Investigating the Effect of Antibiotic Exposure on the Prevalence of Antibiotic-resistant *H. pylori* Infection and the Incidence of Anti-*H. pylori* Treatment Failure in Northern Canadian Communities

by

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Abstract

The frequent and improper use of antibiotics for the treatment of unrelated bacterial infections is believed to influence the development of antibiotic-resistant *H. pylori* infection. Antibiotic-resistant *H. pylori* infection is a major risk factor for the failure of anti-*H. pylori* treatment regimens to eliminate the infection. Considering the limited therapeutic options available to eliminate *H. pylori* infection, it is of public health interest to better understand factors that impact the occurrence of antibiotic resistance in *H. pylori* to inform strategies to prevent its occurrence and improve the effectiveness of anti-*H. pylori* treatment regimens. Northern Canadian Aboriginal communities are disproportionately affected by *H. pylori* infection relative to southern Canadian communities. In response to community concerns regarding *H. pylori*-associated health outcomes, the Canadian North *Helicobacter pylori* (CAN*Help*) Working Group conducts community-driven research to inform public health policy pertaining to control of *H. pylori* infection. One aim of the CAN*Help* Working Group is to improve the clinical management of *H. pylori* infection in northern Canadian communities by identifying factors that limit the effectiveness of anti-*H. pylori* treatment regimens. To better understand factors associated with antibiotic-resistant *H. pylori* infection and anti-*H. pylori* treatment effectiveness, this thesis aims to estimate among CAN*Help* project participants the effect of exposure to antibiotics on the prevalence of antibiotic-resistant *H. pylori* infection and incidence of anti-*H. pylori* treatment failure.

I completed a systematic review of the literature published during 2010 to 2014 to synthesize current evidence of the prevalence of antibiotic-resistant *H. pylori* infection worldwide and antibiotic resistance mechanisms in *H. pylori* infection. To estimate the

effect of antibiotic exposure on the prevalence of antibiotic-resistant *H.* pylori infection and the incidence of anti-*H. pylori* treatment failure, I developed a chart review tool to collect antibiotic exposure histories from participant medical charts for the 5-year period before enrollment in a CAN*Help* community project for *H. pylori*-positive participants who either had samples of *H. pylori* cultured from gastric biopsies or who had completed a post-treatment breath test to classify their post-treatment infection status. I used logistic regression to estimate odds ratios and 95% confidence intervals (CIs) for the effect of antibiotic exposure on the prevalence of antibiotic-resistant *H. pylori* infection among individuals who had *H. pylori* organisms cultured from gastric biopsy samples. I used robust Poisson regression and binomial regression to estimate risk ratios with 95% CIs and risk differences with 95% CIs, respectively, for the effect of antibiotic exposure on the average risk of treatment failure among individuals who completed a post-treatment breath test. I also completed a qualitative analysis of semi-structured interviews to describe participants' perspectives on the value and potential harms of using antibiotics to treat infections and factors that influence their adherence to prescribed regimens.

Results of my quantitative analysis provide evidence that suggest a higher frequency of antibiotic exposure was associated with the prevalence odds of antibiotic-resistant *H. pylori* infection as well as the risk of anti-*H. pylori* treatment failure in CAN*Help* communities. Additionally, the results suggest that previous exposure to anti-*H. pylori* treatment regimens is associated with both the prevalence odds of antibiotic-resistant *H. pylori* infection and the average risk of anti-*H. pylori* treatment failure in CAN*Help* communities. Results of my qualitative analysis provide evidence of widespread knowledge among participants of the importance of adherence to prescribed

antibiotics regimens. Factors that influence adherence to prescribed antibiotics most frequently identified by participants included the occurrence of adverse events and forgetfulness. Given that poor adherence to prescribed antibiotic regimens is known to influence the occurrence of antibiotic-resistant bacterial infections, strategies that support individuals to manage adverse events and remember to take their medications may be useful to improve the effectiveness of anti-*H. pylori* treatment regimens, which would decrease the frequency of repeated exposure to such regimens and, in turn, decrease the occurrence of resistant *H. pylori* infection.

Preface

This thesis is an original work by Kathleen Williams. The research conducted by the CAN*Help* Working Group, of which this thesis is a part, received research ethics approval from the University of Alberta Research Ethnics Board under the Project Name "Addressing Community Concerns about Risks from *H. pylori* Infection in the Circumpolar North" (No. Pro00007868) on October 6, 2015. Additionally, the research conducted by the CAN*Help* Working Group, of which this thesis is a part, is approved by the Northwest Territories (Licence No. 15785) and Yukon (Licence No. 16-13S&E) research licensing authorities.

The prevalence of antibiotic-resistant *H. pylori* infection and the effectiveness of anti-*H. pylori* treatment regimens among CAN*Help* project participants referred to in chapter 2 were estimated from data previously collected by project staff in CAN*Help* project communities. The literature review presented in chapter 1, the data analysis presented in chapter 2 and the qualitative analysis presented in chapter 3 are my original works. I developed the chart review tool used to collect antibiotic exposure histories in chapter 2 and the semi-structured questionnaire used to conduct interviews in chapter 3. I collected the antibiotic exposure history data from northern community health centre medical charts using the chart review tool, with the help of a research assistant, and I collected the semi-structured questionnaire data by interviewing research participants.

No part of this thesis has been previously published.

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Chapter 1. Introduction

Helicobacter pylori is a bacterium that colonizes the lining of the stomach and/or duodenum.[1] Chronic *H. pylori* infection generally results in chronic inflammation of the stomach lining, known as gastritis, which is a risk factor for peptic ulcer disease and gastric cancer. *H. pylori*-associated gastritis is often asymptomatic; symptomatic *H. pylori*-associated disease most commonly occurs in adults following long-term infection.[2–4] *H. pylori* was identified in 1982 by Barry Marshall and Robin Warren following isolation of the bacterium from gastric biopsy samples.[2,3] For their discovery, Marshall and Warren were awarded the 2005 Nobel prize for physiology and medicine. *H. pylori* was classified as a class I carcinogen in 1994 by the World Health Organization following evaluation of evidence in the literature supporting the carcinogenicity to humans of *H. pylori* organisms and the association between *H. pylori* infection and gastric malignancies.[4] It is not known how frequently *H. pylori* spreads through various potential pathways, but initial infection is thought to occur most frequently during childhood, and evidence suggests it is most often transmitted directly from one person to another through contact with digestive fluids.[5]

Prevalence of H. pylori *Infection*

Around 1995-2000, *H. pylori* was estimated to infect approximately half of the world's population.[6–8] Brown *et al.* (2000) estimated the prevalence of *H. pylori* infection to be 70% in developing regions of the world and 40% in developed regions.[9] In recent years, a decline in *H. pylori* prevalence has been observed in developed regions while the prevalence remains high in both developing regions and marginalized groups within developed regions.[10–12] *H. pylori* prevalence has been observed to vary according to ethnicity, socioeconomic status, geographic region and age.[9,13]

The prevalence of *H. pylori* infection has been estimated in major urban centres across Canada. Estimates published during 1994-2007 for the prevalence of *H. pylori* infection in southern Canadian adults range from 23-38%.[14–17] Similar estimates have been reported in a review published in 2014 conducted by Sierra *et al*. for the prevalence of *H. pylori* infection in western countries.[5] In contrast to southern Canada, previous

estimates for the prevalence of *H. pylori* infection are relatively high for northern Canadian Aboriginal communities and demonstrate a disproportionate burden of *H. pylori* infection in these populations. Estimates for the prevalence of *H.* pylori infection in northern Canadian Aboriginal populations published during 1999 & 2002 range from 51%-95%.[18–20]

Anti-H. pylori *Treatment Regimens*

Successful elimination of *H. pylori* infection has been observed to improve the prognosis of *H. pylori*-associated disease outcomes; the successful elimination of *H. pylori* infection has been observed to improve peptic ulcer healing, decrease peptic ulcer recurrence and reduce the risk of gastric cancer.[21,22] Additionally, other *H. pylori*-associated diseases such as atrophic gastritis and intestinal metaplasia have been observed to improve following successful elimination of *H. pylori* infection.[23,24]

Clinical guidelines recommend treatment to eliminate *H. pylori* infection for individuals who are known to be *H. pylori*-positive,[25–27] and in particular, *H. pylori*-positive individuals with duodenal ulcers and/or gastric ulcers.[25,26] Treatment to eliminate *H. pylori* infection requires multi-drug regimens composed of a PPI with two or more antibiotics for a duration of 7-14 days. PPIs prevent acid production in the stomach and are used in anti-*H. pylori* treatment regimens to improve the stability of antibiotics inhibited by low pH.[28] A limited number of antibiotics are effective at eliminating *H. pylori* infection *in vivo* due to factors including: the antibiotic concentration achievable at the gastric mucus layer;[29] the instability and degradation of antibiotics at the low pH found in the stomach;[30] and the slow growth rate of *H. pylori* which inhibits the efficacy of antibiotics dependent on bacterial growth.[31] Antibiotics primarily used for the initial treatment of *H. pylori* infection include clarithromycin, metronidazole and amoxicillin. In the presence of antibiotic-resistant *H. pylori* infection or following the failure of an anti-*H. pylori* treatment regimen, commonly used alternative antibiotics include tetracycline, ciprofloxacin, levofloxacin, nitrofurantoin and rifampicin. Regardless of an antibiotic's *in vitro* antimicrobial activity against *H. pylori* organisms, there are no antibiotics that are effective at eliminating *H. pylori* infection when prescribed as a monotherapy.[32] Additionally, another drug commonly used in anti-*H. pylori* treatment regimens is

bismuth subsalicylate. When included in anti-*H. pylori* treatment regimens, bismuth subsalicylate has been observed to improve treatment effectiveness.[33,34] The effectiveness of anti-*H. pylori* treatment regimens is defined as the proportion of treated individuals that eliminate the infection.[35]

Standard triple therapy has been the most commonly recommended anti-*H. pylori* treatment regimen for the initial treatment of *H. pylori* among clinical guidelines worldwide.[25,26,36–38] Standard triple therapy combines twice daily oral doses of a PPI with clarithromycin and amoxicillin or metronidazole for 10-14 days. Other regimens used to eliminate *H. pylori* infection include: bismuth-based quadruple therapy; non-bismuth quadruple therapy; sequential therapy; and hybrid therapy. Bismuth-based quadruple therapy combines oral doses of a PPI twice daily, tetracycline four times daily, metronidazole four times daily, and bismuth subsalicylate (two tablets) four times daily for 10-14 days. In regions where bismuth is not available, non-bismuth quadruple therapy is substituted; it combines twice daily oral doses of a PPI, clarithromycin, amoxicillin, and metronidazole. However, the effectiveness of either quadruple therapy is greatly influenced by adherence to the regimen due to the complexity of these regimens and the associated occurrence of adverse events.[39] Sequential therapy combines twice daily oral doses of a PPI and amoxicillin for days 1 to 5 of a 10-day regimen and twice daily oral doses of a PPI, metronidazole and clarithromycin for days 6 to 10. Hybrid therapy combines components of sequential and non-bismuth quadruple therapies; it combines twice daily oral doses of a PPI with amoxicillin for days 1-7 of a 14-day regimen and twice daily oral doses of a PPI, amoxicillin, metronidazole and clarithromycin for days 8-14.[40]

Effectiveness of Anti-H. pylori *Treatment Regimens*

Clinical guidelines for the treatment of *H. pylori* infection in various geographic regions recommend as an acceptable standard that anti-*H. pylori* treatment regimens should be successful in ≥80% of individuals treated.[27] The guidelines recommend the use of alternative therapies when the standard initial therapy no longer successfully eliminates *H. pylori* infection in ≥80% of individuals treated. Meta-analysis published from 2003 to 2015 on the effectiveness of commonly prescribed anti-*H. pylori* treatment

regimens, such as standard triple therapy, bismuth-based quadruple therapy, non-bismuth quadruple therapy, sequential therapy, hybrid therapy, and levofloxacin-based triple therapies, present evidence that the effectiveness of these regimens was generally >80%.[41–48] However, studies conducted in various geographic regions have presented evidence that the effectiveness of standard triple therapy is <80% in many settings.[49–51] The effectiveness of anti-*H. pylori* treatment regimens is influenced by several factors, including antibiotic resistance. Variation in the prevalence of antibiotic-resistant *H. pylori* infection appears to be related to the variation in the effectiveness of standard triple therapy across geographic regions.[52] Evidence shows, in particular, that when *H. pylori* organisms are resistant to the antimicrobial activity of antibiotics commonly used in anti-*H. pylori* treatment regimens, the effectiveness of some anti-*H. pylori* treatment regimens is reduced.[53]

*Canadian Guidelines for Anti-*H. pylori *Treatment Regimens*

In 1998, Canadian clinical guidelines recommended standard triple therapy for the initial treatment of *H. pylori* infection.[37] A 2004 update to the Canadian clinical guidelines added bismuth-based quadruple therapy as a recommended regimen for the initial treatment of *H. pylori* infection.[25,27] However, following review of the current literature regarding the effectiveness of standard triple therapy to eliminate *H. pylori* infection in Canada, 2016 Canadian clinical guidelines no longer recommend standard triple therapy, but now recommend instead the use of either non-bismuth or bismuth-based quadruple therapy for the initial treatment of *H. pylori* infection.[54] Additionally, the 2016 Canadian clinical guidelines now recommend extending treatment for all prescribed anti-*H. pylori* treatment regimens from 10 days to 14 days.[54] If initial therapy fails to eliminate the infection, bismuth-based quadruple therapy is recommended for the second anti-*H. pylori* treatment attempt if not initially prescribed, as long as the individual had no prior exposure to metronidazole.[54] Following two failed anti-*H. pylori* treatment attempts, or following failure of initial therapy to eliminate the infection among individuals with previous metronidazole exposure, a triple therapy combining a PPI, levofloxacin and amoxicillin is recommended.[54] If a third treatment attempt fails to

eliminate *H. pylori* infection, a triple therapy combining a PPI, rifabutin and amoxicillin is recommended.[54]

*Impact of Antibiotic Resistance on the Effectiveness of Anti-***H. pylori** *Treatment Regimens*

The impact of antibiotic-resistant *H. pylori* infection on the effectiveness of anti-*H. pylori* treatment regimens varies according to factors including: the antibiotic combination prescribed; the antibiotic susceptibility of *H. pylori* to the antibiotic combination prescribed; the dose of antibiotics prescribed; and the duration of antibiotic treatment. In particular, the effectiveness of anti-*H. pylori* treatment regimens that include clarithromycin are greatly reduced when *H. pylori* organisms are resistant to clarithromycin. Even though clarithromycin has the greatest *in vitro* bactericidal effect against *H. pylori* organisms compared to other antibiotics with antimicrobial activity against *H. pylori* organisms,[55] estimates of the effectiveness of standard triple therapy in individuals with clarithromycin-resistant *H. pylori* infection range from 18 to 44%.[53,56,57]

The prevalence of *H. pylori* resistance to specific antibiotics is highest for metronidazole across geographic regions. However, resistance of *H. pylori* organisms to metronidazole does not reduce the effectiveness of anti-*H. pylori* treatment regimens as much as clarithromycin resistance does.[6,52,58,59] In addition to clarithromycin and metronidazole, resistance to other antibiotics used in anti-*H. pylori* treatment regimens, including tetracycline, amoxicillin, levofloxacin, cirprofloxacin, nitrofurantoin and rifampicin, also contribute to the reduced effectiveness of anti-*H. pylori* treatment regimens, although less frequently.[60–63]

Antibiotic Resistance

Antibiotic resistance refers to reduced susceptibility of a microorganism to an antibiotic drug that was previously effective at eliminating infection caused by the microorganism.[64] The rate at which antibiotic resistance develops in bacterial species is determined by factors including the bacterial chromosomal mutation rate and the size of the bacterial population.[65,66] The spread and stability of antibiotic resistance within bacterial populations is determined by the presence of selective pressures and the effect

of a mutation event on bacterial fitness.[67] Antibiotic-resistant *H. pylori* infection generally develops from small existing populations of resistant organisms that are selected for by pressures such as antibiotic exposure.[68]

The number of *H. pylori* organisms present in the stomach of an infected individual may be large and a small population of resistant organisms can generate through spontaneous mutation.[49,68] Spontaneous mutations can make bacteria resistant to a wide range of antibiotics *in vitro,* with the incidence of resistant mutations varying according to the bacterial species and the antibiotic of interest.[69] The average spontaneous mutation rate of *H. pylori* isolates has been estimated to range between 10^{-5} and 10^{-7} mutations/bacterium[67] which is several orders of magnitude higher than the average mutation rate of *Escherichia coli* but comparable to the mutation rate of *E. coli* strains that express a mutator phenotype.[70] Research investigating the stability of antibiotic-resistant *H. pylori* infection *in vitro*, based on observing the maintenance of a resistant phenotype during cellular growth and division, has produced inconsistent results. However, it remains unclear how the results of *in vitro* studies examining the stability of antibiotic-resistant *H. pylori* infection compares to *in vivo* infection.

Antibiotic Susceptibility Testing of H. pylori

Antibiotic resistance in bacteria can be defined by either phenotypic or genotypic characteristics. The phenotypic characteristics of a bacterium include the physical expression of a particular trait whereas the genotypic characteristics of a bacterium include the genes that encode a particular trait. Phenotypic bacterial resistance is commonly defined by the minimum inhibitory concentration (MIC) of an antibiotic: the concentration of an antibiotic that inhibits bacterial growth.[71] Phenotypic bacterial resistance is generally identified in a laboratory setting by obtaining an antibiotic MIC value *in vitro* for each *H. pylori* organism isolated by culture of gastric biopsy tissue. The antibiotic MIC value for each *H. pylori* isolate is compared to a standard value that represents the limit of bacterial susceptibility for a given antibiotic. Alternatively, genotypic bacterial resistance is defined by the expression of antibiotic resistance genes within bacterial organisms.[71] Genotypic bacterial resistance is generally assessed with the use of molecular methods in a laboratory setting.

Risk Factors for Antibiotic-resistant H. pylori *Infection*

Exposure to antibiotics at the population level is believed to be a major factor associated with the prevalence of antibiotic-resistant *H. pylori* infection.[53,72,73] Epidemiological studies have generated evidence of an association between the widespread use of antibiotics in defined populations and the prevalence of antibiotic-resistant *H. pylori* infection in those populations.[73–75] Differences in the observed prevalence of antibiotic-resistant *H. pylori* infection by factors such as age, sex and geographic region may also occur due to varying frequencies of antibiotic use for the treatment of unrelated bacterial infections at the population level.[76–78] Additionally, exposure to unsuccessful anti-*H. pylori* treatment regimens is reported to be a major risk factor for antibiotic-resistant *H. pylori* infection.[79–81]

Some evidence suggests that *H. pylori* genes hypothesized to confer virulence are associated with the susceptibility of *H. pylori* infection to antibiotics.[82] Virulence genes encode bacterial products that enable bacteria to cause more severe disease.[82] Two identified *H. pylori* virulence genes include the cytotoxin-associated gene A (*cagA*) and the vacuolating-associated gene A (*vacA*), which encode products that enable the bacteria to persistently infect an individual in the acidic environment of the stomach and inflict injury on the stomach lining.[83] Studies have presented evidence that virulent *H. pylori* genotypes have a lower frequency of antibiotic resistance than less virulent strain types,[55,84] and are also associated with a lower incidence of anti-*H. pylori* treatment failure.[85–88]

*Additional Risk Factors for Anti-*H. pylori *Treatment Failure*

Aside from antibiotic resistance, other factors that are reported to be associated with anti-*H. pylori* treatment failure include adherence to prescribed treatment regimens and treatment-related factors.

Adherence

Poor adherence to anti-*H. pylori* treatment regimens is widely regarded as a major risk factor for failure of the regimen to eliminate *H. pylori* infection.[89] Low adherence to

an antibiotic treatment regimen results in decreased antibiotic concentration at the site of infection. Graham *et al*. (1992) conducted a regression analysis investigating factors associated with the effectiveness of bismuth-based triple therapy and reported that treatment effectiveness was 96% among individuals who completed 60% or more of the prescribed therapy and 69% among individuals who completed less than 60% of the prescribed therapy.[90] Adverse effects occur in approximately 5-20% of individuals prescribed an anti-*H. pylori* treatment regimen, and contribute to reduced treatment adherence.[91] Adverse effects of anti-*H. pylori* treatment regimens commonly include: nausea; vomiting; diarrhea; and a metallic taste in the mouth. Education regarding the importance of completing all prescribed medication is important for the effectiveness of anti-*H. pylori* treatment regimens.[92] In large multi-centre studies investigating the effectiveness of anti-*H. pylori* treatment regimens, patients are generally educated regarding treatment dosing schedules, adherence and potential side effects. A small randomized controlled trial conducted by Al-Eidan *et al.* (2002) investigating the effect of counseling and follow-up evaluation on the effectiveness of anti-*H. pylori* treatment regimens reported evidence of more frequent infection elimination among individuals receiving counseling and follow-up (intervention group) compared to those who only received a standard treatment advice sheet (control group).[93] Both the intervention and control groups were prescribed the same anti-*H. pylori* treatment regimen: PPI, amoxicillin and clarithromycin for 1 week.[93] While both intervention groups reported adverse events during treatment (19/38 individuals in the intervention group and 17/38 individuals in the control group) both treatment effectiveness and adherence was higher among individuals in the intervention group. Treatment effectiveness was 95% in the intervention group and 74% in the control group (95% CI for the difference between intervention and control estimates: 5%, 36%).[93] The proportion with perfect adherence to the prescribed regimen was 92% in the intervention group and 24% in the control group (95% CI of difference between intervention and control estimates: 52%, 85%).[93]

Treatment Related Factors

Specific details of anti-*H. pylori* treatment regimens also influence treatment effectiveness; these details include: the specific antibiotics prescribed; the dosage of the

antibiotics; and the duration of therapy. In particular, treatment effectiveness of nitroimidazole-containing *H. pylori* treatment regimens against metronidazole-resistant *H. pylori* infection is reported to be influenced by the components of the treatment regimen and the duration of therapy. In a meta-analysis conducted by van der Wouden *et al*. (1999), triple therapies consisting of a PPI, clarithromycin and metronidazole were more effective at eliminating metronidazole-resistant *H. pylori* infection than triple therapies containing a PPI, amoxicillin and metronidazole.[59] Similarly, bismuth-based triple therapies that contained tetracycline were more effective at eliminating metronidazole-resistant *H. pylori* infection than bismuth-based triple therapies with amoxicillin.[59] Bismuth-based quadruple therapies were reported to be more effective than bismuth-based triple therapies at eliminating metronidazole-resistant *H. pylori* infection.[59] Finally, increasing the duration of nitroimidazole-containing anti-*H. pylori* treatment regimens has been observed to increase treatment effectiveness against metronidazole-resistant *H. pylori* infection.[59] However, unlike metronidazole-resistant *H. pylori* infection, increasing the duration of treatment has not been observed to improve the effectiveness of anti-*H. pylori* treatment regimens against *H. pylori* infection resistant to the antimicrobial activity of macrolides, fluoroquinolones or rifamycins.[92]

The Canadian North Helicobacter pylori (*CAN*Help) *Working Group*

The Canadian North *Helicobacter pylori* (CAN*Help*) Working Group came together in 2006 in response to concerns expressed by community leaders in northern Canadian Aboriginal communities regarding health risks associated with *H. pylori* infection. The CAN*Help* Working Group conducts community-driven research in northern Canada and links northern Canadian communities, health care providers, regional health authorities and University of Alberta researchers from a variety of disciplines to investigate *H. pylori*-associated health outcomes and effective treatment in order to improve clinical management of this infection and inform public health policy pertaining to its control.

The first CAN*Help* community project, the Aklavik *H. pylori* Project, was launched in 2007 in Aklavik, NT. Subsequent projects included the Old Crow *H. pylori* Project established in 2010 in Old Crow, YT, the Inuvialuit Settlement Region (ISR) *H.*

pylori Project established in 2011 in Tuktoyaktuk, NT, and the Fort McPherson *H. pylori* Project established in 2012 in Fort McPherson, NT. According to the 2006 Statistics Canada census, all four CAN*Help* projects are based in small communities that primarily identify as Aboriginal: the population of Aklavik was 594 with approximately 92% of individuals identifying as either Inuvialuit or Gwich'in, First Nations; the population of Old Crow was 253 with approximately 85% identifying as Vuntut Gwitchin; the population of Tuktoyaktuk, NT was 870 with approximately 84% identifying as either Inuvialuit or First Nations; and the population of Fort McPherson, NT was 776 with approximately 92% identifying as either Inuvialuit, Métis or First Nations.[94]

Across CAN*Help* Working Group community projects in Aklavik, Old Crow, Tuktoyaktuk, and Fort McPherson the prevalence of *H. pylori* infection has been estimated to range from 57% to 68%: the prevalence of *H. pylori* infection among tested CAN*Help* project participants is 58% (193/332) in Aklavik, 68% (128/189) in Old Crow, 57% (59/103) in Tuktoyaktuk, and 59% (124/209) in Fort McPherson. Microbiologic investigation of *H. pylori* cultured from gastric biopsy samples have produced estimates of the frequencies of antibiotic-resistant *H. pylori* infection in CAN*Help* Working Group community projects. Due to a very small sample size, estimates of the prevalence of antibiotic-resistant *H. pylori* infection are not reported for Tuktoyaktuk, NT. The estimated prevalence of clarithromycin resistance among *H. pylori*-positive project participants is 8% (10/120) in Aklavik, 25% (13/25) in Old Crow, and 29% (8/28) in Ft. McPherson. The estimated prevalence of metronidazole resistance among *H. pylori*-positive project participants is 28% (34/120) in Aklavik, 42% (22/53) in Old Crow, and 46% (13/28) in Ft. McPherson.

