitroimidazole resistance on the efficacy of anti-\(H.\) pylori regimens. Possible explanations for these conflicting data may be the method of testing antimicrobial resistance, the use of different MIC-cut off values to define resistant strains, the use of different medications or different dosages, duration or combinations of medications that may overcome nitroimidazole resistance, and differences in patient populations. One should also consider whether or not heterogeneity in susceptibility to antimicrobials is taken into account. In the human stomach you may find different \(H.\) pylori strains, and some institutions test multiple \(H.\) pylori strains while other centers only test one strain.\footnote{73} There is no consensus as to which MIC cut-off level should be used to define nitroimidazole-resistant versus -susceptible strains. Perhaps only strains with a high level of resistance may be of clinical concern.\footnote{74}

Because these regimens are very effective, with 80-90\% eradication rates, it is difficult to test the impact of nitroimidazole resistance, especially when the prevalence of resistance is low. Despite all these problems in studying nitroimidazole resistance, most studies find a clear drop in efficacy of dual therapies and of bismuth-based triple therapies in the case of nitroimidazole resistance.\footnote{75,76} Most studies show a decrease in the efficacy of a combination therapy of a PPI, nitroimidazole, and amoxicillin in the presence of nitroimidazole-resistant \(H.\) pylori strains.\footnote{76} The clinical relevance of nitroimidazole-resistance for \(H.\) pylori eradication rates in patients treated with a PPI, nitroimidazole and clarithromycin is still controversial. To date, a few studies have found a significant drop in efficacy with this regimen,\footnote{44,77,82} while several others were unable to detect a difference in efficacy.\footnote{83-90}

In quadruple therapy, given for one or two weeks, five studies to date have found a drop in efficacy in case of nitroimidazole-resistant strains\footnote{91-94}; however, this was only significant in the largest published series by Van der Hulst et al.\footnote{95} All other studies, in which mostly only a few patients with nitroimidazole-resistant strains were treated with quadruple therapy, have not found a drop in efficacy in case of nitroimidazole-resistance.\footnote{96-106}

Data on the effect of nitroimidazole resistance in the case of therapy combining ranitidine bismuth citrate combined with clarithromycin and nitroimidazole are still scarce, with only one published patient-group\footnote{67}, which yielded large, overlapping 95\% confidence intervals.

Many studies have been published without data on antimicrobial resistance, often showing high overall eradication rates. Since nitroimidazole-resistant strains still have a considerable chance of eradication, the overall effectiveness of an eradication regimen is only jeopardized when the prevalence of resistance is high. Besides, the effect of nitroimidazole resistance cannot be thought of as a 'yes' or 'no' phenomena and can at least be partly overcome.\footnote{88,108} It seems logical that, when the MIC is higher, the chance of eradication decreases, as was reported for PPI-triple therapy by Kist et al.\footnote{44}

**Clarithromycin resistance**

The prevalence of clarithromycin resistance varies from country to country, and the highest reported prevalence comes from France, which is now around 10-15\%.\footnote{64} In most countries, it is still below 5\%, but is expected to rise over the next years due to the increasingly widespread
use of macrolides. In treating *H. pylori* infections, clarithromycin is the most commonly prescribed macrolide because of its excellent *in vitro* activity and because it is less affected by a decrease in pH. Resistance to macrolides appears to be a stable phenomenon with cross-resistance to other macrolides. A mutation of the 23S rRNA gene, causing diminished binding of the antibiotic to the ribosome, seems the most significant mechanism of macrolide resistance. There is a clear bimodal distribution between clarithromycin-susceptible and -resistant *H. pylori* strains. Therefore, the method of measuring clarithromycin resistance is not crucial. Since the prevalence of clarithromycin resistance is low, only a few patients have been studied. However, when the MIC data are correlated to the outcome of clinical trials using clarithromycin, most studies have found decreased efficacy in the case of clarithromycin resistance. 

**Secondary resistance**

Secondary or acquired resistance is defined as susceptible strains that become resistant after exposure to an antibiotic. Whether this is truly an acquired resistance or merely a selection of strains already present in the stomach is, at present, unknown. Previous eradication failures increase the possibility of the development of secondary resistance. Several agents have been reported to prevent development of secondary resistance. For example, co-administration of amoxycillin is suggested to result in a relatively low rate of ‘only’ 30% secondary clarithromycin resistance in PPI triple therapy. Also, bismuth is suggested to prevent the development of secondary resistance; however, the evidence for this claim remains scarce. The best way to prevent secondary resistance is to prescribe effective therapies, which has also been shown to be the most cost-effective approach.