As a component of each community project, the CAN*Help* Working Group conducted community treatment trials to investigate the effectiveness of standard triple therapy, sequential therapy and quadruple therapy among project participants. Participants were randomly assigned to one of two treatment regimens when enrolled in a community treatment trial. The Aklavik *H. pylori* Project treatment trial observed a failure frequency of 41% (20/49) for standard triple therapy and 30% (12/40) for sequential therapy.[23] Given the high failure frequency of standard therapy in the Aklavik

H. pylori Project treatment trial, subsequent CAN*Help* treatment trials did not use this regimen. The observed failure frequency of sequential therapy was 40% (8/20) in the Old Crow treatment trial and 17% (5/29) in the Fort McPherson treatment trial. The observed failure frequency of quadruple therapy was 9% (2/23) in the Old Crow treatment trial and 0% (0/17) in the Fort McPherson treatment trial.

Significance of Research

Given the limited number of antibiotics available to eliminate bacterial infections, antibiotic-resistant bacteria are a threat to health considering the impact of resistance on the effectiveness of therapeutic options. In particular, the reduced effectiveness of anti-*H. pylori* treatment regimens is an important public health issue, considering there is no effective vaccine or other public health measures for preventing *H. pylori* infection. Therefore, a better understanding of factors associated with the occurrence of antibiotic-resistant *H. pylori* infection and anti-*H. pylori* treatment failure may contribute to strategies aimed at improving treatment effectiveness and reducing health risks from chronic *H. pylori* infection.

Research Aims

The specific aims of this MSc thesis are to:

1) conduct a systematic review of the literature on: A) antibiotic resistance mechanisms in *H. pylori* organisms; and B) the prevalence of antibiotic-resistant *H. pylori* infection worldwide;
2) estimate the effect among CAN*Help* project participants of exposure to antibiotics on two health outcome: A) the prevalence of antibiotic-resistant *H. pylori* infection; and B) the incidence of anti-*H. pylori* treatment failure; and
3) describe participants' perspectives on the value and potential harms of using antibiotics to treat infections and factors that influence adherence to prescribed antibiotic regimens through qualitative analysis of semi-structured interviews.

Thesis Structure

The remainder of this thesis contains three papers and a conclusion. The paper titled "Review of antibiotic resistance mechanisms in *H. pylori* organisms and prevalence of antibiotic-resistant *H. pylori* infection worldwide during 2010 to 2014" synthesizes current evidence on antibiotic resistance mechanisms of *H. pylori* and the prevalence of antibiotic-resistant *H. pylori* infection worldwide. The paper entitled "Estimating the effect of antibiotic exposure on antibiotic-resistant *H. pylori* infection and anti-*H. pylori* treatment failure among participants in CAN*Help* community projects in northern Canada" presents the results of statistical analysis aimed at estimating the effect of antibiotic exposure on the prevalence of antibiotic-resistant *H. pylori* infection and the incidence of anti-*H. pylori* treatment failure. The paper entitled "Describing participant's perspectives regarding antibiotic use and factors that influence regimen adherence among participants in the Aklavik *H. pylori* Project: a qualitative analysis" presents the results of qualitative investigation of CAN*Help* project participants' perspectives on the value and potential harms of using antibiotics to treat infection and factors influencing adherence to prescribed antibiotic regimens. The conclusion distills the lessons learned from this thesis research.

Chapter 2. Review of antibiotic resistance mechanisms in H. pylori organisms and prevalence of antibiotic-resistant H. pylori infection worldwide during 2010 to 2014

Introduction

Helicobacter pylori are bacteria that infect the lining of the stomach and/or duodenum.[1] *H. pylori* infection often persists long term, nearly always accompanied by chronic inflammation of the stomach lining, known as gastritis, which is a risk factor for peptic ulcer disease and gastric cancer.[95–98] *H. pylori*–associated gastritis is often asymptomatic, but it may occur with non-specific symptoms of dyspepsia. Peptic ulcer disease and, more rarely, gastric cancer are believed to result from long-term infection with the bacterium.[2–4] In 2000, Brown *et al.* estimated the prevalence of *H. pylori* infection to be around 40% in developed regions of the world and 70% in developing regions, with prevalence varying according to geographic region, ethnicity, socioeconomic status and age.[9] Within recent years, however, the prevalence of *H. pylori* infection has been observed to decrease overall in developed regions while remaining high among marginalized groups.[10–12]

Treatment to eliminate *H. pylori* infection requires a multi-drug regimen that generally combines a proton-pump inhibitor with two to three antibiotics for a duration of 7-14 days. The anti-*H. pylori* treatment regimen used most commonly worldwide is frequently referred to as standard triple therapy, which combines twice daily oral doses of a PPI with clarithromycin and amoxicillin or metronidazole for 10 to 14 days.[26,27,49] Other commonly prescribed anti-*H. pylori* treatment regimens, used especially when standard treatment fails to eliminate the infection, are referred to in the literature as bismuth-based quadruple therapy, concomitant therapy, sequential therapy and hybrid therapy.[49,99–101] These anti-*H. pylori* treatment regimens use various multi-drug combinations with generally more complex dosing schedules to eliminate the infection. Bismuth-based quadruple therapy, used commonly in areas where the infection is harder to cure, consists of a PPI with tetracycline (or amoxicillin), metronidazole and bismuth subsalicylate for 10 to 14 days.[49] In comparison to standard triple therapy, bismuth-based quadruple

therapy has a more complicated dosing schedule and is associated with a higher incidence of side effects.[101] A large number of clinical trials have estimated the effectiveness of various anti-*H. pylori* treatment regimens, with effectiveness defined as the proportion of individuals prescribed treatment to eliminate *H. pylori* infection who are shown to be *H. pylori* negative after treatment. In a meta-analysis conducted by Rodgers and van Zanten (2007) of Canadian clinical trials that estimated the effectiveness of standard triple therapy and bismuth-based quadruple therapy in adults with or without previous exposure to an anti-*H. pylori* treatment regimen, the summary estimates of effectiveness of standard triple therapy consisting of a PPI, clarithromycin and amoxicillin and standard triple therapy consisting of a PPI, clarithromycin and metronidazole were 84% (95% confidence interval [CI]: 79%, 90%) and 82% (95% CI: 76%, 88%), respectively, while the summary estimate of effectiveness of bismuth-based quadruple therapy was 87% (95% CI: 80%, 95%).[41] Other meta-analyses of randomized trials that estimated the effectiveness of standard triple therapy and bismuth-based quadruple therapy have reported similar results.[42,43]

Evidence from the literature shows, however, that the effectiveness of anti-*H. pylori* treatment regimens varies across geographic regions. In particular, the effectiveness of standard triple therapy to eliminate *H. pylori* infection has been low in various regions worldwide over recent years and the use of alternative anti-*H. pylori* treatment regimens has increased.[6] The reduced effectiveness of anti-*H. pylori* treatment regimens in various regions is likely influenced by the local prevalence of antibiotic-resistant *H. pylori* infection.[52] Consensus guidelines, including the 2012 Maastricht IV/Florence Consensus Report have recommended the use of quadruple therapy for the initial treatment of *H. pylori* when the prevalence of clarithromycin-resistant *H. pylori* infection is >15-20% and the effectiveness of standard triple therapy does not meet the widely accepted standard of >80%.[26,27] Additionally, the 2016 Canadian Consensus guidelines recommend the use of bismuth-based quadruple therapy or non-bismuth quadruple therapy for the initial treatment of *H. pylori* infection, regardless of the prevalence of clarithromycin-resistant *H. pylori* infection in a given region, due to the low observed effectiveness of standard triple therapy to eliminate infection in many areas.[54]

Chapter 2. Review of antibiotic resistance mechanisms in *H. pylori* organisms and prevalence of antibiotic-resistant *H. pylori* infection worldwide during 2010 to 2014

Antibiotic resistance is defined as a reduced susceptibility of a bacterial organism to an antibiotic.[68] In *H. pylori*, antibiotic resistance generally develops from spontaneous mutation.[68] The frequency of spontaneous mutations in *H. pylori* organisms is estimated to range from 10^{-5} to 10^{-7} mutations/bacterium.[67] The spontaneous mutation rate of *H. pylori* is similar to the mutation rate of *Escherichia coli* isolates that express a mutator phenotype and several orders of magnitude greater than the mutation rate of *E. coli* that do not express a mutator phenotype.[70] The spread and stability of the resistance mutations in *H. pylori* organisms is believed to be determined by exposure to selective pressures, such as antibiotics, and the impact of the mutation on bacterial growth and survival.[67]

Across geographic regions worldwide, the prevalence of antibiotic-resistant *H. pylori* infection varies. In a systematic review conducted by De Francesco *et al.* (2010) of studies published from 2006 to 2009, the average prevalence of clarithromycin-resistant *H. pylori* infection among *H. pylori*-positive individuals with no history of previous treatment for *H. pylori* infection ranged from 11% to 29% across America, Asia, Africa and Europe.[102] De Francesco *et al.* also reported that the average prevalence of metronidazole-resistant *H. pylori* infection among *H. pylori*-positive individuals with no history of previous treatment for *H. pylori* infection ranged from 17% to 92% across America, Asia, Africa, and Europe.[102] The widespread use of antibiotics for the treatment of unrelated infections in the general population is believed to have increased the occurrence of antibiotic-resistant *H. pylori* infection globally.[53,72,73]

Additionally, evidence shows that the unsuccessful use of anti-*H. pylori* treatment regimens is a major risk factor for the development of antibiotic-resistant *H. pylori* infection.[79–81,103] Romano *et al.* (2008) reported the prevalence of clarithromycin-resistant *H. pylori* infection to be higher among individuals with previous unsuccessful anti-*H. pylori* treatment attempts compared to treatment naïve individuals: the prevalence of clarithromycin-resistant *H. pylori* infection was 18% and 46%, respectively, among 109 individuals with no previous exposure to an anti-*H. pylori* treatment regimen and 104 individuals who remained *H. pylori*-positive following one or more anti-*H. pylori* treatment attempts.[79] Heep *et al*. (2000) estimated the prevalence of clarithromycin-resistant *H. pylori* infection among individuals with one or more unsuccessful anti-*H.*

pylori treatment attempts to be high, ranging from 49% to 58%.[81] Similarly, Heep *et al.* estimated the prevalence of metronidazole-resistant *H. pylori* infection to range from 66% to 75% among individuals who had one or more unsuccessful anti-*H. pylori* treatment attempts.[81] Additionally, McMahon *et al.* (2003) reported previous antibiotic use as a risk factor for antibiotic-resistant *H. pylori* infection.[104] However, because the studies by Romano *et al.* and Heep *et al.* do not prospectively show evidence of the development of resistance following treatment, the reported association between previous anti-*H. pylori* treatment regimens and antibiotic-resistant *H. pylori* infection is ambiguous with respect to cause and effect: the presence of an antibiotic-resistant *H. pylori* isolate could result from the failure of the prescribed anti-*H. pylori* treatment to eliminate the infection.

Meta-analyses published from 1999 to 2007 have reported that the estimated effectiveness of anti-*H. pylori* treatment regimens is lower when prescribed for the treatment of antibiotic-resistant *H. pylori* infection.[52,58,59] In their 2000 meta-analysis, Dore *et al.* reported that the effectiveness of anti-*H. pylori* treatment regimens containing clarithromycin was lower by 55% (95% CI: 33%, 78%) when used to treat clarithromycin-resistant *H. pylori* infection compared to clarithromycin-susceptible *H. pylori* infection.[58] Similarly, a meta-analysis conducted by Fischbach and Evans (2007) estimated that the effectiveness of standard triple therapy (PPI, clarithromycin, metronidazole) was lower by 35% (95% CI: 26%, 47%) for eliminating clarithromycin-resistant compared to clarithromycin-susceptible *H. pylori* infection.[52] Dore *et al.* reported that resistance to metronidazole was associated with reduced effectiveness of various anti-*H. pylori* treatment regimens containing metronidazole by an average of 38% (95% CI: 30, 46%) when compared to metronidazole-susceptible *H. pylori*.[58] Additionally, Fischbach and Evans estimated that the effectiveness of standard triple therapy (PPI, clarithromycin, and metronidazole) was lower by 18% (95% CI: 13, 23%) when prescribed for the treatment of metronidazole-resistant compared to metronidazole-susceptible *H. pylori* infection.[52] Fischbach and Evans also reported, however, that the use of bismuth-based quadruple therapy was effective at eliminating clarithromycin- or metronidazole-resistant *H. pylori* infection (in >90% of individuals) but was not effective at eliminating *H. pylori* infection that is simultaneously resistant to both clarithromycin

and metronidazole (successful in <50% of individuals).[52] The reduced effectiveness of anti-*H. pylori* treatment regimens to eliminate antibiotic-resistant *H. pylori* infection is evidence for the use of alternative anti-*H. pylori* treatment regimens that consist of antibiotics to which the infection is susceptible.

The susceptibility of *H. pylori* isolates is assessed with laboratory techniques that can be divided into culture-based and nucleic acid-based assays which identify the phenotypic and genotypic characteristics, respectively, of a bacterial isolate. Phenotypic methods are primarily used for the detection of antibiotic-resistant *H. pylori* infection and include agar dilution, broth dilution, breakpoint susceptibility testing, disk diffusion, and the epsilometer test (Etest®).[105] Phenotypic methods commonly yield a quantitative result: minimum inhibitory concentration (MIC) expressed in μg/mL or mg/L, which represents the lowest concentration of an antibiotic that inhibits the growth of a bacterial organism.[105] The agar dilution method and the broth dilution method assess the antibiotic susceptibility of *H. pylori* grown on agar plates or in broth containing two-fold dilutions of an antibiotic, respectively.[106] The broth dilution method can be performed as a microbroth dilution which involves the addition of a small volume of bacterial suspension cultured in broth into the wells of a microplate (such as a 96 well plate) that contains two-fold concentration increments of an antibiotic.[107] However, due to the difficulty of growing *H. pylori* in broth, the broth dilution method is not commonly used.[105] The breakpoint susceptibility method is a simplified protocol of either the agar or broth dilution method; for the simplified protocol, *H. pylori* grown on agar plates or in broth are exposed to a predetermined concentration of an antibiotic and the susceptibility of the bacteria is classified by assessing whether bacterial growth is inhibited upon antibiotic exposure.[106] Alternatively, the disk diffusion method identifies the amount of bacterial growth inhibited around an antibiotic infused disk placed on an agar plate cultured with *H. pylori*.[108] The diameter of the inhibition zone is compared to pre-determined laboratory standards for each antimicrobial agent and the results are classified as susceptible, intermediate, or resistant.[108] The Etest® is a quantitative variant of the disk diffusion method; it classifies the antibiotic susceptibility of *H. pylori* organisms by assessing the zone of bacterial growth inhibited around a test strip infused with an antibiotic concentration gradient placed on an agar plate cultured with *H. pylori*.[106]

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Published results of previous studies that compare the accuracy of distinct tests that detect antibiotic-resistant *H. pylori* infection are generally in agreement but have shown inconsistent results for the detection of metronidazole resistance.[109–120] The agar dilution method is the standard method approved by the Clinical and Laboratory Standards Institute (CLSI) for classifying the susceptibility of *H. pylori* but is more expensive and difficult to perform than the disk diffusion method and Etest®.[121] Antibiotic susceptibility results determined by Etest® correlate well with the agar dilution method for all antibiotics except metronidazole.[122] Previous studies have observed that some *H. pylori* isolates are classified by the Etest® as metronidazole-resistant and are classified by the agar dilution method as metronidazole-susceptible.[123] Discordant results across susceptibility methods may occur because the Etest® can classify metronidazole-resistant *H. pylori* infection by an intermediate MIC value and may therefore detect a higher prevalence of resistance due to detection of MIC values lower than what is detectable by the agar dilution method.[123] In general, phenotypic methods for the detection of antibiotic-resistant *H. pylori* infection are time consuming: culture-based susceptibility tests may take up to two weeks to complete due to the time required to prepare *H. pylori* cultures from gastric biopsy samples.[106]

Alternatively, genotypic methods for *H. pylori* susceptibility testing include: real-time polymerase chain reaction (PCR); PCR restriction fragment length polymorphism (PCR-RFLP); allele specific PCR (ASP-PCR); fluorescent *in situ* hybridization; and GenoType® HelicoDR analysis.[53,124–128] Real-time PCR is a molecular technique that quantitatively detects the presence of genetic mutations within DNA sequences amplified by PCR methods that are associated with antibiotic resistance.[124] Alternatively, PCR-RFLP amplifies DNA sequences of interest with PCR methods and uses enzymes to cut the DNA at specific nucleotides sequences.[129] The DNA fragments produced following enzymatic cleavage are then investigated with gel electrophoresis, a technique that separates the DNA fragments by size within a gel medium through the application of an electric charge.[129] Nucleotide changes, including mutations associated with antibiotic resistance, will prevent enzymatic cleavage at the targeted nucleotide sequences and generate DNA fragments of differing lengths.[129,130] Similarly, ASP-PCR is used to amplify a specific genetic allele (a variant form of a gene) to identify single nucleotide

polymorphisms in DNA samples.[131–133] Fluorescent *in situ* hybridization identifies antibiotic-resistant *H. pylori* isolates with fluorescently labelled oligonucleotide probes (small molecules composed of short nucleotide sequences that bind to complementary DNA sequences) designed to bind to *H. pylori* DNA sequences known to contain mutations associated with antimicrobial resistance.[134] GenoType® HelicoDR is a method that uses PCR methods to amplify DNA segments and hybridization (the use of probes that bind to specific DNA sequences) to identify the presence of specific genetic mutations associated with antibiotic resistance.[128] Compared to phenotypic susceptibility methods, genotypic methods are relatively faster to conduct and do not require live bacteria.[106,135] However, genotypic methods are expensive and require specific expertise, equipment and prior knowledge of chromosomal mutations associated with antibiotic-resistant *H. pylori* infection. Additionally, genotypic methods are an ineffective method for identifying antibiotic-resistant *H. pylori* strains resulting from novel gene sequences or mutations.[106,135] Continuing research on molecular mechanisms that confer antibiotic-resistance in *H. pylori* isolates permits the development of improved genetic susceptibility techniques.

Currently in Canada, as in most countries, antibiotic susceptibility testing is not routinely completed prior to the initial prescription of an anti-*H. pylori* treatment regimen. Due to the time, expense and expertise required to conduct susceptibility testing, it is usually not completed before one or more failed anti-*H. pylori* treatment attempts. Therefore, prescription practices pertaining to anti-*H. pylori* treatment regimens are generally guided by estimates of the local prevalence of antibiotic-resistant *H. pylori* infection, when known, or an individual's antibiotic exposure history.[49] The Maastricht IV/Florence Concensus Report recommends avoiding the use of clarithromycin and metronidazole when the prevalence of clarithromycin-resistant and metronidazole-resistant *H. pylori* infection exceed 15-20% and >40%, respectively, in a given region.[26] This recommendation assumes that knowledge of the prevalence of antibiotic-resistant *H. pylori* infection can be used to guide local anti-*H. pylori* prescription patterns in a manner that reduces the frequency of anti-*H. pylori* treatment failure and prevents increases in the frequency of resistant *H. pylori* infection.

Chapter 2. Review of antibiotic resistance mechanisms in *H. pylori* organisms and prevalence of antibiotic-resistant *H. pylori* infection worldwide during 2010 to 2014

The aim of this literature review is to systematically review the literature and synthesize current evidence regarding: 1) antibiotic resistance mechanisms in *H. pylori*; and 2) estimates of the prevalence of antibiotic-resistant *H. pylori* infection worldwide. Information regarding antibiotic resistance mechanisms in *H. pylori* and the prevalence of antibiotic-resistant *H. pylori* infection was synthesized for antibiotics commonly used in anti-*H. pylori* treatment regimens including: clarithromycin; metronidazole; amoxicillin; tetracycline; levofloxacin; ciprofloxacin; rifampicin; and nitrofurantoin. Estimates for the prevalence of antibiotic-resistant *H. pylori* infection are presented for individuals with: 1) no history of treatment for *H. pylori* infection; 2) history of previous treatment for *H. pylori* infection; and 3) either mixed or unidentified anti-*H. pylori* treatment histories.

Methods

I conducted a comprehensive, systematic review of the literature using a literature review search strategy developed with the guidance of a health sciences librarian. Two databases, EMBASE and Medline, were selected for inclusion in this literature search. EMBASE and Medline were accessed through the University of Alberta online library resources. The search terms used included '*exp Helicobacter pylori/*' combined with the AND operator with the following four search term strings connected with the OR operator: *exp Helicobacter pylori/ AND* ((**anti-bacterial agents/ or *amoxicillin/ or *clarithromycin/ or *fluoroquinolones/ or *tetracycline/ or *rifampin/*) OR (*exp *Drug Resistance, Bacterial/*) OR (**Anti-Infective Agents/ or exp *Metronidazole/ or exp *Ciprofloxacin/ or nitroflurantoin*.mp. or exp *Macrolides/ or exp *beta-Lactams/ or exp *Nitroimidazoles/ or exp *Rifamycins/ or exp *Polyketides/ or exp *Nitrofurans/*) OR ((*amoxicillin or clarithromycin or metronidazole or ciprofloxacin or nitrofurantoin or rifampicin or tetracycline or levofloxacin or macrolide* or beta lactam* or nitroimaidazole* or fluroquinolone* or rifamycin* or polyketide* or nitrofuran*).ti*)). The initial online library search was restricted to articles published in English. Search results were further limited to articles that: were published during January 2010 through December 2014; were not conference abstracts; and were accessible through the University of Alberta library resources. I reviewed the title and abstract of all articles identified by the online library search to select articles that presented estimates of the

frequency of antibiotic-resistant *H. pylori* infection, by searching for keywords such as 'antibiotic resistance', 'antibiotic susceptibility', 'resistance frequency', or mention of specific anti-*H. pylori* treatment regimens, bacterial susceptibility methods, or antibiotics commonly used in anti-*H. pylori* treatment regimens. To ensure that the search strategy identified all relevant articles, I compared articles selected by the online library search with the references included in relevant review articles and primary research articles. Relevant articles identified among published references lists not initially identified through the online library search were added to the final search results. Data I extracted from each article included: the geographic region of study; the size of the study population; the time period during which biopsy samples for susceptibility testing were collected from the study population; the mean age and age range of the study population; the detection methods used to classify the antibiotic susceptibility of *H. pylori* isolates; and the estimated prevalence of resistance to each antibiotic investigated. Additionally, during the review of article titles and abstracts identified by this search strategy, articles presenting evidence of antibiotic resistance mechanisms in *H. pylori* were selected for inclusion to provide perspective for synthesizing the current literature. The recommendations outlined by the Meta-analysis of Observational studies in Epidemiology (MOOSE) guidelines for reporting review methods were followed to the extent to which they applied to this qualitative systematic literature review.[136]

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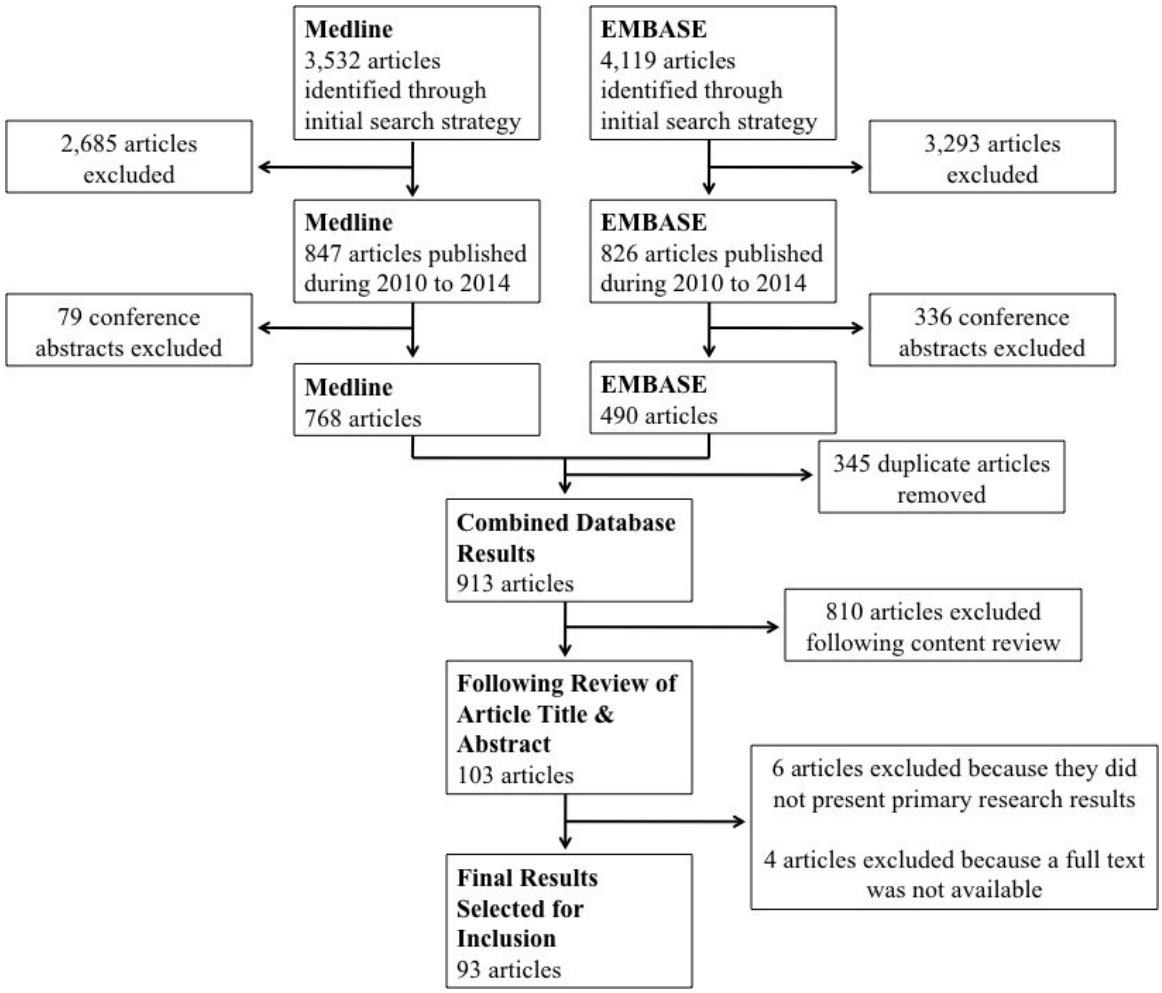
Figure 2.1. The total number of articles identified by the search strategy and justification for article exclusion.