**Heterogeneity of *H. pylori* and its relation to clinical outcome**

With molecular techniques, such as DNA fingerprinting and restriction fragment length polymorphism, *H. pylori* appears to be highly diverse at a chromosomal and extra-chromosomal level, to a degree that is seldom found in other bacteria. This microorganism inhabits the human stomach for many bacterial generations and evolves separately in each host such that it is unusual to find two identical strains in different hosts. Several *H. pylori* genes code for so-called virulence factors that have been associated with different clinical manifestations. For example, the cytotoxin-associated gene (CagA) expresses a high molecular weight protein and is associated with more severe clinical outcome, like peptic ulcer disease and gastric carcinoma. Similarly, VacA type s1 strains have been related to ulcer disease, whereas CagA-negative and VacA type s2 strains have been found to be related to functional dyspepsia. The reason for these associations is not known, however evidence has emerged that suggests pathways by which these virulence factors might cause these diseases. For example persons who
carry CagA-positive strains have, on average, a higher bacterial density and significantly higher acute and chronic inflammation, compared to persons carrying CagA-negative H. pylori.\textsuperscript{123,130} CagA-positivity has also been related to higher production of cytokines and to higher proliferation of gastric epithelial cells, which has been suggested to be a risk factor for the development of gastric adenocarcinoma.\textsuperscript{132,133}

Several factors confuse this relation between virulence factors and disease. In the first place, host-related factors also exert an influence on clinical outcome (e.g. acid production, sex, age, blood group, smoking\textsuperscript{134-136}). Secondly, none of the currently identified H. pylori virulence factors show any disease specificity. Furthermore, there is a remarkable geographic variability in the prevalence of certain strains or virulence factors\textsuperscript{137-140}, and associations between virulence factors and specific diseases can be different in different geographical regions. For example, in Asian populations most H. pylori-positive persons carry CagA-positive strains, and the association with clinical outcome is not readily apparent.\textsuperscript{138,141}

Patients are mostly infected with one predominant H. pylori strain, although more than one strain may co-exist in the human stomach at any time.\textsuperscript{142-146} Harborbing more than one H. pylori strain is reported ranging from a rare occurrence\textsuperscript{142} to occurring in more than 75\% of patients\textsuperscript{143}. It has been reported that some patients can harbor up to 6 variant strains.\textsuperscript{142} The reported variation in the rate of harboring multiple strains may be another example of regional differences in H. pylori infections.

An interesting hypothesis is that different strains may react differently to antimicrobial therapy. Several authors have reported higher cure rates for the more virulent strains, all with relatively ineffective therapies. Van der Hulst et al reported a higher efficacy of dual therapy in patients infected with CagA+ strains, than in patients infected with CagA- H. pylori.\textsuperscript{147} Marais et al showed in a multicenter study that H. pylori eradication rates were achieved in 87\% of patients\textsuperscript{54/62} infected with CagA+ strains, when treated with one-week PPI-based triple therapy, compared to 69 \%\textsuperscript{47/68} of those harboring CagA- strains.\textsuperscript{148} Van Doorn et al\textsuperscript{149} reported a trend to a higher cure rate for VacA type s1 strains than the VacA type s2 strains with one-day quadruple therapy. Go et al\textsuperscript{150} reported the same trend with partly dual-, partly triple-therapy. Van Doorn et al postulated that these strains are easier to eradicate since antibiotics achieve higher concentrations in inflamed tissue, and bacteria in the growth phase are more readily killed. In contrast, VacA type s2 strains are more often found in functional dyspepsia, produce no toxin, induce less inflammation, grow slowly, and are usually in a stationary phase and are therefore more difficult to eradicate in vivo.\textsuperscript{149}

Clinical outcome may, in general, be related to the host, to the strain(s) infecting the host, to environmental factors, or to a combination of these factors. Differences in strains undoubtedly have an impact on clinical outcome. However, because of the considerable heterogeneity and geographical distribution of strains, the association of H. pylori with a disease, obtained from one geographic region, should be confirmed in other regions before this association can be accepted as potentially meaningful.\textsuperscript{151} Similarly, the results of possibly successful therapies should be confirmed in different geographic areas.\textsuperscript{152}
**Treatment of H. pylori infection**

At present, the only well established indication for treating *H. pylori* are peptic ulcer disease and low-grade B-cell gastric lymphoma\(^45,153,154\) *H. pylori* eradication in functional dyspepsia is still controversial \(^155-156\); however, the consensus meetings become increasingly liberal with respect to eradicating *H. pylori* in patients with functional dyspepsia in whom no other somatic causes of upper abdominal discomfort can be found.\(^43\)

Most anti-*H. pylori* therapies consist of an acid inhibitor and/ bismuth or ranitidine bismuth citrate in combination with an antimicrobial. Effective antimicrobials are nitroimidazole, tetracycline, clarithromycin, amoxycillin and azithromycin.