Results

Mechanism of Action of Antibiotics

Antibiotics have various mechanisms of action against bacterial cells that include inhibition of cell wall synthesis, interference with nucleic acid synthesis (deoxyribonucleic acid [DNA] and ribonucleic acid [RNA]), inhibition of metabolic pathways such as the folate synthesis pathway, and inhibition of protein synthesis.[137,138] The location of antibiotic mechanisms of action within a bacterial cell are depicted in Figure 2.2. Mechanisms of action of antibiotics on bacterial cells are classified as either

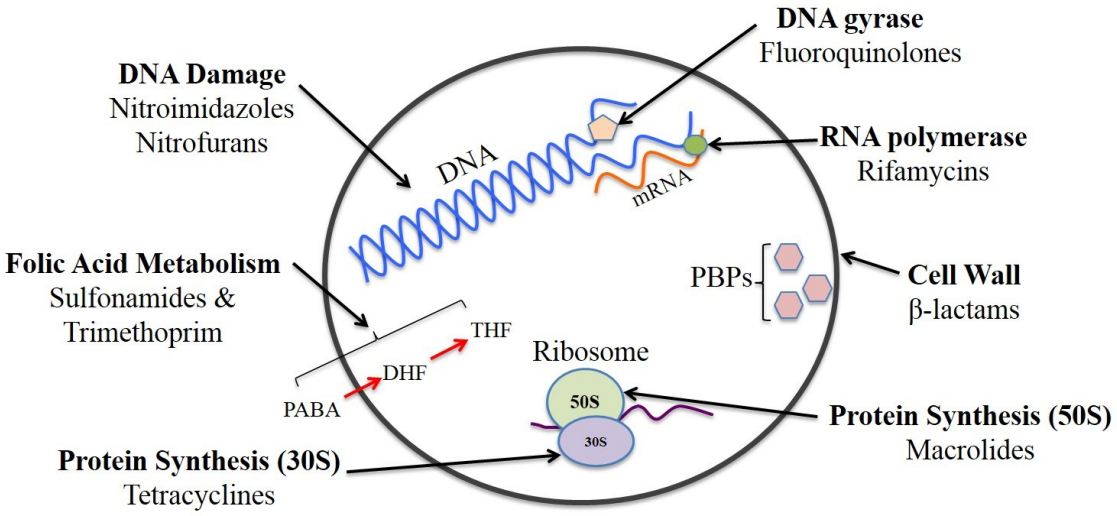
bactericidal (inducing bacterial cell death) or bacteriostatic (inhibiting bacterial cell growth).[64]

Bactericidal antibiotics include antibiotics that disrupt bacterial cell wall synthesis and bacterial membrane structure. Cell wall synthesis is essential for cellular growth, division, and maintenance of cell structure.[139] The cell wall is composed of peptidoglycan molecules: polysaccharide (sugar) chains joined by peptide bonds.[140] Within bacterial cells, penicillin binding proteins (PBPs) are essential for the synthesis of peptidoglycan molecules.[140] Antimicrobial agents that inhibit the enzymatic activity of PBPs disrupt bacterial cell wall synthesis and inhibit the structural integrity of the bacterial cell wall which results in cell lysis (the destruction of the cell wall) and cell death.[140] Another bactericidal antibiotic mechanism of action includes the interference of nucleic acid synthesis. DNA gyrase is an enzyme involved in cell growth and division that controls the folding and supercoiling of chromosomal DNA during DNA replication.[140] The antibiotic inhibition of DNA gyrase is lethal to bacterial cells because the inhibition of DNA gyrase commonly results in the generation of double-stranded DNA breaks during DNA replication.[141] Similarly, DNA-dependent RNA polymerase is an enzyme that catalyzes the synthesis of RNA from chromosomal DNA.[142] The inhibition of DNA-dependent RNA polymerase by an antibiotic inhibits essential cellular functions including protein synthesis and cellular growth.[142,143] Finally, the synthesis of DNA and RNA requires folate (folic acid): a vitamin synthesized within bacteria.[142] The inhibition of metabolic pathways, such as the folate synthesis pathway, by antimicrobial agents is therefore a bactericidal antibiotic mechanism of action because it inhibits the synthesis of DNA and RNA.[144]

A bacteriostatic antibiotic mechanism of action includes the inhibition of protein synthesis. Proteins are large molecules consisting of one or more amino acid chains that are synthesized by ribosomes within the bacterial cell.[142] Ribosomes are cellular organelles that are composed of two major subunits: the 30S subunit and 50S subunit.[140] The 30S ribosomal subunit translates messenger RNA (mRNA).[140] mRNA are genetic copies of the bacterium's chromosomal DNA that encodes for various cellular proteins.[140] Once the mRNA sequence is translated, the 30S ribosomal subunit signals to amino acid-

carrying transfer RNA (tRNA) to bind to the 30S and 50S subunits of the ribosome.[140] Once tRNA is bound to the ribosome, peptidyl transferase (located on the 50S subunit of the ribosome) catalyzes the formation of amino acid chains joined by peptide bonds according to the translated mRNA sequence.[142] Antibiotics that inhibit the function of the ribosome at the 30S or 50S subunit inhibit the synthesis of proteins required for bacterial cell growth.[142]

Figure 2.2. Location of the mechanism of action of antimicrobial agents by antibiotic class in a bacterial cell. DNA, deoxyribonucleic acid; RNA, ribonucleic acid; mRNA, messenger RNA; PBPs, penicillin-binding proteins; PABA, *p*-aminobenzoic acid; DHFA, dihydrofolic acid; THFA, tetrahydrofoic acid.



Adapted from Bbosa *et al.*, Lewis, and Brown.[145–147]

Antibiotic Resistance Mechanisms

Bacteria have evolved diverse mechanisms of antibiotic resistance that include: antibiotic target site modification; enzymatic destruction or modification of antibiotic compounds; decreased permeability of antibiotics across the bacterial cell wall; and increased efflux of antibiotics from bacterial cells.[138,148] Bacteria may be inherently resistant to an antibiotic or may acquire antibiotic resistance to one or more antibiotic compounds. Inherent bacterial resistance to an antibiotic may occur due to genetic alterations that confer resistance encoded in chromosomal DNA that are maintained through growth and division events.[140] Alternatively, antimicrobial resistance can be

acquired through genetic events that alter bacterial chromosomal DNA, such as point mutations (a chromosomal mutation that alters a single nucleotide in a gene sequence), gene amplification (the replication of a specific gene sequence), or genetic transfer (the transfer of genes between bacterial organisms).[64,138] Genetic transfer of genes between or within bacterial species can occur through the transfer of transposons (mobile DNA elements that move from one position in the chromosomal sequence to another), plasmids (circular segments of DNA that replicate independently of chromosomal DNA) or integrons (DNA elements that integrate genes into specific genomic sites).[64,138]

Antibiotic resistance mechanisms in *H. pylori* have not been completely elucidated. Evidence suggests that antibiotic-resistant *H. pylori* infection occurs primarily due to *de novo* mutations.[53,106,149] However, genetic exchanges between *H. pylori* isolates, and therefore the transmission of antibiotic resistance, is possible.[53,150] Plasmids, which commonly include antibiotic resistance genes in many pathogenic bacteria, have been identified in *H. pylori* organisms.[151] However, the role of plasmids and their significance in the development of antibiotic-resistant *H. pylori* infection remains uncertain.[151] The following sections discuss antibiotic resistance mechanisms in *H. pylori* for antibiotics commonly used in anti-*H. pylori* treatment regimens including: clarithromycin; metronidazole; amoxicillin; tetracycline; levofloxacin; ciprofloxacin; rifampicin; and nitrofurantoin.

Clarithromycin Resistance Mechanisms

Clarithromycin is a bacteriostatic antibiotic belonging to the macrolide class of antibiotics.[152] Clarithromycin inhibits the activity of bacterial ribosomes by binding to 23S ribosomal RNA: a component of the 50S subunit of bacterial ribosomes.[152] Upon binding, clarithromycin inhibits the translocation step of protein synthesis by preventing tRNA from moving from the ribosomal tRNA acceptor site to the site of peptidyl transferase activity and, therefore, prevents the formation of peptide bonds between amino acids to form proteins.[152] In Gram-negative bacteria, resistance to clarithromycin develops most commonly due to modification of the antibiotic binding site on the ribosome that prevents the binding of clarithromycin to the ribosome or through the active efflux of clarithromycin from bacterial cells.[153] Modification of the 23S rRNA

binding site can arise from point mutations in the gene encoding 23S rRNA or by the enzymatic addition of a methyl group (a carbon atom bonded with three hydrogen atoms) which structurally hinders the binding of clarithromycin.[153] In *H. pylori*, clarithromycin resistance most commonly occurs due to point mutations in the peptidyl transferase region of domain V of 23S rRNA gene that prevent the binding of clarithromycin to the bacterial ribosome.[154,155] Previous studies using genetic susceptibility testing methods estimate approximately ≥90% of clarithromycin-resistant *H. pylori* strains possess one or more of the following point mutations in the peptidyl transferase region in domain V of 23S rRNA: 1) substitution of adenine to guanine at position 2143 (A2143G); 2) substitution of adenine to guanine at position 2142 (A2142G); and 3) substitution of adenine to cytosine at position 2142 (A2142C).[53,106,124,129,156] Other point mutations in the peptidyl transferase region of 23S rRNA have been observed in clarithromycin-resistant *H. pylori* strains but their significance remains uncertain.[106,156–158] To date, it remains unclear whether mutated genes outside of the peptidyl transferase region of 23S rRNA are associated with clarithromycin resistance in *H. pylori* strains. However, clarithromycin-resistant *H. pylori* strains have been identified without mutations in 23S rRNA which suggests that there are resistance mechanisms in a small number of clarithromycin-resistant *H. pylori* isolates that remain to be identified.[159]

Metronidazole Resistance Mechanisms

Metronidazole is a bactericidal antibiotic that belongs to the nitroimidazole class of antibiotics.[160] Metronidazole is administered as an inactive prodrug: a compound that requires structural modification by enzymatic reduction to become pharmacologically activated within the bacterial cell.[161] Following activation, the metronidazole compounds produced from the reduction reaction are toxic, highly reactive intermediates that include imidazole intermediates, cytotoxic short-lived radicals, and other reactive compounds that can damage bacterial DNA and thereby induce bacterial cell death.[160] Metronidazole resistance in various bacteria, including *H. pylori,* has been commonly associated with the poor reduction of the metronidazole prodrug due to reduced enzymatic activity of bacterial reductases.[162] Goodwin *et al*. (1998) reported metronidazole resistance in *H. pylori* infection to be associated with the activity of nicotinamide adenine dinucleotide

phosphate (NADPH) nitroreductase: an enzyme that reduces metronidazole in *H. pylori.*[163] NADPH nitroreductase is encoded for by the *rdxA* gene in *H. pylori;* several studies have reported an association between metronidazole-resistant *H. pylori* infection and chromosomal mutations in *rdxA* that inhibit the enzymatic activity of NADPH nitroreductase.[163–167] Overall, the inactivation of the *rdxA* gene by genetic mutation is widely viewed as the most common mechanism of metronidazole resistance in *H. pylori* organisms. More recent studies, however, have reported identical *rdxA* sequences in both metronidazole-resistant and metronidazole-susceptible *H. pylori* isolates.[167,168] Additionally, metronidazole-resistant *H. pylori* isolates without the presence of mutations in the *rdxA* gene sequence have been identified.[165,169,170] Therefore, although mutations in *rdxA* may contribute to reduced metronidazole susceptibility in *H. pylori* isolates, additional mechanisms of resistance may contribute to metronidazole susceptibility in *H. pylori* isolates. Kwon *et al*. (2000) presented evidence of an association between mutations in the NADPH flavin oxidoreductase (*frxA*) gene and metronidazole-resistant *H. pylori* infection.[171] Additional analysis suggests that mutations in *frxA*, in the absence of *rdxA* mutations, result in low levels of metronidazole resistance in *H. pylori*[172] whereas mutations in both *rdxA* and *frxA* result in high levels of metronidazole resistance in *H. pylori*.[166,170–173] However, metronidazole-resistant *H. pylori* isolates without mutations in either *rdxA* or *frxA* genes have been identified.[174] It may be that other mechanisms of metronidazole resistance have yet to be identified. Additional reducing factors present in *H. pylori* organisms, including pyruvate ferredoxin oxidoreductase and ferredoxin-like protein (FdxB) may be associated with metronidazole-resistant *H. pylori* infection.[170,171,173,175] Tsugawa *et al*. (2011) also reported evidence of the presence of efflux systems, responsive to metronidazole, that may contribute to antibiotic resistance in *H. pylori* organisms.[176]

Amoxicillin Resistance Mechanisms

Amoxicillin is a bactericidal antibiotic belonging to the β-lactam class of antibiotics.[139] Amoxicillin induces bacterial cell death by inhibiting the activity of PBPs and thereby inhibiting bacterial cell wall synthesis and inducing cell lysis.[106] In Gram-negative bacteria, resistance to amoxicillin, and other penicillin antibiotics, is mediated

primarily through the activity of β-lactamase: an enzyme that breaks apart the β-lactam ring of amoxicillin and thereby inactivates its antimicrobial activity.[177] However, in *H. pylori,* amoxicillin resistance most commonly arises due to mutations in PBPs rather than β-lactamase activity.[178–182] Structural modifications to PBPs due to genetic mutations in chromosomal DNA inhibit the binding of β-lactams to PBPs and result in decreased amoxicillin susceptibility in bacteria.[105] Some cases of amoxicillin-resistant *H. pylori* infection has been observed to have mutational changes within genetic sequences encoding two of the nine PBPs that have previously been identified in *H. pylori* organisms: PBP1 and PBP4.[183] In a study conducted by Gerrits *et al*. (2006) that investigated seven amoxicillin-resistant *H. pylori* isolates, various mutational changes in PBP1A were found to mediate amoxicillin resistance.[183] Aside from mutational changes to PBPs, other possible mechanisms of amoxicillin resistance that have been investigated in *H. pylori* organisms include reduced *H. pylori* membrane permeability to amoxicillin and active efflux of amoxicillin.[106] The results of previous studies investigating alternative mechanisms of amoxicillin-resistant *H. pylori* infection suggest that amoxicillin resistance in *H. pylori* may occur in part due to reduced permeability of amoxicillin into the bacterial cell due to alterations in the composition of outer membrane proteins.[181,184] Additionally, Tseng *et al*. (2009) demonstrated that the presence of β-lactamase activity can mediate amoxicillin resistance in *H. pylori* isolates.[185] Previous studies have reported the presence of β-lactamase-like genes in *H. pylori* isolates, including the *H. pylori* cysteine-rich protein A (HcpA).[186,187] The production of beta-lactamase activity by proteins, such as HcpA, in *H. pylori* isolates may reduce bacterial susceptibility to amoxicillin.[185] In a review of the current knowledge regarding resistance mechanisms that confer amoxicillin resistance in *H. pylori* isolates, De Francesco *et al.* (2011) suggest that multiple concomitant mutational changes or resistance mechanisms may be required to confer a high level of amoxicillin resistance.[156] Compared to the prevalence of *H. pylori* infection with resistance to other commonly used antibiotics in anti-*H. pylori* treatment regimens, particularly metronidazole and clarithromycin, the relatively low prevalence of amoxicillin-resistant *H. pylori* infection may be due to the requirement of multiple concurrent genetic mutations to confer amoxicillin resistance.

Tetracycline Resistance Mechanisms

Tetracycline is a bacteriostatic antibiotic belonging to the tetracycline class of antibiotics.[152] Tetracycline exerts antimicrobial activity by inhibiting bacterial protein sysnthesis.[152,188] Tetracycline binds to the 30S subunit of bacterial ribosomes and blocks the binding of tRNA.[148,152,189] Different bacterial species express various mechanisms of tetracycline resistance including active efflux of tetracycline from bacterial cells, enzymatic inactivation of tetracycline, alteration of the tetracycline binding site on bacterial ribosomes, and ribosomal protection proteins.[189] Ribosomal protection proteins are proteins present in the cytoplasm of the bacterial cell that reduce the susceptibility of ribosomes to tetracycline by interfering with the tetracycline binding site.[189] Current evidence suggests that ribosomal protection proteins likely reduce bacterial susceptibility to tetracycline by binding to the ribosome, and upon binding, induce the alteration of the ribosomal structure so that tetracycline can no longer bind to the 30S subunit.[189] Mutations in the gene encoding 16S rRNA (the chromosomal DNA that encodes for the binding site of tetracycline on the bacterial ribosome) have been observed among tetracycline-resistant *H. pylori* isolates.[190–192] Mutations in the gene encoding 16S rRNA inhibit the binding of tetracycline to bacterial ribosomes.[190,192] However, tetracycline-resistant *H. pylori* infection without any mutational changes in the 16S rRNA gene sequence has been identified.[191,193] Aside from alterations to the 16S rRNA gene sequence, possible mechanisms of tetracycline resistance in *H. pylori* may include mutations in porin genes (which alter the permeability of the cell wall to tetracycline) or efflux pumps.[191] In *H. pylori* isolates, Kutschke *et al.* (2005) observed the efflux pump, HefABC, to be responsive to tetracycline; mutations decreasing the activity of HefABC may, therefore, improve bacterial susceptibility to tetracycline in *H. pylori* organisms.[194] Additionally, Wu *et al*. (2005) presented evidence of *H. pylori* isolates with reduced intracellular tetracycline accumulation that did not possess genetic mutations in the 16S rRNA gene sequence. Wu *et al*. (2005), therefore, provides evidence of a mechanism of tetracycline resistance in *H. pylori* organisms that may include the active efflux of tetracycline or reduced membrane permeability to tetracycline.[193] Dailidiene *et al.* (2002) suggest that tetracycline resistance in *H. pylori* is not commonly observed because *in vivo*

tetracycline resistance may require multiple simultaneous mutations to confer a high level of tetracycline resistance.[191]

Levofloxacin & Ciprofloxacin Resistance Mechanisms

Levofloxacin and ciprofloxacin are bactericidal antibiotics that belong to the fluoroquinolone class of antibiotics.[144] Levofloxacin and ciprofloxacin share a mechanism of action in which antimicrobial activity is exerted through the inhibition of DNA gyrase.[106] DNA gyrase is essential for bacterial cell growth because it catalyzes the relaxation of DNA nucleic acid strands for the purposes of replication, recombination, and transcription of bacterial DNA.[148] DNA gyrase is composed of four subunits, two A subunits and two B subunits, that are respectively encoded for by the genes *gyrA* and *gyrB*.[195] The mechanism of levofloxacin resistance and ciprofloxacin resistance in Gram-negative bacteria involves mutations within the quinolone-resistance-determining region (QRDR) of the *gyrA* gene and the active efflux of levofloxacin and ciprofloxacin from bacterial cells through efflux pumps.[196,197] Levofloxacin and ciprofloxacin resistance in *H. pylori* isolates is attributed primarily to mutations in *gyrA*.[195,198] However, Moore *et al.* (1995) reported the absence of mutations in the QRDR region of *gyrA* when investigating a ciprofloxacin-resistant *H. pylori* isolate, which suggests that additional unidentified mechanisms of fluoroquinolone resistance may be present in *H. pylori* organisms.[195] The results of previous studies investigating the contribution of efflux pumps in fluoroquinolone-resistant *H. pylori* isolates suggest that efflux pumps do not largely contribute to fluoroquinolone resistance.[194,199] Bina *et al*. (2000) reported that the active efflux of ciprofloxacin does not contribute to the intrinsic resistance of *H. pylori* isolates following the investigation of the *in vitro* susceptibility of *H. pylori* isolates.[199] Similarly, Kutschke *et al*. (2005) observed that ciprofloxacin was not susceptible to efflux from *H. pylori* organisms when investigating the role of HefABC-mediated efflux in the susceptibility of *H. pylori* isolates to various antibiotics.[194]

Rifampicin Resistance Mechanisms

Rifampicin is a bactericidal antibiotic that belongs to the rifamycin class of antibiotics.[200] Rifampicin inhibits RNA transcription and protein synthesis by binding to

the β-subunit of DNA-dependent RNA polymerase.[200] Upon binding to DNA-dependent RNA polymerase, rifampicin inhibits the activity of DNA-dependent RNA polymerase and therefore inhibits RNA synthesis from chromosomal DNA.[200] The mechanism of resistance to rifamycins in various bacterial species, including *H. pylori,* involves mutations in the gene encoding the β-subunit of DNA-dependent RNA polymerase: *rpoB*.[201,202] Several studies have reported evidence of specific point mutations in the *rpoB* gene that confer rifampicin resistance in *H. pylori* isolates.[201–205]

Nitrofurantoin Resistance Mechanisms

Nitrofurantoin is a bactericidal antibiotic belonging to the nitrofuran class of antibiotics.[144] Similar to the mechanism of action of metronidazole, nitrofurantoin is administered as a prodrug that requires enzymatic reduction by oxygen-insensitive nitroreductase to become pharmacologically activated within the bacterial cell.[106,144] Following enzymatic reduction of nitrofurantoin within bacterial cells, the intermediate compounds produced from the reduction reaction induce bacterial DNA damage.[206,207] However, although the mechanism of antimicrobial activity of nitrofurantoin is similar to metronidazole, evidence suggests that resistance to nitrofurantoin does not occur by the same mechanism as resistance to metronidazole.[208] Kwon *et al.* (2001) investigated nitrofurantoin resistance mechanisms in *H. pylori* and reported that *H. pylori* isolates with inactivated *rdxA* and *frxA* genes maintained susceptibility to nitrofurantoin. The findings reported by Kwon *et al.* suggest that nitrofurantoin resistance in *H. pylori* does not occur by the same mechanism as metronidazole resistance in *H. pylori* because inactivation of *rdxA* and *frxA* is widely understood to be an important mechanism of resistance to metronidazole in *H. pylori*.[163,165,166,170,171,173,208] While the exact mechanism of nitrofurantoin resistance in *H. pylori* isolates remain uncertain, nitrofurantoin-resistant *H. pylori* infection may be due to the enzymatic activity of bacterial nitroreductases, aside from those encoded for by *rdxA* and *frxA,* such as pyruvate-flavodoxin oxidoreductase and 2-oxoglutarate oxidoreductase.[208,209]

Chapter 2. Review of antibiotic resistance mechanisms in *H. pylori* organisms and prevalence of antibiotic-resistant *H. pylori* infection worldwide during 2010 to 2014

Prevalence of Antibiotic-resistant *H. pylori* Infection

Prevalence of Clarithromycin-resistant H. pylori *Infection*

The literature search identified 93 articles published during 2010 through 2014 that reported estimates of the prevalence of antibiotic-resistant *H. pylori* infection. Among the 93 articles identified, 84 reported results of studies that estimated the prevalence of clarithromycin-resistant *H. pylori* infection among individuals with *H. pylori* infection who had samples of *H. pylori* tested for antibiotic susceptibility in distinct geographic regions: 43 from Asian populations,[210–252] 26 from European populations,[103,253–277] 6 from African populations,[154,278–282] 5 from South American populations,[283–287] 3 from North American populations,[288–290] and 1 from an Australian population.[291]

Prevalence of Clarithromycin-resistant H. pylori *Infection in Individuals with No History of Antibiotic Treatment to Eliminate* H. pylori *Infection*

Of the 84 articles estimating the prevalence of clarithromycin-resistant *H. pylori* infection among individuals with *H. pylori* infection who had samples of *H. pylori* tested for clarithromycin susceptibility, 48 reported estimates of individuals with no history of antibiotic treatment to eliminate *H. pylori* infection (Table 2.1). The susceptibility of *H. pylori* organisms to clarithromycin was estimated by Etest® in 31 studies,[103,154,216,225,228,235–237,239,242,247,250,251,254,259–261,264–269,272,274,275,277,279,286,287,291] disk diffusion method in 5,[238,240,241,249,271] agar dilution method in 5,[224,244–246,248] PCR/RFLP analysis in 2,[252,285] ASP-PCR analysis in 2,[234,276] TaqMan® real-time PCR analysis in 1,[270] breakpoint susceptibility testing in 1,[273] and the microdilution method in 1.[243] Across the identified studies in this subgroup, the estimated prevalence of clarithromycin-resistant *H. pylori* infection among individuals with *H. pylori* infection who had no history of antibiotic treatment to eliminate *H. pylori* infection and who had samples of *H. pylori* tested for clarithromycin susceptibility ranged from 0% to 85%. Separating studies of children and adults, this range had many individual values that were much higher in studies of individuals who were <18 years of age (7% to 85%) than in studies of individuals who were ≥18 years of age (1% to 45%).

Chapter 2. Review of antibiotic resistance mechanisms in *H. pylori* organisms and prevalence of antibiotic-resistant *H. pylori* infection worldwide during 2010 to 2014

Table 2.1. Prevalence of clarithromycin-resistant *H. pylori* infection among people with *H. pylori* infection who had no history of treatment to eliminate *H. pylori* infection and who had samples of *H. pylori* tested for clarithromycin susceptibility.

Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Sample Size (n)	Prevalence of Resistance (%)
Adults ≥18 years of age					
Africa					
Senegal, 2013, Seck[154]	2007 – 2009	18-93, [45.3]	Etest®	108	1.0
Tunisia, 2010, Ben Mansour[279]	March 2005 – Aug 2007	18-88, [38.3]	Etest®	225	14.6
Asia					
China, 2014, Song[235]	March 2008 – Feb 2012	18-75, [42.5]	Etest®	600	37.5
Thailand, 2013, Vilaichone[236]	Jan 2004 – Dec 2012	>18	Etest®	400	3.7
Italy, 2012, Saracino[237]	Jan 2010 – Dec 2011	24-83	Etest®	145	35.2
Northern Iran, 2011, Abadi[238]	Jan – July 2009	18-74, [40.7]	Disk diffusion	197	45.2
Iran, 2012, Talebi Bezmin Abadi[239]	Oct 2009 – July 2010	21-70, [38.6]	Etest®	150	34.0
Europe					
Portugal, 2014, Almeida[261]	Sept 2009 – Oct 2013	19-77, [41.6]	Etest®	103	21.4
Germany, 2014, Wuppen-horst[265]	2001-2012 Subinterval: 2001-2002 2003-2004 2005-2006 2007-2008 2009-2010 2011-2012	≥18	Etest®	902 166 205 176 120 134 101	6.7 4.8 4.9 7.4 4.2 9.7 10.9

Chapter 2. Review of antibiotic resistance mechanisms in *H. pylori* organisms and prevalence of antibiotic-resistant *H. pylori* infection worldwide during 2010 to 2014

Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Sample Size (n)	Prevalence of Resistance (%)
Southern Poland, 2012, Karczewska [266]	Jan 2009 – Dec 2011	18-75, [45.6]	Etest®	43	18.6
Poland, 2014, Biernat[267]	2008 – 2011	19-89	Etest®	50	24.0
18 European Countries, 2013, Megraud[268]	April 2008 – June 2009	19-99	Etest®	1893	17.5
Finland, 2011, Kostamo[269]	2000 – 2008	18-92, [62]	Etest®	505	8.0
Italy, 2011, De Francesco [270]	Jan – Sept 2010	>18, [51.5]	TaqMan® real-time PCR	253	9.9
Belgium, 2011, Miendje Deyi[271]	Jan 1990 – Dec 2009	18.0-98.9	Disk diffusion	7903	5.2
Oceania/Australia					
New Zealand, 2013, Hsiang[291]	Feb – Oct 2012	22-93.5, [59.8]	Etest®	73	16.4
South America					
Brazil, 2011, Eisig[286]	-	22-72	Etest®	39	7.7
Brazil, 2013, Suzuki[285]	Feb 2003 – Dec 2006	19-91	PCR/RFLP	488	2.5
Mixed Age Groups – Adults & Children					
Asia					

Chapter 2. Review of antibiotic resistance mechanisms in *H. pylori* organisms and prevalence of antibiotic-resistant *H. pylori* infection worldwide during 2010 to 2014

Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Sample Size (n)	Prevalence of Resistance (%)
China, 2010, Gao[216]	2000 – 2009 Subinterval: 2000 2001 2002-2003 2004-2005 2006-2007 2008 2009	13-83, [49]	Etest®	290 47 63 22 24 71 39 24	23.8 12.8 12.7 9.1 20.8 38.0 38.5 25.0
Iran, 2013, Zendedel[240]	Jan – March 2008	19-82 Age Group: <30 30-50 >50	Disk diffusion	82 25 38 19	17.1 15.8 18.4 16.0
India, 2014, Pandya[241]	Feb 2008 – Aug 2011	10-90	Disk diffusion	80	58.8
Europe					
Poland, 2014, Gosciniak[272]	2008 – 2012	4-89 Age Group: 4-18 19-89	Etest®	165 105 60	30.3 33.3 25.0
France, 2010, Raymond[254]	2004 – 2007	16-87, [48.8]	Etest®	455	19.1
Bulgaria, 2010, Boyanova[273]	2007 – 2009	18-87, [39.4] & 5-17, [12.9]	Breakpoint susceptibility testing	501	19.4
Children <18 Years of Age					
Africa					
Tunisia, 2010, Ben Mansour[279]	March 2005 – Aug 2007	2-14, [8.75]	Etest®	48	18.8
Asia					
South Korea, 2013, Seo[224]	1990 – 1994 2005 – 2009	3.4-17.8 2.4-15.8	Agar dilution	58 33	6.9 18.2

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Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Sample Size (n)	Prevalence of Resistance (%)
China, 2011, Liu[242]	Jan 1 1009 – Jun 30 2010	3-16, [10]	Etest®	73	84.9
Japan, 2010, Kato[243]	Total Subinterval: 1999-2002 2003-2007	4-18, [12.6]	Microdilution method	61 34 27	36.1 32.4 40.7
Israel, 2010, Zevit[225]	Jan 2005 – Aug 2007	2-17, [13]	Etest®	36	25.0
Europe					
Belgium, 2011, Miendje Deyi[271]	Jan 1990 – Dec 2009	0.2-17.9	Disk diffusion	1527	7.3
South America					
Brazil, 2010, Garcia[287]	2008 – 2009	1-18, [10]	Etest®	45	26.7
Age Range of Study Population Not Specified					
Asia					
Japan, 2011, Yamade[234]	June 2006 – Feb 2009	[56.9]	ASP-PCR	103	38.8
Korea, 2010, Hwang[244]	Total Subinterval: 2003-2005 2007-2009	[55.4] [53.1] [56.4]	Agar dilution	222 66 156	32.0 16.7 38.5
Korea, 2012, Chung[245]	2004 & 2007 Subinterval: 2004 2007	[50.7]	Agar dilution	185 83 102	10.8 10.8 10.8
Korea, 2013, Lee[246]	2003 – 2012 Subinterval: 2003-2005 2006-2008 2009-2012	 [53.4] [56.2] [56.7]	Agar dilution	347 64 169 114	 17.2 21.4 23.7
Southern Iran, 2010, Farshad[247]	Oct 2008 – Oct 2009	-	Etest®	121	5.0

Chapter 2. Review of antibiotic resistance mechanisms in *H. pylori* organisms and prevalence of antibiotic-resistant *H. pylori* infection worldwide during 2010 to 2014

Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Sample Size (n)	Prevalence of Resistance (%)
Iran, 2011, Shokrzadeh [248]	April 2007 – Dec 2008	26 males [45] & 18 females [38]	Agar dilution	42	14.3
Iran, 2013, Sadeghifard [249]	Jan 2009 – March 2010	-	Disk diffusion	50	32.0
Malaysia, 2011, Ahmad[250]	Sept 2004 – 2007	-	Etest®	187	1.1
Malaysia, 2014, Teh[251]	-	-	Etest®	102	6.8
Malaysia, 2010, Ho[252]	March – June 2002	[49.3]	PCR/RFLP	105	2.9
Malaysia, 2011, Goh[228]	Jan – Aug 2009	[50.5]	Etest®	90	0
Europe					
Germany, 2013, Selgrad[103]	Jan 2005 – May 2012	[51.7]	Etest®	159	7.5
Germany, 2014, Selgrad[264]	Jan 2010 – Oct 2012	[52.54]	Etest®	29	6.9
Lithuania, 2013, Kupcinskas [274]	1998 2001 2007-2008	[46.9] [48.1] [46.6]	Etest®	89 81 90	1.1 3.7 3.3
Poland, 2011, Karczewska [259]	Dec 2006 – Dec 2008	Adults	Etest®	90	21.1
Ireland, 2010, O'Connor [260]	2007 – 2008	[46]	Etest®	182	9.3
Norway, 2013, Larsen[275]	2008 – 2009	-	Etest®	102	5.9

Chapter 2. Review of antibiotic resistance mechanisms in *H. pylori* organisms and prevalence of antibiotic-resistant *H. pylori* infection worldwide during 2010 to 2014

Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Sample Size (n)	Prevalence of Resistance (%)
Greece, 2014, Karamanolis [276]	2000 2010	-	ASP-PCR	50 57	30.0 42.1
Southern Croatia, 2012, Tonkic[277]	Jan 2008 – Dec 2010	adults	Etest®	345	21.2

Prevalence of Clarithromycin-resistant H. pylori *Infection in Individuals Previously Treated for* H. pylori *Infection*

Of the 84 articles estimating the prevalence of clarithromycin-resistant *H. pylori* infection among individuals with *H. pylori* infection who had samples of *H. pylori* tested for clarithromycin susceptibility, 14 reported estimates of the prevalence of clarithromycin-resistant *H. pylori* infection among individuals who had previously been treated with antibiotics to eliminate *H. pylori* infection (Table 2.2). The susceptibility of *H. pylori* organisms to clarithromycin was estimated by Etest® in 11 studies,[103,216,225,251,254,259–261,264–266] disk diffusion in 1,[271] agar dilution in 1,[246] and ASP-PCR analysis in 1.[234] Across the identified literature in this subgroup, the prevalence of clarithromycin-resistant *H. pylori* infection among individuals with *H. pylori* infection who had previously been treated with antibiotics to eliminate *H. pylori* infection and who had samples of *H. pylori* tested for clarithromycin susceptibility was reported to range from 9% to 90%.

Table 2.2. Prevalence of clarithromycin-resistant *H. pylori* infection among individuals with *H. pylori* infection who had previously been treated for *H. pylori* infection and who had samples of *H. pylori* tested for clarithromycin susceptibility.

Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Subgroup	Sample Size (n)	Prevalence of Resistance (%)
Adults ≥18 years of age						
Europe						
Portugal, 2014, Almeida[261]	Sept 2009 – Oct 2013	18-85, [45.8]	Etest®		77	88.3
Germany, 2014, Wuppen-horst[265]	2001 – 2012	≥18	Etest®	one prior treatment ≥2 prior treatments	359 262	58.5 78.2
Belgium, 2011, Miendje Devi[271]	Jan 1990 – Dec 2009	18.0-90.2	Disk diffusion		1209	8.5
Southern Poland, 2012, Karczewska[266]	Jan 2009 – Dec 2011	18-75, [45.6]	Etest®		8	37.5
Mixed Age Groups – Adults & Children						
Asia						
China, 2010, Gao[216]	2000 – 2009 Subinterval: 2000 2001 2002-2003 2004-2005 2006-2007 2008 2009	13-83, [49]	Etest®		84 7 8 5 3 9 24 28	83.3 28.6 50.0 80.0 100.0 88.9 87.5 100.0
Children <18 Years of Age						
Asia						
Israel, 2010, Zevit[225]	Jan 2005 – Dec 2009	2-17, [13]	Etest®		19	42.1
Europe						

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Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Subgroup	Sample Size (n)	Prevalence of Resistance (%)
Belgium, 2011, Miendje Devi[271]	Jan 1990 – Dec 2009	2.3-17.8	Disk diffusion		162	14.2
Range of Study Population Age Not Specified						
Asia						
Korea, 2013, Lee[246]	2003 – 2012 Subinterval: 2003-2005 2006-2008 2009-2012	 [57.3] [55.9] [53.7]	Agar dilution		86 6 32 48	 83.3 46.9 68.8
Malaysia, 2014, Teh[251]	-	-	Etest®		8	87.5
Japan, 2011, Yamade[234]	June 2006 – Feb 2009	[52.5]	ASP-PCR		50	90.0
Europe						
Germany, 2013, Selgrad[103]	Jan 2005 – May 2012	[51.7]	Etest®	one prior treatment ≥2 prior treatments	106 171	63.2 75.4
Germany, 2014, Selgrad[264]	Jan 2010 – Oct 2012	[52.54]	Etest®	one prior treatment ≥2 prior treatments	13 24	53.8 83.3
Poland, 2011, Karczewska[259]	Dec 2006 – Dec 2008	adults	Etest®		25	80.0
France, 2010, Raymond[254]	2004 – 2007	18-87, [51.7]	Etest®		75	68.0

Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Subgroup	Sample Size (n)	Prevalence of Resistance (%)
Ireland, 2010, O'Connor [260]	2007 – 2008	[46]	Etest®		37	32.4

Prevalence of Clarithromycin-resistant H. pylori *Infection in Study Populations with Mixed or Unidentified Anti-*H. pylori *Antibiotic Treatment Histories*

Of the 84 articles estimating the prevalence of clarithromycin-resistant *H. pylori* infection among individuals with *H. pylori* infection who had samples of *H. pylori* tested for clarithromycin susceptibility, 44 reported estimates of the prevalence of clarithromycin-resistant *H. pylori* among individuals from a study population that either included individuals: 1) who had or had not been previously treated with antibiotics to eliminate *H. pylori* infection; or 2) whose previous exposure history to anti-*H. pylori* treatment regimens was not specified (Table 2.3). The susceptibility of *H. pylori* organisms to clarithromycin was estimated by Etest® in 28 studies,[210–214,216,217,219,222,225–227,229,254–264,282,283,288,289] agar dilution in 9,[230–233,253,278,280,284,290] disk diffusion in 3,[218,220,221] GenoType® HelicoDR analysis in 2, microbroth dilution method in 1,[215] and ASP-PCR analysis in 1.[234] Across the studies in this subgroup, the estimated prevalence of clarithromycin-resistant *H. pylori* infection among individuals with *H. pylori* infection who had *H. pylori* samples tested for clarithromycin susceptibility ranged from 0% to 67%.

Chapter 2. Review of antibiotic resistance mechanisms in *H. pylori* organisms and prevalence of antibiotic-resistant *H. pylori* infection worldwide during 2010 to 2014

Table 2.3. Prevalence of clarithromycin-resistant *H. pylori* infection among individuals with *H. pylori* infection who had *H. pylori* samples tested for clarithromycin susceptibility in study populations with either mixed or unidentified anti-*H. pylori* treatment histories.

Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Subgroup	Sample Size (n)	Prevalence of Resistance (%)
Adults ≥18 years of age						
Asia						
Pakistan, 2013, Yakoob[210]	April 2008 – June 2010	18-77, [41]	Etest®		47	36.2
Pakistan, 2014, Rasheed[211]	July 2011 – March 2012	19-80	Etest®		46	47.8
United Arab Emirates, 2010, Alfaresi[212]	July 2008 – Jan 2009	19-80, [40]	Etest®		26	19.2
Israel, 2014, Peretz[213]	June 2011 – April 2012	19-79, [48]	Etest®		44	22.3
North America						
Cuba, 2010, Llanes[288]	Sept – Dec 2005	19-68	Etest®		40	10.0
South America						
Southern Brazil, 2014, Picoli[283]	Jan 2011 – Jan 2012	18-80	Etest®		54	11.1
Mixed Age Groups – Adults & Children						
Africa						
The Gambia, 2013, Secka[278]	-	1.5-70, [30]	Agar dilution		64	0
Asia						
Malaysia, 2014, Alfizah[214]	May 2004 – Dec 2007	17-89	Etest®		161	1.2

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Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Subgroup	Sample Size (n)	Prevalence of Resistance (%)
Japan, 2014, Okamura[215]	Sept 2000 – Aug 2013	2-88, [53.6]	Microbroth dilution		1073	31.1
	<u>Subinterval:</u>	Age group:				
	2000-2001	<30			10	10.0
	2012-2013				38	57.9
	2000-2001	31-50			17	11.8
	2012-2013				29	34.5
	2000-2001	>50			86	25.6
	2012-2013				114	35.1
China, 2010, Gao[216]	2000 – 2009	13-83	Etest®		374	37.2
	Subinterval:					
	2000				54	14.8
	2001				71	16.9
	2002-2003				27	22.2
	2004-2005				27	29.6
	2006-2007				80	43.8
	2008				63	57.1
	2009				52	65.4
Vietnam, 2013, Binh[217]	2008	14-83	Etest®		103	33
Iran, 2010, Siavoshi[218]	2005 – 2008	16-83	Disk diffusion		110	7.3
Iran, 2010, Tomatari[219]	2006 – 2007	17-65	Etest®		128	23.5
Iran, 2010, Sirous[220]	Oct 2007 – June 2008	15-75	Disk diffusion		33	0
Iran, 2012, Milani[221]	Dec 2010 – Nov 2011	adults [46] & children 3-14, [5]	Disk diffusion		112	14.3
Iran, 2013, Mirzaei[222]	March – June 2011	15-58	Etest®		48	14.6
Pakistan, 2012, Rajper[223]	June 2009 – July 2010	9-75	GenoType® HelicoDR		162	37.0
Europe						

Chapter 2. Review of antibiotic resistance mechanisms in *H. pylori* organisms and prevalence of antibiotic-resistant *H. pylori* infection worldwide during 2010 to 2014

Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Subgroup	Sample Size (n)	Prevalence of Resistance (%)
Eastern Russia, 2012, Reva[253]	2004 – 2009	15-80	Agar dilution		170	7.6
France, 2010, Raymond [254]	2004 – 2007	16-87	Etest®		530	26.0
Turkey, 2012, Kalem[255]	Jan 2006 – Dec 2009	[50.2]	Etest®		28	28.5
North America						
Mexico, 2011, Ayala[289]	Jan 2002 – Dec 2004	≥15	Etest®	Biopsies from: Antrum Corpus	90	 5.5 8.9
Alaska, 2011, Tveit[290]	Jan 1, 2000 – Dec 31 2008	3-96, [51]	Agar dilution		531	29.9
South America						
Brazil, 2013, Ogata[284]	Feb 2008 – Aug 2009	3-20, [11.1]	Agar dilution		77	19.5
Children <18 years of age						
Asia						
Israel, 2010, Zevit[225]	Jan 2005 – Aug 2007	2-17, [13]	Etest®		55	30.9
Israel, 2014, Peretz[213]	June 2011 – April 2012	7-17, [12]	Etest®		41	24.3
Vietnam, 2012, Nguyen[226]	May 2005 – Feb 2006	3-15	Etest®		222	50.9
Europe						
Austria, 2012, Prechtl[256]	2002 – 2009 Subinterval: 2002-2005 2006-2009	3-18 [11.7] [12.56]	Etest®		 38 36	 18.4 25.0

Chapter 2. Review of antibiotic resistance mechanisms in *H. pylori* organisms and prevalence of antibiotic-resistant *H. pylori* infection worldwide during 2010 to 2014

Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Subgroup	Sample Size (n)	Prevalence of Resistance (%)
Age Range of Study Population Not Specified						
Africa						
Kenya, 2010, Kimang'a [282]	-	children and adults	Etest®		65	0
South Africa, 2010, Tanih[280]	-	-	Agar dilution		200	20.0
South Africa, 2013, Tanih[281]	-	-	GenoType® HelicoDR		78	15.4
Asia						
Bhutan, 2013, Vilaichone [227]	Dec 6-9 2010	[36.8]	Etest®		111	0
Iran, 2010, Talebi Bezmin Abadi[229]	2007 – 2010	[45.8]	Etest®		132	30.0
China, 2010, Sun[230]	2000 2005 2009	[44.3] [45.2] [42.5]	Agar dilution		58 100 135	8.6 9.0 20.7
China, 2013, Su[231]	Jan 2010 – April 2012	-	Agar dilution		17731	21.5
South Korea, 2012, Seo[232]	1985 – 1989 1990 – 1994 1995 – 1999 1995 – 1999	[30.0] [33.2] [22.7] [42.0]	Agar dilution	Jinju, South Korea Cheongju, South Korea	53 70 47 23	1.9 5.7 6.4 0
Korea, 2013, An[233]	July 2009 – Dec 2010 June 2011 – Dec 2012	- -	Agar dilution		71 94	7.0 16.0

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Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Subgroup	Sample Size (n)	Prevalence of Resistance (%)
Japan, 2011, Yamade[234]	June 2006 – Feb 2009	[55.4]	ASP-PCR		153	55.6
Europe						
Northern Spain, 2012, Cuadrado-Lavin[257]	Feb – Dec 2010	-	Etest®		68	14.7
Spain, 2011, Agudo[258]	May – Dec 2008	children and adults	Etest®		118	35.6
Poland, 2011, Karczewska[259]	Dec 2006 – Dec 2008	-	Etest®		115	34.0
Ireland, 2010, O'Connor[260]	2007 – 2008	[46]	Etest®		219	13.2
Portugal, 2014, Almeida[261]	Sept 2009 – Oct 2013	[43.3]	Etest®		180	50.0
Bulgaria, 2014, Boyanova[262]	2012 – 2013	[50.5]	Etest®		50	22.0
Germany, 2013, Wueppen-horst[263]	Jan 2006 – Dec 2011	-	Etest®		5296	67.1
Germany, 2014, Selgrad[264]	Jan 2010 – Oct 2012	[52.54]	Etest®		66	43.9

Prevalence of Metronidazole-resistant H. pylori Infection

Of the 93 articles published during 2010 to 2014 identified in the literature search, 77 reported estimates of the prevalence of metronidazole-resistant *H. pylori* infection among individuals with *H. pylori* infection who had samples of *H. pylori* tested for metronidazole susceptibility in distinct geographic regions: 38 from Asian populations,[211,213–222,224–233,235,236,238–251,292] 27 from European populations,[103,237,253–257,259–265,267–269,271–275,277,293–296] 5 from African populations,[154,278–280,282] 3 from South American populations,[284,286,287] 3 from North American populations,[288–290] and 1 from an Australian population.[291]

Prevalence of Metronidazole-resistant H. pylori *Infection in Individuals with No History of Antibiotic Treatment to Eliminate* H. pylori *Infection*

Of the 77 identified articles that reported estimates of the prevalence of metronidazole-resistant *H. pylori* infection among individuals with *H. pylori* infection who had samples of *H. pylori* tested for antibiotic susceptibility, 45 reported estimates for individuals with no history of antibiotic treatment to eliminate *H. pylori* infection (Table 2.4). The method used to classify susceptibility of *H. pylori* organisms to metronidazole was Etest® in 32 studies,[103,154,216,225,228,235–237,239,242,247,250,251,254,259–261,264,265,267–269,272,274,275,277,279,286,291,294–296] disk diffusion method in 6,[238,240,241,249,271,287] agar dilution method in 5,[224,244–246,248] breakpoint susceptibility testing in 1,[273] and microdilution method in 1.[243] Across the 45 studies in this subgroup, the prevalence of metronidazole-resistant *H. pylori* infection among individuals with *H. pylori* infection who had no history of antibiotic treatment to eliminate *H. pylori* infection and who had samples of *H. pylori* tested for metronidazole susceptibility was estimated to range from 7% to 88%. Separating studies of children and adults, this range had many individual values that were higher in studies of individuals who were ≥18 years of age (22% to 85%) than in studies of individuals who were <18 years of age (17% to 62%).

Table 2.4. Prevalence of metronidazole-resistant *H. pylori* infection among individuals with *H. pylori* infection who had no history of treatment for *H. pylori* infection and who had samples of *H. pylori* tested for metronidazole susceptibility.

Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Sample Size (n)	Prevalence of Resistance (%)
Adults ≥18 years of age					
Africa					
Senegal, 2013, Seck[154]	2007 – 2009	18-93, [45.3]	Etest®	108	85.2
Tunisia, 2010, Ben Mansour[279]	March 2005 – Aug 2007	18-88, [38.3]	Etest®	225	56.8
Asia					
China, 2014, Song[235]	March 2008 – Feb 2012	18-75, [42.5]	Etest®	600	67.2
Northern Iran, 2011, Abadi[238]	Jan – July 2009	18-74, [40.7]	Disk diffusion	197	65.5
Iran, 2012, Talebi Bezmin Abadi[239]	Oct 2009 – July 2010	21-70, [38.6]	Etest®	150	78.6
Thailand, 2013, Vilaichone[236]	Jan 2004 – Dec 2012	>18	Etest®	400	36.0
Europe					
Germany, 2014, Wueppen-horst[265]	2001 – 2012 Subinterval: 2001-2002 2003-2004 2005-2006 2007-2008 2009-2010 2011-2012	≥18	Etest®	902 166 205 176 120 134 101	29.4 22.3 24.9 33.0 34.2 31.3 35.6
Poland, 2014, Biernat[267]	2008 – 2011	19-89	Etest®	50	42.0
Portugal, 2014, Almeida[261]	Sept 2009 – Oct 2013	19-77, [41.6]	Etest®	103	29.1

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Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Sample Size (n)	Prevalence of Resistance (%)
18 European countries, 2013, Megraud[268]	April 2008 – June 2009	19-99	Etest®	1893	34.9
Italy, 2012, Saracino[237]	Jan 2010 – Dec 2011	24-83	Etest®	145	59.3
Finland, 2011, Kostamo[269]	2000 – 2008	18-92, [62]	Etest®	505	41.0
Belgium, 2011, Miendje Deyi[271]	Jan 1990 – Dec 2009	18.0-98.9	Disk diffusion	7903	26.1
Oceania					
New Zealand, 2013, Hsiang[291]	Feb 2012 – Oct 2012	22-93.5, [59.8]	Etest®	73	49.3
South America					
Brazil, 2011, Eisig[286]	-	22-72	Etest®	39	51.3
Mixed Age Groups – Adults & Children					
Asia					
China, 2010, Gao[216]	2000 – 2009 Subinterval: 2000 2001 2002-2003 2004-2005 2006-2007 2008 2009	13-83, [49]	Etest®	290 47 63 22 24 71 39 24	56.6 12.8 12.7 9.1 20.8 38.0 38.5 25.0
Japan, 2010, Kato[243]	1999 – 2007 Subinterval: 1999-2002 2003-2007	4-18, [12.6]	Microdilution method	61 34 27	14.8 17.6 7.4

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Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Sample Size (n)	Prevalence of Resistance (%)
Iran, 2013, Zendedel[240]	Jan – March 2008	19-82 Age Group: <30 30-50 >50	Disk diffusion	82 25 38 19	64.6 88.0 57.9 47.3
India, 2014, Pandya[241]	Feb 2008 – Ag 2011	10-90	Disk diffusion	80	83.8
Europe					
Germany, 2014, Selgrad[264]	Jan 2010 – Oct 2012	[52.54]	Etest®	29	17.2
Poland, 2014, Gosciniak[272]	2008 – 2012	4-89 Age Group: 4-18 19-89	Etest®	165 105 60	53.9 44.8 70.0
Bulgaria, 2010, Boyanova[273]	2007 – 2009	18-87, [39.4] & 5-17, [12.9]	Breakpoint susceptibility testing	501	25.7
Austria, 2010, Vecsei[294]	March 1 2002 – Feb 28 2008	0.5-20.9	Etest®	153	22.9
Croatia, 2012, Hojsak[295]	Jan 2001 – Dec 2010	1.08-18.8	Etest®	168	10.1
France, 2010, Raymond[254]	2004 – 2007	16-87, [48.8]	Etest®	455	58.9
Portugal, 2011, Oleastro[296]	Jan 2000 – Dec 2009	0.33-18, [10.17]	Etest®	1115	13.9
South America					
Brazil, 2010, Garcia[287]	2009 – 2009	1-18, [10]	Disk diffusion	45	13.1
Children <18 years of age					
Africa					

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Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Sample Size (n)	Prevalence of Resistance (%)
Tunisia, 2010, Ben Mansour[279]	March 2005 – Aug 2007	2-14, [8.75]	Etest®	48	25.0
Asia					
South Korea, 2013, Seo[224]	1990 – 1994 2005 – 2009	3.4-17.8 & 2.4-15.8	Agar dilution	58 33	32.8 27.3
China, 2011, Liu[242]	Jan 1 2009 – June 30 2010	3-16, [10.0]	Etest®	73	61.6
Israel, 2010, Zevit[225]	Jan 2005 – Aug 2007	2-17, [13]	Etest®	36	19.4
Europe					
Belgium, 2011, Miendje Deyi[271]	Jan 1990 – Dec 2009	0.2-17.9	Disk diffusion	1527	17.4
Age Range of Study Population Not Specified					
Asia					
Korea, 2010, Hwang[244]	total Subinterval: 2003-2005 2007-2009	[55.4] [53.1] [56.4]	Agar dilution	222 66 156	29.7 34.8 27.6
Korea, 2012, Chung[245]	2004 & 2007 Subinterval: 2004 2007	[50.7]	Agar dilution	185 83 102	30.3 42.2 20.6
Southern Iran, 2010, Farshad[247]	Oct 2008 – Oct 2009	-	Etest®	121	43.8
Iran, 2011, Shokrzadeh[248]	April 2007 – Dec 2008	24 males [45] & 18 females [38]	Agar dilution	42	40.5
Iran, 2013, Sadeghifard[249]	Jan 2009 – March 2010	-	Disk diffusion	50	88.0

Chapter 2. Review of antibiotic resistance mechanisms in *H. pylori* organisms and prevalence of antibiotic-resistant *H. pylori* infection worldwide during 2010 to 2014

Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Sample Size (n)	Prevalence of Resistance (%)
Korea, 2013, Lee[246]	2003-2012 Subinterval: 2003-2005 2006-2008 2009-2012	 [53.4] [56.2] [56.7]	Agar dilution	347 64 169 114	 35.9 26.8 32.5
Malaysia, 2011, Ahmad[250]	Sept 2004 – 2007	-	Etest®	187	35.3
Malaysia, 2011, Goh[228]	Jan – Aug 2009	[50.5]	Etest®	90	75.5
Malaysia, 2014, Teh[251]	-	-	Etest®	102	32.3
Europe					
Germany, 2013, Selgrad[103]	Jan 2005 – May 2012	[51.7]	Etest®	159	32.7
Lithuania, 2013, Kupcinskas[274]	1998 2001 2007-2008	[46.9] [48.1] [46.6]	Etest®	89 81 90	24.7 33.3 35.6
Ireland, 2010, O'Connor[260]	2007 – 2008	[46]	Etest®	182	29.1
Poland, 2011, Karczewska[259]	Dec 2006 – Dec 2008	adults	Etest®	90	37.0
Norway, 2013, Larsen[275]	2008 – 2009	-	Etest®	102	15.7
Southern Croatia, 2012, Tonkic[277]	Jan 2008 – Dec 2010	adults	Etest®	345	10.2

Prevalence of Metronidazole-resistant H. pylori *Infection in Individuals Previously Treated for* H. pylori *Infection*

From the 77 articles identified by the literature search that reported estimates of the prevalence of metronidazole-resistant *H. pylori* infection among indiviudals with *H. pylori* infection who had samples of *H. pylori* tested for metronidazole susceptibility, 13 reported estimates for the prevalence of metronidazole-resistant *H. pylori* infection among individuals who had previously been treated with antibiotics to eliminate *H. pylori* infection (Table 2.5). The susceptibility of *H. pylori* organisms to metronidazole was estimated by Etest® in 11 studies,[103,216,225,251,254,259–261,264,265,295] disk diffusion in 1,[271] and agar dilution in 1.[246] Across the 13 articles in this subgroup, the prevalence of metronidazole-resistant *H. pylori* infection among individuals with *H. pylori* infection who had been previously treated for *H. pylori* infection and who had samples of *H. pylori* tested for metronidazole susceptibility ranged from 38% to 89%.

Table 2.5. Prevalence of metronidazole-resistant *H. pylori* infection among individuals with *H. pylori* infection who had previously been treated for *H. pylori* infection and who had samples of *H. pylori* tested for metronidazole susceptibility.

Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Subgroup	Sample Size (n)	Prevalence of Resistance (%)
Adults ≥18 years of age						
Europe						
Portugal, 2014, Almeida[261]	Sept 2009 – Oct 2013	18-85, [45.8]	Etest®		77	41.6
Germany, 2014, Wuppen-horst[265]	2001 – 2012	≥18	Etest®	one prior treatment ≥2 prior treatments	359 262	48.2 80.9
Belgium, 2011, Miendje Deyi[271]	Jan 1990 – Dec 2009	18.0-90.2	Disk diffusion		1209	49.0
Mixed Age Groups – Adults & Children						
Asia						

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Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Subgroup	Sample Size (n)	Prevalence of Resistance (%)
China, 2010, Gao[216]	2000 – 2009 Subinterval: 2000 2001 2002-2003 2004-2005 2006-2007 2008 2009	13-83, [49]	Etest®		84 7 8 5 3 9 24 28	89.3 71.4 75.0 80.0 100.0 88.9 100.0 89.3
Europe						
France, 2010, Raymond[254]	2004 – 2007	18-87, [51.7]	Etest®		75	74.6
Croatia, 2012, Hojsak[295]	Jan 2001 – Dec 2010	1.08-18.8	Etest®		11	63.6
Children <18 years of age						
Asia						
Israel, 2010, Zevit[225]	Jan 2005 – Aug 2007	2-17, [13]	Etest®		19	52.6
Europe						
Belgium, 2011, Miendje Deyi[271]	Jan 1990 – Dec 2009	2.3-17.8	Disk diffusion		162	37.7
Age Range of Study Population Not Specified						
Asia						
Korea, 2013, Lee[246]	2003-2012 Subinterval: 2003-2005 2006-2008 2009-2012	 [57.3] [55.9] [53.7]	Agar dilution		86 6 32 48	 16.7 21.9 43.8
Malaysia, 2014, Teh[251]	-	-	Etest®		8	50.0
Europe						

Chapter 2. Review of antibiotic resistance mechanisms in *H. pylori* organisms and prevalence of antibiotic-resistant *H. pylori* infection worldwide during 2010 to 2014

Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Subgroup	Sample Size (n)	Prevalence of Resistance (%)
Germany, 2013, Selgrad[103]	Jan 2005 – May 2012	[51.7]	Etest®	one prior treatment ≥2 prior treatments	106 171	63.2 80.1
Germany, 2014, Selgrad[264]	Jan 2010 – Oct 2012	[52.54]	Etest®	one prior treatment ≥2 prior treatments	13 24	69.2 83.3
Ireland, 2010, O'Connor[260]	2007 – 2008	[46]	Etest®		37	43.2
Poland, 2011, Karczewska[259]	Dec 2006 – Dec 2008	adults	Etest®		25	72.0

Prevalence of Metronidazole-resistant H. pylori *Infection in Study Populations with Mixed or Unidentified Anti-*H. pylori *Antibiotic Treatment Histories*

Of the 77 articles that reported results for the prevalence of metronidazole-resistant *H. pylori* infection among individuals with *H. pylori* infection who had samples of *H. pylori* tested for metronidazole susceptibility, 39 reported estimates of the prevalence of metronidazole-resistant *H. pylori* infection among individuals from a study population that either included individuals: 1) who had or had not been previously treated with antibiotics to eliminate *H. pylori* infection; or 2) whose previous exposure history to anti-*H. pylori* treatment regimens was not specified (Table 2.6). The susceptibility of *H. pylori* organisms to metronidazole was estimated by Etest® in 25 studies,[211,213,214,216,217,219,222,225–227,229,254–257,259–264,282,288,289,292] agar dilution in 9,[230–233,253,278,280,284,290] disk diffusion in 4,[218,220,221,293] and microbroth dilution method in 1.[215] Across the studies in this subgroup, the estimated prevalence of metronidazole-resistant *H. pylori* infection among individuals with *H. pylori* infection who either had mixed or

unidentified anti-*H. pylori* treatment histories and who had samples of *H. pylori* tested for metronidazole susceptibility ranged from 5% to 96%.

Table 2.6. Prevalence of metronidazole-resistant *H. pylori* infection among individuals with *H. pylori* infection in study populations with either mixed or unidentified anti-*H. pylori* treatment histories who had samples of *H. pylori* tested for metronidazole susceptibility.

Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Subgroup	Sample Size (n)	Prevalence of Resistance (%)
Adults ≥18 years of age						
Asia						
Pakistan, 2014, Rasheed[211]	July 2011 – March 2012	19-80	Etest®		46	73.9
Malaysia, 2013, Alfizah[292]	May 2004 – Dec 2007	18-89. [55.47]	Etest®		95	45.3
Israel, 2014, Peretz[213]	June 2011 – April 2012	19-79, [48]	Etest®		44	70.5
North America						
Cuba, 2010, Llanes[288]	Sept – Dec 2005	19-68	Etest®		40	85.0
Mixed Age Groups – Adults & Children						
Africa						
The Gambia, 2013, Secka[278]	-	1.5-70, [30]	Agar dilution		64	68.8
Asia						
Malaysia, 2014, Alfizah[214]	May 2004 – Dec 2007	17-89	Etest®		161	36.6

Chapter 2. Review of antibiotic resistance mechanisms in *H. pylori* organisms and prevalence of antibiotic-resistant *H. pylori* infection worldwide during 2010 to 2014

Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Subgroup	Sample Size (n)	Prevalence of Resistance (%)
Japan, 2014, Okamura[215]	Sept 2000 – Aug 2013	2-88, [53.6]	Microbroth dilution		1073	40.2
	Subinterval:	Age Group:				
	2000-2001	<30			10	10.0
	2008-2009				30	56.7
	2012-2013				38	31.6
	2000-2001	31-50			17	11.8
	2008-2009				31	67.7
	2012-2013				29	37.9
	2000-2001	>50			86	11.6
	2008-2009				68	48.5
	2012-2013				114	42.1
Vietnam, 2013, Binh[217]	2008	14-83	Etest®		103	69.9
Iran, 2010, Tomatari[219]	2006 – 2007	17-65	Etest®		128	64.1
Iran, 2010, Siavoshi[218]	2005 – 2008	16-90	Disk diffusion		160	55.6
Iran, 2012, Milani[221]	Dec 2010 – Nov 2011	adults [46] & children 3-14, [5]	Disk diffusion		112	76.8
Iran, 2010, Sirous[220]	Oct 2007 – June 2008	15-75	Disk diffusion		33	51.5
Iran, 2013 Mirzaei[222]	March – June 2011	15-58	Etest®		48	56.3
China, 2010, Gao[216]	2000 – 2009	13-83	Etest®		374	63.9
	Subinterval:					
	2000				54	38.9
	2001				71	36.6
	2002-2003				27	59.3
	2004-2005				27	74.1
	2006-2007				80	81.3
	2008				63	79.4
	2009				52	78.8
Europe						

Chapter 2. Review of antibiotic resistance mechanisms in *H. pylori* organisms and prevalence of antibiotic-resistant *H. pylori* infection worldwide during 2010 to 2014

Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Subgroup	Sample Size (n)	Prevalence of Resistance (%)
France, 2010, Raymond [254]	2004 – 2007	16-87	Etest®		530	61.0*
Eastern Russia, 2012, Reva[253]	2004 – 2009	15-80	Agar dilution		170	56.5
Austria, 2012, Prechtl[256]	2002 – 2009 Subinterval: 2002-2005 2006-2009	3-18 [11.7] [12.56]	Etest®		 38 36	 13.2 30.6
North America						
Mexico, 2011, Ayala[289]	Jan 2002 – Dec 2004	≥15	Etest®	Biopsies from: Antrum Corpus	 90 90	 61.1 62.2
Alaska, 2011, Tveit[290]	Jan 1, 2000 – Dec 31 2008	3-96, [51]	Agar dilution		531	41.8
South America						
Brazil, 2013, Ogata[284]	Feb 2008 – Aug 2009	3-20, [11.1]	Agar dilution		77	40.2
Children <18 years of age						
Asia						
Vietnam, 2012, Nguyen[226]	May 2005 – Feb 2006	3-15	Etest®		222	65.3
Israel, 2010, Zevit[225]	Jan 2005 – Aug 2007	2-17, [13]	Etest®		55	30.9
Israel, 2014, Peretz[213]	June 2011 – April 2012	7-17, [12]	Etest®		41	24.3
Europe						
Turkey, 2014, Karabiber [293]	-	2-17	Disk diffusion		51	11.7

Chapter 2. Review of antibiotic resistance mechanisms in *H. pylori* organisms and prevalence of antibiotic-resistant *H. pylori* infection worldwide during 2010 to 2014

Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Subgroup	Sample Size (n)	Prevalence of Resistance (%)
Age Range of Study Population Not Specified						
Africa						
South Africa, 2010, Tanih[280]	-	-	Agar dilution		200	95.5
Kenya, 2010, Kimang'a [282]	-	children and adults	Etest®		65	4.6
Asia						
Bhutan, 2013, Vilaichone [227]	Dec 6-9 2010	[36.8]	Etest®		111	82.9
South Korea, 2012, Seo[232]	1985 – 1989 1990 – 1994 1995 – 1999 1995 – 1999	[30.0] [33.2] [22.7] [42.0]	Agar dilution	Jinju, South Korea Cheongju, South Korea	53 70 47 23	37.7 24.3 21.3 30.4
Korea, 2013, An[233]	July 2009 – Dec 2010 June 2011 – Dec 2012	- -	Agar dilution		71 94	45.1 56.3
Iran, 2010, Talebi Bezmin Abadi[229]	2007 – 2010	[45.8]	Etest®		132	73.4
China, 2010, Sun[230]	2000 2005 2009	[44.3] [45.2] [42.5]	Agar dilution		58 100 135	48.3 49.0 42.2
China, 2013, Su[231]	Jan 2010 – April 2012	-	Agar dilution		17731	95.4
Europe						
Portugal, 2014, Almeida[261]	Sept 2009 – Oct 2013	[43.3]	Etest®		180	34.4

Chapter 2. Review of antibiotic resistance mechanisms in *H. pylori* organisms and prevalence of antibiotic-resistant *H. pylori* infection worldwide during 2010 to 2014

Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Subgroup	Sample Size (n)	Prevalence of Resistance (%)
Germany, 2014, Selgrad[264]	Jan 2010 – Oct 2012	[52.54]	Etest®		66	51.5
Bulgaria, 2014, Boyanova [262]	2012 – 2013	[50.5]	Etest®		50	34.0
Ireland, 2010, O'Connor [260]	2007 – 2008	[46]	Etest®		219	31.5
Germany, 2013, Wueppenhorst[263]	Jan 2006 – Dec 2011	-	Etest®		5296	67.1
Northern Spain, 2012, Cuadrado-Lavin[257]	Feb – Dec 2010	-	Etest®		71	45.1
Poland, 2011, Karczewska [259]	Dec 2006 – Dec 2008	-	Etest®		115	44.3
Turkey, 2012, Kalem[255]	Jan 2006 – Dec 2009	[50.2]	Etest®		28	39.2

Prevalence of Amoxicillin-Resistant H. pylori Infection

Of the 93 articles estimating the prevalence of antibiotic-resistant *H. pylori* infection, 73 reported results of studies that estimated the prevalence of amoxicillin-resistant *H. pylori* infection among individuals with *H. pylori* infection who had samples of *H. pylori* infection tested for amoxicillin susceptibility in distinct geographic regions: 36 from Asian populations,[210,211,213,214,216–222,224–233,235,236,238–247,249–251] 24 from European populations,[253–257,259,261–265,267–269,271–275,277,293–296] 5 from African populations,[154,278–280,282]

4 from South American populations,[283,284,286,287] 3 North American populations,[288–290] and 1 from an Australian population.[291]

Prevalence of Amoxicillin-resistant H. pylori *Infection in Individuals with No History of Antibiotic Treatment to Eliminate* H. pylori *Infection*

Of the 73 identified studies that reported estimates of the prevalence of amoxicillin-resistant *H. pylori* infection among individuals with *H. pylori* infection who had samples of *H. pylori* tested for amoxicillin susceptibility, 40 estimated the prevalence of amoxicillin-resistant *H. pylori* infection in individuals with no history of antibiotic treatment to eliminate *H. pylori* infection (Table 2.7). The susceptibility of *H. pylori* organisms to amoxicillin was estimated by Etest® in 29 studies,[154,216,225,228,235,236,239,242,247,250,251,254,259,261,264,267–269,272,274,275,277,279,286,287,291,294–296] disk diffusion method in 5,[238,240,241,249,271] agar dilution method in 4,[224,244–246] breakpoint susceptibility testing in 1,[273] and the microdilution method in 1.[243] The estimated prevalence of amoxicillin-resistant *H. pylori* infection in individuals with *H. pylori* infection who had no history of antibiotic treatment to eliminate *H. pylori* infection and who had *H. pylori* samples tested for amoxicillin resistance ranged from 0% to 24%, with the exception of one study conducted by Pandya *et al*. (2014) in India that estimated the prevalence of amoxicillin-resistant *H. pylori* infection among 80 *H. pylori*-positive individuals 10 to 90 years of age to be 73%.[241]

Table 2.7. Prevalence of amoxicillin-resistant *H. pylori* infection in individuals with *H. pylori* infections who had no history of treatment for *H. pylori* infection and who had *H. pylori* tested for amoxicillin susceptibility.

Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Sample Size (n)	Prevalence of Resistance (%)
Adults ≥18 years of age					
Africa					
Senegal, 2013, Seck[154]	2007 – 2009	18-93, [45.3]	Etest®	108	0
Tunisia, 2010, Ben Mansour[279]	March 2005 – Aug 2007	18-88, [38.3]	Etest®	225	0

Chapter 2. Review of antibiotic resistance mechanisms in *H. pylori* organisms and prevalence of antibiotic-resistant *H. pylori* infection worldwide during 2010 to 2014

Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Sample Size (n)	Prevalence of Resistance (%)
Asia					
Northern Iran, 2011, Abadi[238]	Jan – July 2009	18-74, [40.7]	Disk diffusion	197	23.9
Iran, 2012, Talebi Bezmin Abadi[239]	Oct 2009 – July 2010	21-70, [38.6]	Etest®	150	10.0
Thailand, 2013, Vilaichone [236]	Jan 2004 – Dec 2012	>18	Etest®	400	5.2
China, 2014, Song[235]	March 2008 – Feb 2012	18-75, [42.5]	Etest®	600	6.8
Europe					
Portugal, 2014, Almeida[261]	Sept 2009 – Oct 2013	19-77, [41.6]	Etest®	103	1.0
Poland, 2014, Biernat[267]	2008 – 2011	19-89	Etest®	50	0
Finland, 2011, Kostamo[269]	2000 – 2008	18-92, [62]	Etest®	505	0
Belgium, 2011, Miendje Deyi[271]	Jan 1990 – Dec 2009	18.0-98.9	Disk diffusion	7903	0
Oceania					
New Zealand, 2013, Hsiang[291]	Feb – Oct 2012	22-93.5, [59.8]	Etest®	73	5.5
South America					
Brazil, 2011, Eisig[286]	-	22-72	Etest®	39	0
Mixed Age Groups – Adults & Children					
Asia					

Chapter 2. Review of antibiotic resistance mechanisms in *H. pylori* organisms and prevalence of antibiotic-resistant *H. pylori* infection worldwide during 2010 to 2014

Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Sample Size (n)	Prevalence of Resistance (%)
China, 2010, Gao[216]	2000 – 2009 Subinterval: 2000 2001 2002-2003 2004-2005 2006-2007 2008 2009	13-83, [49]	Etest®	290 47 63 22 24 71 39 24	0.3 2.1 0 0 0 0 0 0
Japan, 2010, Kato[243]	total Subinterval: 1999-2002 2003-2007	4-18, [12.6]	Microdilution method	61 34 27	0 0 0
Iran, 2013, Zendedel[240]	Jan – May 2008	19-82 Age Group: <30 30-50 >50	Disk diffusion	82 25 38 19	9.8 8.0 10.5 10.5
India, 2014, Pandya[241]	Feb 2008 – Aug 2011	10-90	Disk diffusion	80	72.5
Europe					
Poland, 2014, Gosciniak[272]	2008 – 2012	4-89	Etest®	165	0
France, 2010, Raymond[254]	2004 – 2007	16-87, [48.8]	Etest®	455	0
Croatia, 2012, Hojsak[295]	Jan 2001 – Dec 2010	1.08-18.8	Etest®	168	0.6
Austria, 2010, Vecsei[294]	March 1 2002 – Feb 28 2008	0.5-20.9	Etest®	153	0
Portugal, 2011, Oleastro[296]	Jan 2000 – Dec 2009	0.33-18, [10.17]	Etest®	1115	0

Chapter 2. Review of antibiotic resistance mechanisms in *H. pylori* organisms and prevalence of antibiotic-resistant *H. pylori* infection worldwide during 2010 to 2014

Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Sample Size (n)	Prevalence of Resistance (%)
Bulgaria, 2010, Boyanova [273]	2007 – 2009	18-87, [39.4] & 5-17, [12.9]	Breakpoint susceptibility testing	501	0.4
South America					
Brazil, 2010, Garcia[287]	2008 – 2009	1-18, [10]	Etest®	45	4.4
Children <18 years of age					
Africa					
Tunisia, 2010, Ben Mansour[279]	March 2005 – Aug 2007	2-14, [8.75]	Etest®	48	0
Asia					
South Korea, 2013, Seo[224]	1990-1994 2005-2009	3.4-17.8 & 2.4-15.8	Agar dilution	58 33	19.0 24.2
China, 2011, Liu[242]	Jan 1 2009 – June 30 2010	3-16, [10]	Etest®	73	0
Israel, 2010, Zevit[225]	Jan 2005 – Aug 2007	2-17, [13]	Etest®	36	0
Europe					
Belgium, 2011, Miendje Deyi[271]	Jan 1990 – Dec 2009	0.2-17.9	Disk diffusion	1527	0
Age Range of Study Population Not Specified					
Asia					
Southern Iran, 2010, Farshad[247]	Oct 2008 – Oct 2009	-	Etest®	121	19.8
Iran, 2013, Sadeghifard [249]	Jan 2009 – March 2010	-	Disk diffusion	50	12.0

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Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Sample Size (n)	Prevalence of Resistance (%)
Korea, 2010, Hwang[244]	Total Subinterval: 2003-2005 2007-2009	[55.4] [53.1] [56.4]	Agar dilution	222 66 156	4.5 6.1 4.8
Korea, 2012, Chung[245]	2004 & 2007 Subinterval: 2004 2007	[50.7]	Agar dilution	185 83 102	2.2 0 3.9
Korea, 2013, Lee[246]	2003 – 2012 Subinterval: 2003-2005 2006-2008 2009-2012	 [53.4] [56.2] [56.7]	Agar dilution	347 64 169 114	 6.3 8.9 14.9
Malaysia, 2011, Goh[228]	Jan – Aug 2009	[50.5]	Etest®	90	0
Malaysia, 2011, Ahmad[250]	Sept 2004 – 2007	-	Etest®	187	0
Malaysia, 2014, Teh[251]	-	-	Etest®	102	0
Europe					
Germany, 2014, Selgrad[264]	Jan 2010 – Oct 2012	[52.54]	Etest®	29	0
18 European countries, 2013, Megraud[268]	April 2008 – June 2009	19-89	Etest®	1893	0.7
Lithuania, 2013, Kupcinskas[274]	1998 2001 2007-2008	[46.9] [48.1] [46.6]	Etest®	89 81 90	0 0 0
Poland, 2011, Karczewska[259]	Dec 2006 – Dec 2008	adults	Etest®	90	0

Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Sample Size (n)	Prevalence of Resistance (%)
Norway, 2013, Larsen[275]	2008 – 2009	-	Etest®	102	10.8
Southern Croatia, 2012, Tonkic[277]	Jan 2008 – Dec 2010	adults	Etest®	345	0

Prevalence of Amoxicillin-resistant H. pylori *Infection in Individuals Previously Treated for* H. pylori *Infection*

Among the 73 articles that reported estimates of amoxicillin-resistant *H. pylori* infection among individuals with *H. pylori* infection who had samples of *H. pylori* tested for amoxicillin susceptibility, 10 estimated the prevalence of amoxicillin-resistant *H. pylori* infection in individuals who had previously been treated with antibiotics to eliminate *H. pylori* infection (Table 2.8). The susceptibility of *H. pylori* organisms to amoxicillin was estimated by Etest® in 8 studies,[216,225,251,254,259,261,264,295] disk diffusion in 1,[271] and agar dilution in 1.[246] Across the identified literature, the prevalence of amoxicillin-resistant *H. pylori* infection among individuals with *H. pylori* infection who were previously treated for *H. pylori* and who had *H. pylori* samples tested for amoxicillin susceptibility was reported to be zero with the exception of 1 article by Lee *et al.* (2013) in Korea that reported the prevalence of amoxicillin-resistant *H. pylori* infection among 48 *H. pylori*-positive individuals previously treated for *H. pylori* infection to be 27%.[246]

Table 2.8. Prevalence of amoxicillin-resistant *H. pylori* infection in individuals with *H. pylori* infection who were previously treated for *H. pylori* infection and who had samples of *H. pylori* tested for amoxicillin susceptibility.

Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Subgroup	Sample Size (n)	Prevalence of Resistance (%)
Adults ≥18 years of age						
Europe						
Portugal, 2014, Almeida[261]	Sept 2009 – Oct 2013	18-85, [45.8]	Etest®		77	0
France, 2010, Raymond [254]	2004 – 2007	18-87, [51.7]	Etest®		75	0
Belgium, 2011, Miendje Deyi[271]	Jan 1990 – Dec 2009	18.0-90.2	Disk diffusion		1209	0
Mixed Age Groups – Adults & Children						
Asia						
China, 2010, Gao[216]	2000 – 2009 Subinterval: 2000 2001 2002-2003 2004-2005 2006-2007 2008 2009	13-83, [49]	Etest®		84 7 8 5 3 9 24 24	0 0 0 0 0 0 0 0
Europe						
Croatia, 2012, Hojsak[295]	Jan 2001 – Dec 2010	1.08-18.8	Etest®		11	0
Children <18 years of age						
Asia						
Israel, 2010, Zevit[225]	Jan 2005 – Aug 2007	2-17, [13]	Etest®		19	0
Europe						

Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Subgroup	Sample Size (n)	Prevalence of Resistance (%)
Belgium, 2011, Miendje Deyi[271]	Jan 1990 – Dec 2009	2.3-17.8	Disk diffusion		162	0
Age Range of Study Population Not Specified						
Asia						
Korea, 2013, Lee[246]	2003 – 2012 Subinterval: 2003-2005 2006-2008 2009-2012	 [57.3] [55.9] [53.7]	Agar dilution		86 6 32 48	 16.7 12.5 27.1
Malaysia, 2014, Teh[251]	-	-	Etest®		8	0
Europe						
Germany, 2014, Selgrad[264]	Jan 2010 – Oct 2012	[52.54]	Etest®	one prior treatment ≥2 prior treatments	13 24	0 0
Poland, 2011, Karczewska[259]	Dec 2006 – Dec 2008	adults	Etest®		25	0

Prevalence of Amoxicillin-resistant H. pylori *Infection in Study Populations with Mixed or Unidentified Anti-*H. pylori *Antibiotic Treatment Histories*

Of the 73 identified articles that reported estimates of the prevalence of amoxicillin-resistant *H. pylori* infection among individuals with *H. pylori* infection who had samples of *H. pylori* tested for amoxicillin susceptibility, 39 estimated the prevalence of amoxicillin-resistant *H. pylori* among individuals from a study population that either included individuals: 1) who had or had not been previously treated with antibiotics to eliminate *H. pylori* infection; or 2) whose previous exposure history to anti-*H. pylori* treatment regimens was not specified (Table 2.9). Antibiotic susceptibility of *H. pylori*

organisms to amoxicillin was estimated by Etest® in 26 studies,[210,211,213,214,216,217,219,222,225–227,229,254–257,259,261–265,282,283,288,289] agar dilution in 9,[230–233,253,278,280,284,290] and disk diffusion in 4.[218,220,221,293] Across the 39 studies in this subgroup, the estimated prevalence of amoxicillin-resistant *H. pylori* infection among individuals with *H. pylori* infection who had samples of *H. pylori* tested for amoxicillin susceptibility ranged from 0% to 54%.

Table 2.9. Prevalence of amoxicillin-resistant *H. pylori* infection among individuals with *H. pylori* infection who had samples of *H. pylori* tested for amoxicillin susceptibility in study populations with either mixed or unidentified anti-*H. pylori* treatment histories.

Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Subgroup	Sample Size (n)	Prevalence of Resistance (%)
Adults ≥18 years of age						
Asia						
Pakistan, 2013, Yakoob[210]	April 2008 – June 2010	18-77, [41]	Etest®		47	4.3
Pakistan, 2014, Rasheed[211]	July 2011 – March 2012	19-80	Etest®		46	54.3
Israel, 2014, Peretz[213]	June 2011 – April 2012	19-79, [48]	Etest®		44	2.3
Europe						
Germany, 2014, Wuppen-horst [265]	2001 – 2012	≥18	Etest®		1651	0
North America						
Cuba, 2010, Llanes[288]	Sept – Dec 2005	19-68	Etest®		40	0
South America						
Southern Brazil, 2014, Picoli[283]	Jan 2011 – Jan 2012	18-80	Etest®		54	1.9
Mixed Age Groups – Adults & Children						
Africa						

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Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Subgroup	Sample Size (n)	Prevalence of Resistance (%)
The Gambia, 2013, Secka[278]	-	1.5-70, [30]	Agar dilution		64	0
Asia						
Malaysia, 2014, Alfizah[214]	May 2004 – Dec 2007	17-89	Etest®		161	0
Vietnam, 2013, Binh[217]	2008	14-83	Etest®		103	0
China, 2010, Gao[216]	2000 – 2009 Subinterval: 2000 2001 2002-2003 2004-2005 2006-2007 2008 2009	13-83, [49]	Etest®		374 54 71 27 27 80 63 52	0.3 1.9 0 0 0 0 0 0
Iran, 2010, Tomatari[219]	2006 – 2007	17-65	Etest®		112	2.5
Iran, 2010, Siavoshi[218]	2005 – 2008	16-90	Disk diffusion		110	7.3
Iran, 2010, Sirous[220]	Oct 2007 – June 2008	15-75	Disk diffusion		33	0
Iran, 2012, Milani[221]	Dec 2010 – Nov 2011	adults [46] children 3-14, [5]	Disk diffusion		112	28.6
Iran, 2013, Mirzaei[222]	March – June 2011	15-58	Etest®		48	4.2
Europe						
Eastern Russia, 2012, Reva[253]	2004 – 2009	15-80	Agar dilution		170	0
France, 2010, Raymond[254]	2004 – 2007	16-87	Etest®		530	0

Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Subgroup	Sample Size (n)	Prevalence of Resistance (%)
Austria, 2012, Prechtl[256]	2002 – 2009 Subinterval: 2002-2005 2006-2009	3-18 [11.7] [12.56]	Etest®		 38 36	 0 0
Turkey, 2012, Kalem[255]	Jan 2006 – Dec 2009	[50.2]	Etest®		28	0
North America						
Mexico, 2011, Ayala[289]	Jan 2002 – Dec 2004	≥15	Etest®	Biopsies from: Antrum Corpus	90	 0 0
Alaska, 2011, Tveit[290]	Jan 1, 2000 – Dec 31 2008	3-96, [51]	Agar dilution		531	1.9
South America						
Brazil, 2013, Ogata[284]	Feb 2008 – Aug 2009	3-20, [11.1]	Agar dilution		77	10.4
Children <18 years of age						
Asia						
Israel, 2010, Zevit[225]	Jan 2005 – Aug 2007	2-17, [13]	Etest®		55	0
Israel, 2014, Peretz[213]	June 2011 – April 2012	7-17, [12]	Etest®		41	12.2
Vietnam, 2012, Nguyen[226]	May 2005 – Feb 2006	3-15	Etest®		222	0.5
Europe						
Turkey, 2014, Karabiber[293]	-	2-17	Disk diffusion		51	11.8
Age Range of Study Population Not Specified						
Africa						

Chapter 2. Review of antibiotic resistance mechanisms in *H. pylori* organisms and prevalence of antibiotic-resistant *H. pylori* infection worldwide during 2010 to 2014

Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Subgroup	Sample Size (n)	Prevalence of Resistance (%)
South Africa, 2010, Tanih[280]	-	-	Agar dilution		200	2.5
Kenya, 2010, Kimang'a [282]	-	Adults and children	Etest®		65	0
Asia						
Bhutan, 2013, Vilaichone [227]	Dec 6-9 2010	[36.8]	Etest®		111	0
China, 2010, Sun[230]	2000 2005 2009	[44.3] [45.2] [42.5]	Agar dilution		58 100 135	0 0 0
China, 2013, Su[231]	Jan 2010 – April 2012	-	Agar dilution		17731	0.1
South Korea, 2012, Seo[232]	1985-1989 1990-1994 1995-1999 1995-1999	[30.0] [33.2] [22.7] [42.0]	Agar dilution	Jinju, South Korea Cheongju, South Korea	53 70 47 23	7.5 24.3 12.8 21.7
Korea, 2013, An[233]	July 2009 – Dec 2010 June 2011 – Dec 2012	-	Agar dilution		71 94	2.8 2.1
Iran, 2010, Talebi Bezmin Abadi[229]	2007 – 2010	[45.8]	Etest®		132	6.8
Europe						
Germany, 2013, Wueppen-horst[263]	Jan 2006 – Dec 2011	-	Etest®		5296	0
Germany, 2014, Selgrad[264]	Jan 2010 – Oct 2012	[52.54]	Etest®		66	0

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Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Subgroup	Sample Size (n)	Prevalence of Resistance (%)
Portugal, 2014, Almeida[261]	Sept 2009 – Oct 2013	[43.3]	Etest®		180	0.6
Bulgaria, 2014, Boyanova [262]	2012 – 2013	[50.5]	Etest®		50	2.0
Northern Spain, 2012, Cuadrado-Lavin[257]	Feb – Dec 2010	adults	Etest®		71	1.4
Poland, 2011, Karczewska [259]	Dec 2006 – Dec 2008	adults	Etest®		115	0

Prevalence of Tetracycline-resistant H. pylori Infection

Of the 93 studies published during 2010 through 2014 that published estimates for the prevalence of antibiotic-resistant *H. pylori* infection, 62 studies estimated the prevalence of tetracycline-resistant *H. pylori* infection among individuals with *H. pylori* infection who had samples of *H. pylori* tested for tetracycline susceptibility in distinct geographic regions: 30 from Asian populations,[211,213,214,216–221,224,225,227,229,230,232,233,235,236,238–240,242,244–251] 22 from European populations,[103,253,254,256,257,261,262,264,265,267–269,271–275,277,293,294,296,297] 3 from African populations,[154,278,280] 4 from South American populations,[284,286,287,298] 2 from North American populations,[288,290] and 1 from an Australian population.[291]

Prevalence of Tetracycline-resistant H. pylori *Infection in Individuals with No History of Antibiotic Treatment to Eliminate* H. pylori *Infection*

Of the 62 identified articles that reported estimates of the prevalence of tetracycline-resistant *H. pylori* infection among individuals with *H. pylori* infection who

had samples of *H. pylori* tested for tetracycline susceptibility, 37 reported estimates for the prevalence of tetracycline-resistant *H. pylori* infection among individuals who had no history of antibiotic treatment to eliminate *H. pylori* infection (Table 2.10). Tetracycline susceptibility of *H. pylori* organisms was estimated by Etest® in 25 studies,[103,154,216,225,235,236,239,242,247,250,251,254,261,264,267–269,272,274,275,277,286,287,291,294] disk diffusion method in 5,[238,240,249,271,296] agar dilution method in 6,[224,244–246,248,298] and breakpoint susceptibility testing in 1.[273] Across the 37 studies that investigated the prevalence of tetracycline-resistant *H. pylori* infection in individuals with *H. pylori* infection who had no history of antibiotic treatment to eliminate *H. pylori* infection and who had samples of *H. pylori* tested for tetracycline susceptibility, estimates ranged from 0% to 54%.

Table 2.10. Prevalence of tetracycline-resistant *H. pylori* infection in individuals with *H. pylori* infection who had no history of treatment for *H. pylori* infection and who had samples of *H. pylori* tested for tetracycline susceptibility.

Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Sample Size (n)	Prevalence of Resistance (%)
Adults ≥18 years of age					
Africa					
Senegal, 2013, Seck[154]	2007 – 2009	18-93, [45.3]	Etest®	108	0
Asia					
Northern Iran, 2011, Abadi[238]	Jan – July 2009	18-74, [40.7]	Disk diffusion	197	37.1
Iran, 2012, Talebi Bezmin Abadi[239]	Oct 2009 – July 2010	21-70, [38.6]	Etest®	150	9.3
Thailand, 2013, Vilaichone[236]	Jan 2004 – Dec 2012	>18	Etest®	400	1.7
China, 2014, Song[235]	March 2008 – Feb 2012	18-75, [42.5]	Etest®	600	3.5
Europe					

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Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Sample Size (n)	Prevalence of Resistance (%)
18 European Countries, 2013, Megraud[268]	April 2008 – June 2009	19-99	Etest®	1893	0.9
Finland, 2011, Kostamo[269]	2000 – 2008	18-92, [62]	Etest®	505	0.2
Belgium, 2011, Miendje Deyi[271]	Jan 1990 – Dec 2009	18-98.9	Disk diffusion	7903	<0.01
Poland, 2014, Biernat[267]	2008 – 2011	19-89	Etest®	50	0
Portugal, 2014, Almeida[261]	Sept 2009 – Oct 2013	19-77, [41.6]	Etest®	103	0
Oceania					
New Zealand, 2013, Hsiang[291]	Feb 2012 – Oct 2012	22-93.5, [59.8]	Etest®	73	0
South America					
Brazil, 2011, Eisig[286]	-	22-72	Etest®	39	0
Mixed Age Groups – Adults & Children					
Asia					
China, 2010, Gao[216]	2006 – 2009 Subinterval: 2006-2007 2008 2009	13-83, [49]	Etest®	104 41 39 24	1.0 0 0 4.2
Iran, 2013, Zendedel[240]	Jan – March 2008	19-82 Age Group: <30 30-50 >50	Disk diffusion	82 25 38 19	0 0 0 0

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Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Sample Size (n)	Prevalence of Resistance (%)
India, 2014, Pandya[241]	Feb 2008 – Aug 2011	10-90	Disk diffusion	80	53.8
Europe					
Bulgaria, 2010, Boyanova[273]	2007 – 2009	18-87, [39.4] 5-17, [12.9]	Breakpoint susceptibility testing	501	3.0
Portugal, 2011, Oleastro[296]	Jan 2000 – Dec 2009	0.33-18, [10.17]	Disk diffusion	1115	0
Austria, 2010, Vecsei[294]	March 2002 – March 2008	0.5-20.9	Etest®	153	0.9
Poland, 2014, Gosciniak[272]	2008 – 2012	4-89	Etest®	165	0
France, 2010, Raymond[254]	2004 – 2007	16-87, [48.8]	Etest®	455	0
South America					
Brazil, 2010, Garcia[287]	2008 – 2009	1-18, [10]	Etest®	45	0
Children <18 years of age					
Asia					
South Korea, 2013, Seo[224]	1990 – 1994 2005 – 2009	3.4-17.8 & 2.4-15.8	Agar dilution	58 33	6.9 12.1
Israel, 2010, Zevit[225]	Jan 2005 – Aug 2007	2-17, [13]	Etest®	36	0
China, 2011, Liu[242]	Jan 1 2009 – June 30 2010	3-16, [10.0]	Etest®	73	0
Europe					

Chapter 2. Review of antibiotic resistance mechanisms in *H. pylori* organisms and prevalence of antibiotic-resistant *H. pylori* infection worldwide during 2010 to 2014

Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Sample Size (n)	Prevalence of Resistance (%)
Belgium, 2011, Miendje Deyi[271]	Jan 1990 – Dec 2009	0.2-17.9	Disk diffusion	1527	0
Age Range of Study Population Not Specified					
Asia					
Southern Iran, 2010, Farshad[247]	Oct 2008 – Oct 2009	-	Etest®	121	3.3
Korea, 2010, Hwang[244]	total Subinterval: 2003-2005 2007-2009	[55.4] [53.1] [56.4]	Agar dilution	222 66 156	25.7 4.5 34.6
Korea, 2012, Chung[245]	2004 & 2007 Subinterval: 2004 2007	[50.7]	Agar dilution	185 83 102	0.5 0 0.5
Korea, 2013, Lee[246]	2003 – 2012 Subinterval: 2003-2005 2006-2008 2009-2012	 [53.4] [56.2] [56.7]	Agar dilution	347 64 169 114	 18.8 32.4 31.0
Iran, 2011, Shokrzadeh[248]	April 2007 – Dec 2008	24 males [45] & 18 females [38]	Agar dilution	42	4.8
Iran, 2013, Sadeghifard[249]	Jan 2009 – March 2010	-	Disk diffusion	50	12.0
Malaysia, 2011, Ahmad[250]	Sept 2004 – 2007	-	Etest®	187	0
Malaysia, 2014, Teh[251]	-	-	Etest®	102	0
Europe					
Norway, 2013, Larsen[275]	2008 – 2009	-	Etest®	102	0

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Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Sample Size (n)	Prevalence of Resistance (%)
Lithuania, 2013, Kupcinskas [274]	1998 2002 2007 – 2008	[46.9] [48.1] [46.6]	Etest®	89 81 90	0 2.5 0
Germany, 2013, Selgrad[103]	Jan 2005 – May 2012	[51.7]	Etest®	94	0
Germany, 2014, Selgrad[264]	Jan 2010 – Oct 2012	[52.54]	Etest®	29	0
Southern Croatia, 2012, Tonkic[277]	Jan 2008 – Dec 2010	adults	Etest®	345	0
South America					
Chile, 2010, Toledo[298]	June 2005 – Oct 2006	-	Agar dilution	41	26.8

Prevalence of Tetracycline-resistant H. pylori *Infection in Individuals Previously Treated for* H. pylori *Infection*

Of the 62 studies estimating the prevalence of tetracycline-resistant *H. pylori* infection among individuals with *H. pylori* infection who had samples of *H. pylori* tested for tetracycline susceptibility, 9 investigated a study population consisting of individuals who had previously been treated with antibiotics to eliminate *H. pylori* infection (Table 2.11). Tetracycline susceptibility of *H. pylori* organisms was estimated by Etest® in 7 studies,[103,216,225,251,254,261,264] disk diffusion in 1,[271] agar dilution in 1.[246] Across the 9 studies that investigated the prevalence of tetracycline-resistant *H. pylori* infection in individuals with *H. pylori* infection who had samples of *H. pylori* tested for tetracycline susceptibility and who had previously been treated for *H. pylori* infection, estimates ranged from 0% to 46%.

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Table 2.11. Prevalence of tetracycline-resistant *H. pylori* infection in individuals with *H. pylori* infection who were previously treated for *H. pylori* infection and who had samples of *H. pylori* tested for tetracycline susceptibility.

Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Subgroup	Sample Size (n)	Prevalence of Resistance (%)
Adults ≥18 years of age						
Europe						
France, 2010, Raymond[254]	2004 – 2007	18-87, [51.7]	Etest®		75	0
Belgium, 2011, Miendje Deyi[271]	Jan 1990 – Dec 2009	18.0-90.2	Disk diffusion		1209	0
Portugal, 2014, Almeida[261]	Sept 2009 – Oct 2013	18-85, [45.8]	Etest®		77	1.3
Mixed Age Groups – Adults & Children						
Asia						
China, 2010, Gao[216]	2006 – 2009 Subinterval: 2006-2007 2008 2009	13-83	Etest®		60 8 24 28	1.7 12.5 0 0
Children <18 years of age						
Asia						
Israel, 2010, Zevit[225]	Jan 2005 – Aug 2007	2-17, [13]	Etest®		19	0
Europe						
Belgium, 2011, Miendje Deyi[271]	Jan 1990 – Dec 2009	2.3-17.8	Disk diffusion		162	0
Age Range of Study Population Not Specified						
Asia						

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Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Subgroup	Sample Size (n)	Prevalence of Resistance (%)
Korea, 2013, Lee[246]	2003 – 2012 Subinterval: 2003-2005 2006-2008 2009-2012	 [57.3] [55.9] [53.7]	Agar dilution		86 6 32 48	 16.7 34.4 45.8
Malaysia, 2014, Teh[251]	-	-	Etest®		8	0
Europe						
Germany, 2013, Selgrad[103]	Jan 2005 – May 2012	[51.7]	Etest®	one prior treatment ≥2 prior treatments	54 103	1.8 0.1
Germany, 2014, Selgrad[264]	Jan 2010 – Oct 2012	[52.54]	Etest®	one prior treatment ≥2 prior treatments	13 24	0 0

Prevalence of Tetracycline-resistant H. pylori *Infection in Study Populations with Mixed or Unidentified Anti-*H. pylori *Antibiotic Treatment Histories*

Of the 62 identified articles that reported estimates of the prevalence of tetracycline-resistant *H. pylori* infection among individuals with *H. pylori* infection who had samples of *H. pylori* tested for tetracycline susceptibility, 30 estimated the prevalence of tetracycline-resistant *H. pylori* infection among individuals from a study population that either included individuals: 1) who had or had not been previously treated with antibiotics to eliminate *H. pylori* infection; or 2) whose previous exposure history to anti-*H. pylori* treatment regimens was not specified (Table 2.12). Tetracycline susceptibility of *H. pylori* organisms was estimated by Etest® in 18 studies,[211,213,214,216,217,219,225,227,229,254,256,257,261,262,264,265,288,297] agar dilution in 8,[230,232,233,253,278,280,284,290] and disk diffusion in 4.[218,220,221,293] The estimated prevalence of tetracycline-resistant *H. pylori* infection among individuals with *H. pylori* infection who

had samples of *H. pylori* tested for tetracycline susceptibility and who had either mixed or unidentified anti-*H. pylori* treatment histories ranged from 0% to 38%.

Table 2.12. Prevalence of tetracycline-resistant *H. pylori* infection among individuals with *H. pylori* infection who had samples of *H. pylori* tested for tetracycline susceptibility from study populations with either mixed or unidentified anti-*H. pylori* treatment histories.

Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Subgroup	Sample Size (n)	Prevalence of Resistance (%)
Adults ≥18 years of age						
Asia						
Pakistan, 2014, Rasheed[211]	July 2011 – March 2012	19-80	Etest®		46	4.3
Israel, 2014, Peretz[213]	June 2011 – April 2012	19-79, [48]	Etest®		44	2.3
Europe						
Germany, 2014, Wueppenhorst[265]	July 2001 – Dec 2012	≥18	Etest®		1651	0.5
North America						
Cuba, 2010, Llanes[288]	Sept – Dec 2005	19-68	Etest®		40	0
Mixed Age Groups – Adults & Children						
Africa						
The Gambia, 2013, Secka[278]	-	1.5-70, [30]	Agar dilution		64	0
Asia						
China, 2010, Gao[216]	2006 – 2009 Subinterval: 2006-2007 2008 2009	13-83, [49]	Etest®		164 49 63 52	1.2 2.0 0 1.9
Iran, 2010, Siavoshi[218]	2005 – 2008	16-90	Disk diffusion		160	38.1
Iran, 2010, Tomatari[219]	2006 – 2007	17-65	Etest®		128	0

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Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Subgroup	Sample Size (n)	Prevalence of Resistance (%)
Iran, 2010, Sirous[220]	Oct 2007 – June 2008	15-75	Disk diffusion		33	0
Iran, 2012, Milani[221]	Dec 2010 – Nov 2011	adults [46] & children 3-14, [5]	Disk diffusion		112	18.7
Vietnam, 2013, Binh[217]	2008	14-83	Etest®		103	5.8
Malaysia, 2014, Alfizah[214]	May 2004 – Dec 2007	17-89	Etest®		161	0
Europe						
Austria, 2012, Prechtl[256]	2002 – 2009 Subinterval: 2002-2005 2006-2009	3-18 [12.56] [13]	Etest®		 38 36	 0 0
France, 2010, Raymond [254]	2004 – 2007	16-87	Etest®		530	0
Eastern Russia, 2012, Reva[253]	2004 – 2009	15-80	Agar dilution		170	2.4
North America						
Alaska, 2011, Tveit[290]	Jan 1, 2000 – Dec 31 2008	3-96, [51]	Agar dilution		531	0
South America						
Brazil, 2013, Ogata[284]	Feb 2008 – Aug 2009	3-20, [11.1]	Agar dilution		77	0
Children <18 years of age						
Asia						
Israel, 2010, Zevit[225]	Jan 2005 – Aug 2007	2-17, [13]	Etest®		55	0

Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Subgroup	Sample Size (n)	Prevalence of Resistance (%)
Israel, 2014, Peretz[213]	June 2011 – April 2012	7-17, [12]	Etest®		41	2.4
Europe						
Turkey, 2014, Karabiber[293]	-	2-17	Disk diffusion		51	7.8
Age Range of Study Population Not Specified						
Africa						
South Africa, 2010, Tanih[280]	-	-	Agar dilution		200	32.5
Asia						
China, 2010, Sun[230]	2000 2005 2009	[44.3] [45.2] [42.5]	Agar dilution		58 100 135	0 1.0 0
Iran, 2010, Talebi Bezmin Abadi[229]	2007 – 2010	[45.8]	Etest®		132	9.0
South Korea, 2012, Seo[232]	1985-1989 1990-1994 1995-1999 1995-1999	[30.0] [33.2] [22.7] [42.0]	Agar dilution	Jinju, South Korean Cheongju, South Korea	53 70 47 23	9.4 17.1 12.8 30.4
Korea, 2013, An[233]	July 2009 – Dec 2010 June 2011 – Dec 2012	- -	Agar dilution		71 94	0 0
Bhutan, 2013, Vilaichone[227]	Dec 6-9 2010	[36.8]	Etest®		111	0
Europe						

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Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Subgroup	Sample Size (n)	Prevalence of Resistance (%)
Northern Spain, 2012, Cuadrado-Lavin[257]	Feb – Dec 2010	adults	Etest®		71	0
Bulgaria, 2014, Boyanova [262]	2012 – 2013	[50.5]	Etest®		50	2.0
Germany, 2014, Selgrad[264]	Jan 2010 – Oct 2012	[52.54]	Etest®		66	0
Portugal, 2014, Almeida[261]	Sept 2009 – Oct 2013	[43.3]	Etest®		180	0.6
Ireland, 2013, O'Connor [297]	2008 – 2009	[46]	Etest®		85	0

Prevalence of Levofloxacin-resistant *H. pylori* Infection

Of the 93 studies identified, 42 estimated the prevalence of levofloxacin-resistant *H. pylori* infection among individuals with *H. pylori* infection who had samples of *H. pylori* tested for levofloxacin susceptibility in distinct geographic regions: 20 from Asian populations,[214,216,217,224,225,227,228,230–233,235,236,239,241,242,244,246,250,251] 19 from European populations,[103,237,253,257,259,261,262,264,266–269,272,275–277,294,297,299] 1 from African populations,[154] 1 from a North American population[290] and 1 from a South American population.[286]

Prevalence of Levofloxacin-resistant H. pylori *Infection in Individuals with No History of Antibiotic Treatment to Eliminate* H. pylori *Infection*

Of the 42 studies that estimated the prevalence of levofloxacin-resistant *H. pylori* infection among individuals with *H. pylori* infection who had samples of *H. pylori* tested

for levofloxacin susceptibility, 30 estimated this prevalence in individuals with no history of antibiotic treatment to eliminate *H. pylori* infection (Table 2.13). Levofloxacin susceptibility of *H. pylori* isolates was estimated by Etest® in 25 studies,[103,154,216,225,228,235–237,239,242,250,251,259,261,264,266–269,272,275,277,286,294,299] disk diffusion method in 1,[241] agar dilution method in 3,[224,244,246] and ASP-PCR analysis in 1.[276] Across the 30 studies that investigated the prevalence of levofloxacin-resistant *H. pylori* infection among individuals with *H. pylori* infection who had samples of *H. pylori* tested for levofloxacin susceptibility and who had no history of antibiotic treatment to eliminate *H. pylori* infection, estimates ranged from 0% to 46%.

Table 2.13. Prevalence of levofloxacin-resistant *H. pylori* infection among individuals with *H. pylori* infection who had samples of *H. pylori* tested for levofloxacin susceptibility and who had no history of treatment for *H. pylori* infection.

Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Subgroup	Sample Size (n)	Prevalence of Resistance (%)
Adults ≥18 years of age						
Africa						
Senegal, 2013, Seck[154]	2007 – 2009	18-93, [45.3]	Etest®		108	14.8
Asia						
Iran, 2012, Talebi Bezmin Abadi[239]	Oct 2009 – July 2010	21-70, [38.6]	Etest®		150	5.3
Thailand, 2013, Vilaichone [236]	Jan 2004 – Dec 2012	>18	Etest®		208	7.2
China, 2014, Song[235]	March 2008 – Feb 2012	18-75, [42.5]	Etest®		600	33.5
Europe						
18 European Countries, 2013, Megraud[268]	April 2008 – June 2009	19-99	Etest®		1893	14.1

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Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Subgroup	Sample Size (n)	Prevalence of Resistance (%)
Poland, 2014, Biernat[267]	2008 – 2011	19-89	Etest®		50	8.0
Southern Poland, 2012, Karczewska[266]	Jan 2009 – Dec 2011	18-75, [45.6]	Etest®		43	11.6
Finland, 2011, Kostamo[269]	2000 – 2008	18-92	Etest®		505	7.0
Italy, 2012, Saracino[237]	Jan 2010 – Dec 2011	24-83	Etest®		145	22.1
Portugal, 2014, Almeida[261]	Sept 2009 – Oct 2013	19-77, [41.6]	Etest®		103	26.2
South America						
Brazil, 2011, Eisig[286]	-	22-72	Etest®		39	0
Mixed Age Groups – Adults & Children						
Asia						
China, 2010, Gao[216]	2006 – 2009 Subinterval: 2006-2007 2008 2009	13-83, [49]	Etest®		103 40 39 24	36.9 25.0 46.2 41.7
India, 2014, Pandya[241]	Feb 2008 – Aug 2011	10-90	Disk diffusion		80	13.8
Europe						
Austria, 2010, Vecsei[294]	March 1 2002 – Feb 28 2008	0.5-20.9	Etest®		153	0
Poland, 2014, Gosciniak[272]	2008 – 2012	4-18 Age Group: 4-18 19-89	Etest®		165 105 60	5.5 1.9 11.7

Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Subgroup	Sample Size (n)	Prevalence of Resistance (%)
Southern Poland, 2014, Karczewska [299]	2006 – 2012	16-87	Etest®		171	5.9
Children <18 years of age						
Asia						
South Korea, 2013, Seo[224]	1990 – 1994 2005 – 2009	3.4-17.8 & 2.4-15.8	Agar dilution		58 33	12.1 12.1
Israel, 2010, Zevit[225]	Jan 2005 – Aug 2007	2-17, [13]	Etest®		36	0
China, 2011, Liu[242]	Jan 1 2009 – Jun 30 2010	3-16, [10.0]	Etest®		73	13.7
Age Range of Study Population Not Specified						
Asia						
Korea, 2010, Hwang[244]	Total Subinterval: 2003-2005 2007-2009	[55.4] [53.1] [56.4]	Agar dilution		222 66 156	22.1 4.5 29.5
Korea, 2013, Lee[246]	2003 – 2012 Subinterval: 2003-2005 2006-2008 2009-2012	 [53.4] [56.2] [56.7]	Agar dilution		347 64 169 114	 4.7 27.2 28.1
Malaysia, 2011, Goh[228]	Jan – Aug 2009	[50.5]	Etest®		90	0
Malaysia, 2011, Ahmad[250]	Sept 2004 – 2007	-	Etest®		187	0.1
Malaysia, 2014, Teh[251]	-	-	Etest®		102	6.8
Europe						

Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Subgroup	Sample Size (n)	Prevalence of Resistance (%)
Poland, 2011, Karczewska [259]	Dec 2006 – Dec 2008	Adults	Etest®		90	2.2
Norway, 2013, Larsen[275]	2008 – 2009	-	Etest®		102	3.9
Germany, 2013, Selgrad[103]	Jan 2005 – May 2012	[51.7]	Etest®		60	11.7
Germany, 2014, Selgrad[264]	Jan 2010 – Oct 2012	[52.54]	Etest®		29	13.8
Greece, 2014, Kara-manolis[276]	2000 2010	- -	ASP-PCR		50 57	0 5.3
Southern Croatia, 2012, Tonkic[277]	Jan 2008 – Dec 2010	adults	Etest®		175	4.6

Prevalence of Levofloxacin-resistant H. pylori *Infection in Individuals Previously Treated for* H. pylori *Infection*

Of the 42 studies estimating the prevalence of levofloxacin-resistant *H. pylori* infection among individuals with *H. pylori* infection who had samples of *H. pylori* tested for levofloxacin susceptibility, 10 estimated the prevalence of levofloxacin-resistant *H. pylori* infection in individuals who had previously been treated with antibiotics to eliminate *H. pylori* infection (Table 2.14). The susceptibility of *H. pylori* organisms to levofloxacin was estimated by Etest® in 9 studies[103,216,225,251,259,261,264,266,299] and agar dilution in 1.[246] Estimates of the prevalence of levofloxacin-resistant *H. pylori* infection among individuals with *H. pylori* infection who had previously been treated for *H. pylori* infection and who had samples of *H. pylori* tested for levofloxacin susceptibility ranged from 0% to 82%.

Table 2.14. Prevalence of levofloxacin-resistant *H. pylori* infection among individuals with *H. pylori* infection who had samples of *H. pylori* tested for levofloxacin susceptibility and who had previously been treated with antibiotics to eliminate *H. pylori* infection.

Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Subgroup	Sample Size (n)	Prevalence of Resistance (%)
Adults ≥18 years of age						
Europe						
Southern Poland, 2012, Karczewska [266]	Jan 2009 – Dec 2011	18-75, [45.6]	Etest®		8	37.5
Portugal, 2014, Almeida[261]	Sept 2009 – Oct 2013	18-85, [45.8]	Etest®		77	44.2
Mixed Age Groups – Adults & Children						
Asia						
China, 2010, Gao[216]	2006 – 2009 Subinterval: 2006-2007 2008 2009	13-83, [49]	Etest®		60 8 24 28	73.3 37.5 75.0 82.1
Europe						
Southern Poland, 2014, Karczewska [299]	2006 – 2012	16-87, [45]	Etest®		39	18.0
Children <18 years of age						
Asia						
Israel, 2010, Zevit[225]	Jan 2005 – Aug 2007	2-17, [13]	Etest®		19	0
Age Range of Study Population Not Specified						
Asia						

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Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Subgroup	Sample Size (n)	Prevalence of Resistance (%)
Korea, 2013, Lee[246]	2003 – 2012 Subinterval: 2003-2005 2006-2008 2009-2012	 [57.3] [55.9] [53.7]	Agar dilution		86 6 32 48	 16.7 28.1 50.0
Malaysia, 2014, Teh[251]	-	-	Etest®		8	25.0
Europe						
Poland, 2011, Karczewska[259]	Dec 2006 – Dec 2008	adults	Etest®		25	16.0
Germany, 2013, Selgrad[103]	Jan 2005 – May 2012	[51.7]	Etest®	one prior treatment ≥2 prior treatments	34 77	17.6 36.4
Germany, 2014, Selgrad[264]	Jan 2010 – Oct 2012	[52.54]	Etest®	one prior treatment ≥2 prior treatments	13 24	23.1 33.3

Prevalence of Levofloxacin-resistant H. pylori *Infection in Study Populations with Mixed or Unidentified Anti*-H. pylori *Antibiotic Treatment Histories*

Of the 42 studies that estimated the prevalence of levofloxacin-resistant *H. pylori* infection among individuals with *H. pylori* infection who had samples of *H. pylori* tested for levofloxacin susceptibility, 17 estimated the prevalence of levofloxacin-resistant *H. pylori* in individuals from a study population that either included individuals: 1) who had or had not been previously treated with antibiotics to eliminate *H. pylori* infection; or 2) whose previous exposure history to anti-*H. pylori* treatment regimens was not specified (Table 2.15). The susceptibility of *H. pylori* organisms to levofloxacin was estimated by Etest® in 12 studies[214,216,217,225,227,257,259,261,262,290,297,299] and agar dilution in 5.[230–233,253] The estimated prevalence of levofloxacin-resistant *H. pylori* infection among individuals with

H. pylori infection who had samples of *H. pylori* tested for levofloxacin susceptibility from a study populations of individuals with either mixed or unidentified anti-*H. pylori* infection ranged from 0% to 64%.

Table 2.15. Prevalence of levofloxacin-resistant *H. pylori* infection among individuals with *H. pylori* infection who had samples of *H. pylori* tested for levofloxacin susceptibility in study populations with either mixed or unidentified anti-*H. pylori* treatment histories.

Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Subgroup	Sample Size (n)	Prevalence of Resistance (%)
Mixed Age Groups – Adults & Children						
Asia						
China, 2010, Gao[216]	2000 – 2009 Subinterval: 2006-2007 2008 2009	13-83, [49]	Etest®		163 48 63 52	50.3 27.1 57.1 63.5
Vietnam, 2013, Binh[217]	2008	14-83	Etest®		103	18.4
Malaysia, 2014, Alfizah[214]	May 2004 – Dec 2007	17-89	Etest®		161	0.6
Europe						
Eastern Russia, 2012, Reva[253]	2004 – 2009	15-80	Agar dilution		170	15.9
Southern Poland, 2014, Karczewska[299]	2006 – 2012	16-87, [45]	Etest®		210	8.1
North America						
Alaska, 2011, Tveit[290]	Aug 2004 – Dec 2008	3-96, [51]	Etest®		155	19.4
Children <18 years of age						
Asia						

Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Subgroup	Sample Size (n)	Prevalence of Resistance (%)
Israel, 2010, Zevit[225]	Jan 2005 – Aug 2007	2-17, [13]	Etest®		55	0
Age Range of Study Population Not Specified						
Asia						
China, 2010, Sun[230]	2000 2005 2009	[44.3] [45.2] [42.5]	Agar dilution		58 100 135	10.3 24.0 32.6
China, 2013, Su[231]	Jan 2010 – April 2012	-	Agar dilution		17731	20.6
South Korea, 2012, Seo[232]	1985-1989 1990-1994 1995-1999 1995-1999	[30.0] [33.2] [22.7] [42.0]	Agar dilution	Jinju, South Korea Cheongju, South Korea	53 70 47 23	9.4 10.0 6.4 26.1
Korea, 2013, An[233]	July 2009 – Dec 2010 June 2011 – Dec 2012	- -	Agar dilution		71 94	26.8 22.3
Bhutan, 2013, Vilaichone[227]	Dec 6-9 2010	[36.8]	Etest®		111	2.7
Europe						
Poland, 2011, Karczewska[259]	Dec 2006 – Dec 2008	adults	Etest®		115	5.2
Northern Spain, 2012, Cuadrado-Lavin[257]	Feb – Dec 2010	adults	Etest®		69	14.5
Bulgaria, 2014, Boyanova[262]	2012 – 2013	[50.5]	Etest®		50	18.0

Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Subgroup	Sample Size (n)	Prevalence of Resistance (%)
Portugal, 2014, Almeida[261]	Sept 2009 – Oct 2013	[43.3]	Etest®		180	33.9
Ireland, 2013, O'Connor[297]	2008 – 2009	[46]	Etest®		85	11.7

Prevalence of Ciprofloxacin-resistant *H. pylori* Infection

The literature search identified 25 articles that reported estimates for the prevalence of ciprofloxacin-resistant *H. pylori* infection among individuals with *H. pylori* infection who had samples of *H. pylori* tested for ciprofloxacin susceptibility in distinct geographic regions: 15 from Asian populations,[211,214,221,224,227,232,236,238,241,244,245,248,250,251,300] 7 from European populations,[253,254,257,271,273,274,296] 1 from an African population,[280] 1 from a South American population,[283] and 1 from a North American population.[288]

Prevalence of Ciprofloxacin-resistant H. pylori *Infection in Individuals with No History of Antibiotic Treatment to Eliminate* H. pylori *Infection*

Among the 25 studies that estimated the prevalence of ciprofloxacin-resistant *H. pylori* infection among individuals with *H. pylori* infection and who had samples of *H. pylori* tested for ciprofloxacin susceptibility, 14 estimated this prevalence in study populations with no history of antibiotic treatment to eliminate *H. pylori* infection (Table 2.16). The susceptibility of *H. pylori* organisms to ciprofloxacin was estimated by Etest® in 5 studies,[236,250,251,254,274] disk diffusion method in 4,[238,241,271,296] agar dilution method in 4,[224,244,245,248] and breakpoint susceptibility testing in 1.[273] The estimated prevalence of ciprofloxacin-resistant *H. pylori* infection in individuals with *H. pylori* infection who had samples of *H. pylori* tested for ciprofloxacin susceptibility and had no history of antibiotic treatment to eliminate *H. pylori* infection ranged from 0.1% to 50%.

Table 2.16. Prevalence of ciprofloxacin-resistant *H. pylori* infection among individuals with *H. pylori* infection who had no history of treatment for *H. pylori* infection and who had samples of *H. pylori* tested for ciprofloxacin susceptibility.

Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Sample Size (n)	Prevalence of Resistance (%)
Adults ≥18 years of age					
Asia					
Northern Iran, 2011, Abadi[238]	Jan – July 2009	18-74, [40.7]	Disk diffusion	197	34.5
Thailand, 2013, Vilaichone [236]	Jan 2004 – Dec 2012	>18	Etest®	208	7.7
Europe					
Belgium, 2011, Miendje Deyi[271]	Jan 1990 – Dec 2009	18-98.9	Disk diffusion	7903	1.5
Mixed Age Groups – Adults & Children					
Asia					
India, 2014, Pandya[241]	Feb 2008 – Aug 2011	10-90	Disk diffusion	80	50.0
Europe					
Bulgaria, 2010, Boyanova [273]	2007 – 2009	18-87, [39.4] & 5-17, [12.9]	Breakpoint susceptibility testing	501	9.2
Portugal, 2011, Oleastro[296]	Jan 2000 – Dec 2009	0.33-18, [10.17]	Disk diffusion	1115	4.6
France, 2010, Raymond [254]	2004 – 2007	16-87, [48.8]	Etest®	455	12.1
Children <18 years of age					
Asia					
South Korea, 2013, Seo[224]	1990 – 1994 2005 – 2009	3.4-17.8 & 2.4-15.8	Agar dilution	58 33	13.8 15.2

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Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Sample Size (n)	Prevalence of Resistance (%)
Europe					
Belgium, 2011, Miendje Deyi[271]	Jan 1990 – Dec 2009	0.2-17.9	Disk diffusion	1527	0.4
Age Range of Study Population Not Specified					
Asia					
Korea, 2010, Hwang[244]	Total Subinterval: 2003-2005 2007-2009	[55.4] [53.1] [56.4]	Agar dilution	222 66 156	29.3 16.7 34.6
Korea, 2012, Chung[245]	2004 & 2007 Subinterval: 2004 2007	[50.7]	Agar dilution	185 83 102	15.7 15.7 15.7
Iran, 2011, Shokrzadeh[248]	April 2007 – Dec 2008	24 males [45] & 18 females [38]	Agar dilution	42	2.4
Malaysia, 2011, Ahmad[250]	Sept 2004 – 2007	-	Etest®	187	0.1
Malaysia, 2014, Teh[251]	-	-	Etest®	102	6.8
Europe					
Lithuania, 2013, Kupcinskas[274]	2007 – 2008	[46.6]	Etest®	90	5.6

Prevalence of Ciprofloxacin-resistant H. pylori *Infection in Individuals Previously Treated for* H. pylori *Infection*

Among the 25 studies that estimated the prevalence of ciprofloxacin-resistant *H. pylori* infection among individuals with *H. pylori* infection who had samples of *H. pylori* tested for ciprofloxacin susceptibility, 3 estimated the prevalence of ciprofloxacin-resistant *H. pylori* infection among individuals who had previously been treated with antibiotics to eliminate *H. pylori* infection (Table 2.17). The susceptibility of *H. pylori* organisms to ciprofloxacin was estimated by Etest® in 2 studies[251,254] and disk diffusion in 1.[271] Across the 3 identified studies, the prevalence of ciprofloxacin-resistant *H. pylori* infection among individuals with *H. pylori* infection who had previously been treated for *H. pylori* infection and who had samples of *H. pylori* tested for ciprofloxacin susceptibility ranged from 0.6% to 25%.

Table 2.17. Prevalence of ciprofloxacin-resistant *H. pylori* infection among individuals with *H. pylori* infection who were previously treated for *H. pylori* infection and who had samples of *H. pylori* tested for ciprofloxacin susceptibility.

Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Sample Size (n)	Prevalence of Resistance (%)
Adults ≥18 years of age					
Europe					
Belgium, 2011, Miendje Deyi[271]	Jan 1990 – Dec 2009	18 – 90.2	Disk diffusion	1209	0.7
Mixed Age Groups – Adults & Children					
Europe					
France, 2010, Raymond[254]	2004 – 2007	18-87, [51.7]	Etest®	75	20.0
Children <18 years of age					
Europe					
Belgium, 2011, Miendje Deyi[271]	Jan 1990 – Dec 2009	2.3-17.8	Disk diffusion	162	0.6

Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Sample Size (n)	Prevalence of Resistance (%)
Age Range of Study Population Not Specified					
Asia					
Malaysia, 2014, Teh[251]	-	-	Etest®	8	25.0

Prevalence of Ciprofloxacin-resistant H. pylori *Infection in Study Populations with Mixed or Unidentified Anti*-H. pylori *Antibiotic Treatment Histories*

Of the 25 studies that estimated the prevalence of ciprofloxacin-resistant *H. pylori* infection among individuals with *H. pylori* infection who had samples of *H. pylori* tested for ciprofloxacin susceptibility, 12 estimated this prevalence in study populations that combined individuals who had or had not been previously treated with antibiotics to eliminate *H. pylori* infection or individuals whose previous exposure history to anti-*H. pylori* treatment regimens was not specified (Table 2.18). The susceptibility of *H. pylori* organisms to ciprofloxacin was estimated by Etest® in 8 studies,[211,214,227,254,257,283,288,300] agar dilution in 3,[232,253,280] and disk diffusion in 1.[221] Across the identified studies that estimated the prevalence of ciprofloxacin-resistant *H. pylori* infection among individuals with *H. pylori* infection who had samples of *H. pylori* tested for ciprofloxacin susceptibility and who had mixed or unidentified anti-*H. pylori* treatment histories, estimates ranged from 0% to 65%.

Table 2.18. Prevalence of ciprofloxacin-resistant *H. pylori* infection among individuals with *H. pylori* infection who had samples of *H. pylori* tested for ciprofloxacin susceptibility among study populations with either mixed or unidentified anti-*H. pylori* treatment histories.

Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Subgroup	Sample Size (n)	Prevalence of Resistance (%)
Adults ≥18 years of age						
Asia						
Pakistan, 2014, Rasheed[211]	July 2011 – March 2012	19-80	Etest®		46	13.0
North America						
Cuba, 2010, Llanes[288]	Sept – Dec 2005	19-68	Etest®		40	22.5
South America						
Southern Brazil, 2014, Picoli[283]	Jan 2011 – Jan 2012	18-80	Etest®		54	5.5
Mixed Age Groups – Adults & Children						
Asia						
Iran, 2012, Milani[221]	Dec 2010 – Nov 2011	adults [46] children 3-14, [5]	Disk diffusion		112	33.0
Malaysia, 2014 Alfizah[214]	May 2004 – Dec 2007	17-89	Etest®		161	1.2
Europe						
France, 2010, Raymond [254]	2004 – 2007	16-87	Etest®		530	13.2
Eastern Russia, 2012, Reva[253]	2004 – 2009	15-80	Agar dilution		170	0
Age Range of Study Population Not Specified						
Africa						

Chapter 2. Review of antibiotic resistance mechanisms in *H. pylori* organisms and prevalence of antibiotic-resistant *H. pylori* infection worldwide during 2010 to 2014

Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Subgroup	Sample Size (n)	Prevalence of Resistance (%)
South Africa, 2010, Tanih[280]	-	-	Agar dilution		200	0
Asia						
South Korea, 2012, Seo[232]	1985-1989 1990-1994 1995-1999 1995-1999	[30.0] [33.2] [22.7] [42.0]	Agar dilution	Jinju, South Korea Cheongju, South Korea	53 70 47 23	9.8 8.6 6.4 17.4
Bhutan, 2013, Vilaichone[227]	Dec 6-9 2010	[36.8]	Etest®		111	2.7
Iran, 2014, Keshavarz Azizi Raftar[300]	2013	-	Etest®		95	65.3
Europe						
Northern Spain, 2012, Cuadrado-Lavin[257]	Feb – Dec 2010	adults	Etest®		70	14.3

Prevalence of Rifampicin-resistant *H. pylori* Infection

Of the 93 articles identified by the literature search, 10 reported estimates of the prevalence of rifampicin-resistant *H. pylori* infection among individuals with *H. pylori* infection who had samples of *H. pylori* tested for rifampicin susceptibility in distinct geographic regions: 5 from Asian populations[221,228,235,242,251] and 5 from European populations.[253,254,262,275,294]

Chapter 2. Review of antibiotic resistance mechanisms in *H. pylori* organisms and prevalence of antibiotic-resistant *H. pylori* infection worldwide during 2010 to 2014

Prevalence of Rifampicin-resistant H. pylori *Infection in Individuals with No History of Antibiotic Treatment to Eliminate* H. pylori *Infection*

Of the 10 studies that estimated the prevalence of rifampicin-resistant *H. pylori* infection among individuals with *H. pylori* infection who had samples of *H. pylori* tested for rifampicin susceptibility, 6 reported estimates for the prevalence of rifampicin-resistant *H. pylori* infection in individuals with no history of antibiotic treatment to eliminate *H. pylori* infection (Table 2.19). Each of the 6 studies estimated the susceptibility of *H. pylori* organisms to rifampicin by Etest®.[228,235,242,251,275,294] The estimated prevalence of rifampicin-resistant *H. pylori* infection among individuals with *H. pylori* infection who had samples of *H. pylori* tested for rifampicin susceptibility ranged from 0% to 14% in individuals with no history of antibiotic treatment to eliminate *H. pylori* infection.