Combinations of these medications are necessary to eradicate *H. pylori*. Treatments which achieve an eradication rate of greater than 80% on a intention-to-treat basis have been recommended by most authorities\(^45,159-161\) The currently advised regimens are PPI triple therapies, quadruple therapies, and ranitidine bismuth citrate combination therapy. We still do not know the optimal treatment duration or dose for any of the currently advised regimens. In the United States, two-week therapies are prescribed most often, while in Europe one-week therapies are more popular. We should realize that the eradication rates in general practice, are probably lower than the eradication rates obtained in clinical trials.\(^162\)

**Monotherapy**

has proven unsuccessful in treating *H. pylori* infections.

**Dual therapy**

Dual therapy, mainly administered as a proton pump inhibitor plus amoxycillin or clarithromycin, has been used for several years, however eradication rates of such dual therapies fluctuate around 50 to 60%.\(^75,163,164\) Therefore, these dual therapies are now considered obsolete. Dual therapy of ranitidine bismuth citrate (pylorid\(^8\)), which combines the antimicrobial effect of bismuth with the acid suppressive effect of ranitidine, given together with clarithromycin yields eradication rates of over 80%.\(^75,165\)

**Triple therapy**

Triple therapy refers to treatment with an antisecretory drug or a bismuth compound in combination with two antibiotics. Bismuth-based triple therapies (e.g. bismuth, amoxycillin plus nitroimidazole or bismuth, tetracycline plus nitroimidazole) for one to two weeks are effective therapies with eradication rates of over 80%.\(^75,163,164\) Drawbacks include the side effects and the clear drop of efficacy to around 50% in the case of nitroimidazole resistance

Proton pump inhibitor-based triple therapies (e.g., proton pump inhibitor, amoxycillin plus nitroimidazole or proton pump inhibitor, clarithromycin plus nitroimidazole) for one to two weeks have been shown to be very effective with consistently reported high eradication rates of 80-95%.\(^75,163,164\) Because of the high efficacy of these regimens and the relatively low side effects,
proton pump inhibitor-based triple therapies are the most frequently recommended treatments world-wide.\textsuperscript{43,153,159,160}

Triple therapy with ranitidine bismuth citrate (usually ranitidine bismuth citrate, clarithromycin and nitroimidazole) for one week has yielded eradication rates in excess of 90\%.\textsuperscript{166,167}

**Quadruple therapy**

Quadruple therapy consists of a combination of an antisecretory drug, a bismuth compound and two antimicrobials (most often a proton pump inhibitor, a bismuth compound, tetracyclcin and nitroimidazole). Quadruple regimens are the most effective therapies currently available, with cure rates above 90\%.\textsuperscript{75,163,164} Drawbacks of quadruple therapy are the complex regimen and a high rate of side effects; however, the reported compliance appears to be mostly excellent.\textsuperscript{168,169}

**Treatment failure**

In little over a decade, anti-*Helicobacter pylori* therapies have been developed which are now achieving successful eradication in 85-90\% of patients. Unfortunately, this therapeutic advance has been rather empirical and lacking in scientific basis, so that when treatment fails, the underlying reasons remain unclear. Compliance and antimicrobial resistance are thought to be the major determinants of treatment failure.\textsuperscript{78,106,170-173}

Other factors that may play a role in treatment failure relate to drug delivery, in other words, to the pharmacology of the gastric mucosa. Factors that may influence outcome of an antimicrobial regimen in this regard are: drug formulation (tablet, liquid, colloid, granule, etc); administration in relation to meals; frequency, dosage and duration of drug administration; type of bismuth salt used (e.g., citrate, nitrate, salicylate); co-therapy to raise the pH; and administration of mucolytics. For example, the concentration of metronidazole is higher in acidic gastric juice than in plasma. Metronidazole is also very stable in gastric juice (its half-life is greater than 800 hours at a pH of 2\textsuperscript{174}) and this, together with its rapid distribution across the gastric mucosa, probably explains why it is so effective in the clinical setting, despite its relatively high *in vitro* MIC for *H. pylori*.\textsuperscript{175} Amoxycillin also appears to cross gastric mucosa by simple diffusion, but does so very poorly\textsuperscript{176,177}, although it is fairly stable in gastric juice at low pH. Unlike metronidazole, no 'trapping' occurs within gastric juice because amoxycillin is a weak acid. Increasing pH and reducing gastric juice volume, results in increased intra-gastric concentrations of amoxycillin.\textsuperscript{176} This may partly explain why acid-suppression is required for this antibiotic to be effective *in vivo*.\textsuperscript{175}

Clarithromycin is concentrated in gastric juice by an active transport process. Clarithromycin is very unstable in gastric acid (with a half-life of less than 1 hour at pH 2)\textsuperscript{174,178} and acid-suppression should therefore always be used concurrently. These overall findings with metronidazole, amoxycillin and clarithromycin partly explain why antimicrobials that are highly effective against *H. pylori in vitro*, fail to be useful in the clinical setting. For example, *H. pylori* is extremely sensitive
to penicillin in vitro (with MICs < 0.003 mg/L), but even if combined with omeprazole, penicillin produces poor eradication rates in clinical trials.

Antimicrobials might work topically against *H. pylori* (i.e., as they pass through the gastric lumen route to the intestine) or systemically (i.e., being transferred into the gastric lumen from the systemic circulation after intestinal absorption). In practice, both topical and systemic routes are probably important, the contribution of each route differing between drugs. Bismuth, for example, is very poorly absorbed and so probably acts predominantly topically. In contrast, metronidazole has almost 100% bioavailability with rapid distribution into gastric juice from the systemic circulation.

Topical delivery would seem ideal as there are only very occasional reports that *H. pylori* live anywhere other than on the luminal side of the gastric mucosa. However, uniform topical delivery throughout the stomach is very difficult to achieve, and the distribution of drugs varies widely with different formulations and with timing of doses relative to meals. No formulation of drugs can achieve significant concentrations in the fundus of the stomach long enough to effect bacterial killing of *H. pylori*. *H. pylori* can therefore evade treatment in this area of the stomach (a so-called 'sanctuary site').

Not all *H. pylori* are the same and certain strains are associated with a higher degree of inflammation, alteration of gastric blood flow, disruption of the mucus layer, and disruption of the normal epithelial barrier. Therefore, there is probably also a difference in drug delivery among patients infected with different strains.

Successful treatment must overcome additional barriers such as the inoculum effect and the biofilm effect. Attachment to a surface may be associated with an increase in the MIC of antibiotics (the biofilm phenomenon). This phenomenon has been shown with *H. pylori* in tissue culture and is also likely to be present in vivo. *H. pylori* exhibits various adhesins; therefore, successful prevention of adhesion is probably difficult, if not impossible.

The inoculum effect describes a phenomenon in which an antibiotic loses its effectiveness when the inoculum of bacteria is large. In the stomach, a tremendous number of bacteria is present (in the range of 10^7 to 10^11), which increases the likelihood that a few naturally resistant mutants are always present.

Patients who have failed an anti-*H. pylori* regimen constitute a different subgroup of patients that are more likely to fail on subsequent therapies. The explanation for this phenomenon may be non-compliance, the emergence of resistant strains, and other factors that are not clearly elucidated. Host factors as well as differences in strains may also attribute to therapy failure.

### Aims and outline of this thesis

The studies in this thesis originated mainly from unsolved clinical questions.

First, in Chapter 1, an overview is given on the current knowledge of *H. pylori*, focussed on topics related to the studies that were performed for this thesis.
In Chapter 2 the impact of metronidazole resistance on the efficacy of PPI triple therapy is studied via a large randomized trial of a metronidazole-containing and a non-metronidazole-containing PPI-based triple therapy.

Current anti-*H. pylori* therapies have become so effective that very large patient numbers are required to determine the impact of factors that may influence the eradication rates. Therefore, a large systematic review, described in Chapter 3, was performed to analyze the impact of metronidazole resistance on eradication rates with currently advised anti-*H. pylori* regimens.

Because the results of possibly successful therapies should be confirmed in different geographic areas, Chapter 4 describes a review of all published studies of *H. pylori* eradication therapies, performed in The Netherlands.

Chapter 5 describes a large study in which *H. pylori* eradication rates were determined for patients with peptic ulcer disease, versus patients with functional dyspepsia, treated with PPI-triple therapies. This study was designed to determine whether the clinical setting had any bearing on the outcome of antimicrobial chemotherapy.

Chapter 6 describes a multicenter study in which the novel technology 13C-Laser Assisted Ratio Analyzer (LARA™) breath test is evaluated as a means to monitor cure after eradication therapy has been administered.

Chapter 7 describes a study in which patients who were *H. pylori* -positive after receiving a PPI triple therapy were randomized to retreatment with another PPI-based triple therapy or quadruple therapy.

Finally this thesis is summarized in Chapter 8.
References

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