Table 2.19. Prevalence of rifampicin-resistant *H. pylori* infection among individuals with *H. pylori* infection who had samples of *H. pylori* tested for rifampicin susceptibility and who had no history of treatment for *H. pylori* infection.

Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Sample Size (n)	Prevalence of Resistance (%)
Adults ≥18 years of age					
Asia					
China, 2014, Song[235]	March 2008 – Feb 2012	18-75, [42.5]	Etest®	600	14.2
Mixed Age Groups – Adults & Children					
Europe					
Austria, 2010, Vecsei[294]	March 1 2002 – Feb 28 2008	0.5-20.9	Etest®	153	0.9
Children <18 years of age					
Asia					
China, 2011, Liu[242]	Jan 1 2009 – June 30 2010	3-16, [10.0]	Etest®	73	6.8

Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Sample Size (n)	Prevalence of Resistance (%)
Age Range of Study Population Not Specified					
Asia					
Malaysia, 2011, Goh[228]	Jan – Aug 2009	[50.5]	Etest® cutpoint: ≥1 µg/mL ≥4 µg/mL ≥16 µg/mL	90	 14.4 2.2 0
Malaysia, 2014, Teh[251]	-	-	Etest®	102	0
Europe					
Norway, 2013, Larsen[275]	2008 – 2009	-	Etest®	102	0

Prevalence of Rifampicin-resistant H. pylori *Infection in Individuals Previously Treated for* H. pylori *Infection*

Of the 10 articles that reported estimates of the prevalence of rifampicin-resistant *H. pylori* infection among individuals with *H. pylori* infection who had samples of *H. pylori* tested for rifampicin susceptibility, just 1 estimated the prevalence of rifampicin-resistant *H. pylori* infection among individuals who had previously been treated with antibiotics to eliminate *H. pylori* infection (Table 2.20). Teh *et al.* (2014) reported all 8 *H. pylori* isolates collected from individuals with *H. pylori* infection who had samples of *H. pylori* tested for rifampicin susceptibility and who had previously been treated with antibiotics to eliminate *H. pylori* infection to be susceptible to rifampicin.[251]

Table 2.20. Prevalence of rifampicin-resistant *H. pylori* infection among individuals with *H. pylori* infection who had samples of *H. pylori* tested for rifampicin susceptibility and who had previously been treated with antibiotics to eliminate *H. pylori* infection.

Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Sample Size (n)	Prevalence of Resistance (%)
Age Range of Study Population Not Specified					
Asia					
Malaysia, 2014, Teh[251]	-	-	Etest®	8	0

Prevalence of Rifampicin-resistant H. pylori *Infection in Study Populations with Mixed or Unidentified Anti-*H. pylori *Antibiotic Treatment Histories*

Of the 10 studies estimating the prevalence of rifampicin-resistant *H. pylori* among individuals with *H. pylori* infection who had samples of *H. pylori* tested for rifampicin susceptibility, 4 estimated the prevalence of rifampicin-resistant *H. pylori* infection from a study population which either combined individuals who had or had not been previously treated with antibiotics to eliminate *H. pylori* infection or included individuals whose previous exposure history to anti-*H. pylori* treatment regimens was not specified (Table 2.21). The susceptibility of *H. pylori* organisms to rifampicin was estimated by Etest® in 2 studies,[254,262] agar dilution in 1,[253] and disk diffusion in 1.[221] The estimated prevalence of rifampicin-resistant *H. pylori* infection among individuals with *H. pylori* infection from study populations with either mixed or unidentified anti-*H. pylori* treatment histories who had samples of *H. pylori* tested for rifampicin susceptibility ranged from 0% to 29%.

Table 2.21. Prevalence of rifampicin-resistant *H. pylori* infection among individuals with *H. pylori* infection in study populations with either mixed or unidentified anti-*H. pylori* treatment histories who had samples of *H. pylori* tested for rifampicin susceptibility.

Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range [mean] (years)	Detection Method	Sample Size (n)	Prevalence of Resistance (%)
Adults ≥18 years of age					
Asia					
Iran, 2012, Milani[221]	Dec 2010 – Nov 2011	adults [46] & children 3-14, [5]	Disk diffusion	112	28.6
Mixed Age Groups – Adults & Children					
Europe					
France, 2010, Raymond[254]	2004 – 2007	16-87	Etest®	530	0.2
Eastern Russia, 2012, Reva[253]	2004 – 2009	15-80	Agar dilution	170	0
Age Range of Study Population Not Specified					
Europe					
Bulgaria, 2014, Boyanova[262]	2012 – 2013	[50.5]	Etest®	50	12.0

Prevalence of Nitrofurantoin-resistant *H. pylori* Infection

Of the 93 articles identified by the literature search, 3 reported estimates of the prevalence of nitrofurantoin-resistant *H. pylori* infection among individuals with *H. pylori* infection who had samples of *H. pylori* tested for nitrofurantoin susceptibility in Asian populations. The susceptibility of *H. pylori* isolates to nitrofurantoin was estimated by Etest® in 2 studies[228,251] and disk diffusion in 1.[221] Teh *et al.* (2014) estimated the prevalence of nitrofurantoin-resistant *H. pylori* infection among individuals with *H. pylori* infection who had samples of *H. pylori* tested for nitrofurantoin susceptibility without separating individuals with or without previous antibiotic treatment to eliminate

H. pylori infection and reported all tested *H. pylori* isolates to be susceptible to nitrofurantoin (Tables 2.22 & 2.23).[251] Goh *et al.* (2011) investigated the prevalence of nitrofurantoin-resistant *H. pylori* infection among individuals with *H. pylori* infection who had samples of *H. pylori* tested for nitrofurantoin susceptibility and who had no history of anti-*H. pylori* treatment and found all tested isolates to be susceptible to nitrofurantoin. Milani *et al.* (2012) estimated the prevalence of nitrofurantoin-resistant *H. pylori* infection among individuals with *H. pylori* infection who had samples of *H. pylori* tested for nitrofurantoin susceptibility to be 11.5% among study participants in Iran without specifying the anti-*H. pylori* treatment history of individuals included in the study population.

Table 2.22. Prevalence of nitrofurantoin-resistant *H. pylori* infection among individuals with *H. pylori* infection with no history of treatment for *H. pylori* infection who had samples of *H. pylori* tested for nitrofurantoin susceptibility.

Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Sample Size (n)	Prevalence of Resistance (%)
Age Range of Study Population Not Specified					
Asia					
Malaysia, 2011, Goh[228]	Jan – Aug 2009	[50.5]	Etest®	90	0
Malaysia, 2014, Teh[251]	-	-	Etest®	102	0

Table 2.23. Prevalence of nitrofurantoin-resistant *H. pylori* infection among individuals with *H. pylori* infection who were previously treated with antibiotics to eliminate *H. pylori* infection and who had samples of *H. pylori* tested for nitrofurantoin susceptibility.

Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Sample Size (n)	Prevalence of Resistance (%)
Age Range of Study Population Not Specified					
Asia					
Malaysia, 2014, Teh[251]	-	-	Etest®	8	0

Table 2.24. Prevalence of nitrofurantoin-resistant *H. pylori* infection among individuals with *H. pylori* infection from study populations with either mixed or unidentified anti-*H. pylori* treatment histories who had samples of *H. pylori* tested for nitrofurantoin susceptibility.

Study Location, Publication Year, First Author	Time Period of Sample Collection	Age Range, [mean] (years)	Detection Method	Sample Size (n)	Prevalence of Resistance (%)
Mixed Age Groups – Adults & Children					
Asia					
Iran, 2012, Milani[221]	Dec 2010 – Nov 2011	adults [46] & children 3-14, [5]	Disk diffusion	112	11.5

Discussion

Antibiotic treatment to eliminate *H. pylori* is the most effective strategy available to prevent the occurrence of *H. pylori*-associated disease, given that no effective vaccine has been developed and no effective strategies for preventing transmission have been identified. However, antibiotic-resistant *H. pylori* infection is recognized globally as a major risk factor for anti-*H. pylori* treatment failure.[52,89] Additionally, previous exposure to anti-*H. pylori* treatment regimens that fail to eliminate infection is an important risk factor for the development of antibiotic-resistant *H. pylori* infection.[79,80]

Chapter 2. Review of antibiotic resistance mechanisms in *H. pylori* organisms and prevalence of antibiotic-resistant *H. pylori* infection worldwide during 2010 to 2014

The results of this literature review demonstrate a general pattern of higher prevalence of antibiotic-resistant *H. pylori* infection among individuals previously treated for *H. pylori* infection compared to individuals with no history of treatment with an anti-*H. pylori* treatment regimen. The higher prevalence of antibiotic resistance among individuals previously treated with an anti-*H. pylori* treatment regimen may occur due to the presence of resistant organisms that persist following exposure to a selective pressure such as antibiotic treatment.[67] However, it remains unclear whether the higher prevalence of antibiotic-resistant *H. pylori* observed among individuals with previous exposure to an anti-*H. pylori* treatment regimen relative to individuals with no previous exposure to an anti-*H. pylori* treatment regimen reflects *H. pylori* organisms that became resistant after exposure to an unsuccessful anti-*H. pylori* treatment regimen or *H. pylori* organisms that were resistant before treatment and decreased the effectiveness of the treatment. In order to clarify whether individuals develop antibiotic-resistant *H. pylori* infection following a failed anti-*H. pylori* treatment attempt, prospective studies comparing the antibiotic susceptibility of *H. pylori* isolates before and after treatment attempt is required. No prospective study designs were identified in this literature review that could provide evidence of the direction of the association between antibiotic-resistant *H. pylori* infection and anti-*H. pylori* treatment failure.

The results of this review also demonstrate that the prevalence of clarithromycin- and metronidazole-resistant *H. pylori* infection exceed 15%-20% and >40%, respectively, of people with *H. pylori* infection in many regions worldwide. The high prevalence of antibiotic-resistant *H. pylori* infection in various geographic regions may be associated with the consumption of antibiotics for unrelated infections at the population level.[53,72,73] An increase in the prevalence of antibiotic-resistant *H. pylori* infection in Europe over recent decades has been observed to mirror increases in the use of antibiotics.[74,268,301,302] Van Boeckel *et al.* (2014) estimated that the general use of antibiotics increased by 36% from 2000 to 2010 when they investigated the sales data of retail and hospital pharmacies in 71 countries worldwide.[303] Increased use of macrolide antibiotics (the antibiotic class that includes clarithromycin), and specifically, increased use of clarithromycin, has been hypothesized to increase the development of clarithromycin resistance in *H. pylori*.[73,104,268]

The results of this review demonstrate that the range for the estimated prevalence of clarithromycin-resistant *H. pylori* infection in *H. pylori*-positive individuals with no history of treatment for *H. pylori* infection was higher among individuals <18 years of age compared to individuals ≥18 years of age. Aside from its use in anti-*H. pylori* treatment regimens, clarithromycin is often used for the treatment of respiratory infections. The higher prevalence of clarithromycin-resistant *H. pylori* infection among individuals <18 years of age may occur due to the higher consumption of clarithromycin, and other macrolide antibiotics, in younger age groups for the treatment of respiratory tract infections.[55,73] In contrast, the results of this review demonstrated that the range for the estimated prevalence of metronidazole-resistant *H. pylori* infection among *H. pylori*-positive individuals with no history of treatment for *H. pylori* infection was higher in the ≥18-year age group compared to the <18-year age group. Metronidazole is often used for the treatment of gynaecological and parasitic infections.[53] The higher observed range of metronidazole-resistant *H. pylori* infection among individuals ≥18 years of age may in part be attributable to the more frequent use of this antibiotic for the treatment of gynaecological infections, such as bacterial vaginosis and trichomoniasis, in adult women.[53,104,260,269,304–306] A higher prevalence of metronidazole-resistant *H. pylori* infection may also be observed in developing regions due to the increased use of this antibiotic in these regions for the treatment of parasitic infection.[53,104,260,269,304–306] In this review, the prevalence of metronidazole-resistant *H. pylori* infection among *H. pylori*-positive individuals ≥18 years of age with no history of anti-*H. pylori* treatment, appear to be higher in Africa than in Asia or Europe.

There are important limitations to published estimates of the prevalence of antibiotic-resistant *H. pylori* infection worldwide. Antibiotic susceptibility testing of *H. pylori* isolates is not routinely conducted and studies investigating the prevalence of antibiotic-resistant *H. pylori* infection often consist of individuals who have been referred for endoscopy and may, therefore, not be representative of a broader population.[307]

Additionally, it is important to consider some of the limitations associated with the susceptibility testing of *H. pylori* isolates. Studies reported in the literature used diverse susceptibility methods that vary in accuracy.[307] Of the 93 unique studies included

in this review, nearly all (86/93) used phenotypic methods to classify the susceptibility of *H. pylori* isolates, but 7 studies used genotypic methods. Unlike phenotypic methods, genotypic susceptibility methods are unable to detect antibiotic-resistant *H. pylori* isolates occurring from novel or unknown genetic mutations and may, therefore, underestimate the true prevalence of antibiotic resistance.

Phenotypic antibiotic susceptibility testing requires the culture of bacteria from biopsy samples collected by endoscopy. Limitations of susceptibility testing that detect organisms in gastric biopsies include the cost of conducting endoscopy procedures and the wait-times associated with the procedure. Phenotypic testing methods require culture of *H. pylori* from gastric biopsies, which is time-consuming: *H. pylori* cultures are slow growing, especially if the biopsies are from individuals with a low bacterial load in the stomach.[308] Also bacterial culture has a sensitivity less than 100% and therefore the susceptibility of *H. pylori* isolates is not identified among all infected individuals who have gastric biopsies taken for culture. A key caution arises from the observation of discordant antibiotic susceptibility results in some cases when the bacterium is cultured from biopsies collected from different anatomical regions of the stomach such as the antrum and the corpus.[110,264,289,309] Discordant antibiotic susceptibility results may occur from the simultaneous infection of an individual with both antibiotic-resistant and antibiotic-susceptible *H. pylori* organisms. The simultaneous infection of an individual with both antibiotic-resistant and antibiotic-susceptible *H. pylori* infection can occur from multiple strains of *H. pylori* or a single strain that undergoes genomic recombination during long-term infection.[310–313] Therefore it may improve accuracy to collect more than one biopsy sample from an infected individual, from differing anatomical regions of the stomach, to avoid the misclassification of antibiotic susceptibility results. It should also be noted that knowledge of antibiotic susceptibility through *in vitro* laboratory testing methods does not always result in successful elimination of the infection in an *H. pylori*-positive individual.[314]

To investigate the quality of the studies included in this review, the author recorded whether or not the following information was included in the study report: age range of the study population; anti-*H. pylori* treatment histories; geographic region;

sample size; and anatomical site of gastric biopsy sampling. Among the 93 studies included in this review, 17 did not include any specific information regarding the age range or mean age of the study participants; 32 did not include information on anti-*H. pylori* treatment histories; 18 provided estimates for the prevalence of antibiotic-resistant *H. pylori* infection in a study population consisting of individuals with or without previous exposure to an anti-*H. pylori* treatment regimen; and all 93 reported the geographic region and sample size. The actual study size is another indicator of quality: of the 93 studies, 54 reported estimates of the prevalence of antibiotic in a sample of <100 *H. pylori* isolates and 25 studies estimated the prevalence of antibiotic resistance in a sample of <50 *H. pylori* isolates. Underrepresentation of some regions in the world is another limitation of the literature: 84% (78/93) of the studies identified were conducted in either Asia or Europe; 45 studies were conducted in Asia, 33 in Europe, 6 in Africa, 5 in South America, 3 in North America, and 1 in Australia. Among the 86 studies that classified the antibiotic susceptibility of *H. pylori* isolates by phenotypic methods, 18 did not specify the anatomical location of gastric biopsy collection; 35 collected gastric biopsies from 2 or more locations from the antrum, corpus and/or fundus; 30 collected gastric biopsies from the antrum; and 3 collected gastric biopsies from the antrum or both the antrum and corpus of study participants.

Overall, the present literature review on the prevalence of antibiotic-resistant *H. pylori* infection presents evidence of the widespread occurrence of antibiotic-resistant *H. pylori* infection worldwide and provides a comprehensive description of the prevalence of antibiotic-resistant *H. pylori* by geographic region and by specific antibiotic drug. The results of this review are valuable to clinical practice because information regarding the prevalence of antibiotic-resistant *H. pylori* infection in a local region can be used to inform effective prescription practices.

Chapter 3. Estimating the effect of antibiotic exposure on antibiotic-resistant H. pylori infection and anti-H. pylori treatment failure among participants in CANHelp community projects in northern Canada

Introduction

Helicobacter pylori is a bacterium that colonizes the lining of the stomach and/or duodenum.[1] Chronic *H. pylori* infection generally results in gastritis, which is inflammation of the stomach lining, a risk factor for peptic ulcer disease and gastric cancer.[95–98] The prevalence of *H. pylori* infection varies according to geographic region, ethnicity, socioeconomic status and age.[9] Northern Canadian Aboriginal communities are disproportionately burdened by *H. pylori* infection, with prevalence estimates ranging from 51% to 95%.[18–20] In southern Canada, in contrast, estimates of the prevalence of *H. pylori* infection range from 20 to 40%.[14–17] Treatment to eliminate *H. pylori* infection has been observed to improve peptic ulcer healing, decrease peptic ulcer recurrence and reduce the risk of gastric cancer.[21,22]

Successful elimination of *H. pylori* infection requires the use of a multi-drug regimen that combines a proton-pump inhibitor (PPI) with two or three antibiotics for a duration of 7-14 days. Canadian expert clinical guidelines published in 2016 recommend either of two 4-drug therapies for a duration of 14 days for the initial treatment of *H. pylori* infection: "bismuth-based quadruple therapy", which combines a PPI with tetracycline, metronidazole, and bismuth subsalicylate and "non-bismuth quadruple therapy", which combines a PPI, amoxicillin, metronidazole and clarithromycin.[54] If initial therapy fails to eliminate the infection, the 2016 Canadian guidelines recommend an additional course of treatment using bismuth-based quadruple therapy if not initially prescribed as long as the individual had no prior exposure to metronidazole; if the quadruple therapies fail to eliminate *H. pylori* infection or the individual had previous exposure to metronidazole, the 2016 Canadian guidelines recommend the use of a fluoroquinolone-containing therapy such as one that uses levofloxacin with amoxicillin and a PPI.[54]

Chapter 3. Estimating the effect of antibiotic exposure on antibiotic-resistant *H. pylori* infection and anti-*H. pylori* treatment failure among participants in CAN*Help* community projects in northern Canada

Before 2016, Canadian clinical guidelines recommended a 3-drug therapy combining a PPI with clarithromycin and either amoxicillin or metronidazole for 7-14 days for the initial treatment to eliminate *H. pylori* infection.[25,37] This therapy, commonly known as standard triple therapy, has been the anti-*H. pylori* treatment regimen most commonly recommended by expert clinical guidelines worldwide for the initial attempt to eliminate *H. pylori* infection.[25,26,36–38] The new 2016 recommendation to use quadruple therapy, for 14 days, rather than standard triple therapy for 7-14 days, was made following reports that the effectiveness of standard triple therapy for eliminating *H. pylori* infection was unacceptably low in many regions worldwide.[43,315] As a quantitative measure, effectiveness of therapy aimed at eliminating an infection is defined as the proportion of treated individuals whose infection is eliminated.[35] According to a commonly used standard, initially prescribed anti-*H. pylori* treatment regimens should eliminate infection in ≥80% of individuals treated.[26,27] Avoiding anti-*H. pylori* treatment regimens with effectiveness below this standard is important to reduce exposure to multiple anti-*H. pylori* treatment regimens and reduce the risk of developing an antibiotic-resistant infection.[54] However, given challenges posed by *H. pylori* infection, finding a treatment regimen with effectiveness ≥80% is often difficult to achieve in practice in a given population.

A major risk factor for reduced anti-*H. pylori* treatment effectiveness is reduced susceptibility of the bacteria to one or more antibiotics, as occurs in antibiotic-resistant infections.[52] Meta-analyses published from 1999 to 2007 have reported that the effectiveness of anti-*H. pylori* treatment regimens to eliminate infection is lower when prescribed for the treatment of antibiotic-resistant *H. pylori* infection than it is when prescribed for antibiotic-susceptible *H. pylori* infection.[52,58,59] Factors that modify the impact of antibiotic resistance on the effectiveness of an anti-*H. pylori* treatment regimens include the combination of antibiotics prescribed, the dose of antibiotics prescribed, the duration of treatment, and the susceptibility of the bacterium to the antibiotic combination prescribed.[59] In particular, resistance to clarithromycin, an antibiotic with high antimicrobial activity against *H. pylori*, notably reduces the

effectiveness of anti-*H. pylori* treatment regimens containing clarithromycin, such as standard triple therapy.[53,56–58]

Antibiotic-resistant *H. pylori* organisms may arise from a spontaneous genetic mutation that persists following exposure to selective pressures such as antibiotics.[68] A major risk factor for antibiotic-resistant *H. pylori* infection is previous exposure to one or more unsuccessful anti-*H. pylori* treatment regimens.[79,80] Additionally, evidence suggests that the prevalence of antibiotic-resistant *H. pylori* infection is associated with the frequency of exposure to antibiotics for the treatment of unrelated infections.[53,72,73] Perez Aldana *et al.* (2002) estimated a fourfold increase in clarithromycin prescriptions between 1993-1994 and 1999-2000, which was mirrored by an increase in the prevalence of clarithromycin-resistant *H. pylori* infection between 1995-1996 and 1999-2000 in the general population of Japan.[73] Similarly, over past decades, fewer restrictions have controlled the use of macrolides (the antibiotic class that contains clarithromycin) in southern European countries relative to northern European countries and several reports have presented evidence that the prevalence of macrolide-resistant *H. pylori* infection is higher in southern European countries where macrolide use is high relative to northern European countries where macrolide use is low.[74,268,301,302] However, although a threefold increase in clarithromycin prescriptions occurred in the Netherlands between 1993 and 1997, an increase in macrolide-resistant *H. pylori* infection was not observed.[74] An absence in increasing antibiotic-resistant *H. pylori* infection following increasing antibiotic consumption in the Netherlands may be attributed to a more prudent use of antibiotics in the Netherlands compared to other countries in the European Union.[75]

The overall consumption of antibiotics in a given population varies according to factors including age, sex, disease status and geographic region.[76–78] Antibiotics commonly used in anti-*H. pylori* treatment regimens, such as clarithromycin, metronidazole and levofloxacin, are widely consumed in the general population for the treatment of common bacterial infections: clarithromycin is often used for the treatment of respiratory tract infections; metronidazole is often used for the treatment of gynaecological and parasitic infections; and levofloxacin is often used for the treatment of urinary tract infections. Differences in prescribing practices or the use of antibiotics for

the treatment of unrelated bacterial infections may contribute to differences in the prevalence of antibiotic-resistant *H. pylori* infection across geographic regions.[316,317]

Additional risk factors for the development of antibiotic-resistant *H. pylori* infection include inadequate adherence to prescribed anti-*H. pylori* treatment regimens and the genotype of the infecting *H. pylori* strain. Evidence shows that inadequate adherence to a prescribed anti-*H. pylori* treatment regimen is associated with reduced anti-*H. pylori* treatment effectiveness.[89] Graham *et al*. (1992) reported treatment adherence to be associated with the effectiveness of bismuth-based triple therapy (combining tetracycline, metronidazole and bismuth): the effectiveness of standard triple therapy among 93 *H. pylori*-positive individuals was 96% for those who completed ≥60% of the prescribed therapy compared to an effectiveness of 69% for those who completed <60% of the prescribed therapy.[90] Adverse effects of treatment are a major contributor to poor adherence and reports of the frequency of their occurrence range from 5% to 20% of individuals prescribed anti-*H. pylori* treatment regimens.[318] Common adverse effects of anti-*H. pylori* treatment regimens include, but are not limited to: nausea; vomiting; diarrhea; and a metallic taste in the mouth. Additionally, previous studies have generated evidence that suggest an association between *H. pylori* genotypes hypothesized to confer virulence and successful elimination of the infection with anti-*H. pylori* treatment regimens.[85–88,319] Virulence genes encode bacterial products that enable bacteria to induce disease,[82] by allowing the bacteria to survive in the acidic environment of the stomach, to reach the gastric mucus layer, and/or to persistently infect an individual.[83] Two genes that have been identified as *H. pylori* virulence factors are the cytotoxin-associated gene A (*cagA*) and vacuolating-associated gene A (*vacA*). However, studies of the association between *H. pylori* virulence factors and anti-*H. pylori* treatment effectiveness have produced inconsistent results.[293,320] It has also been suggested that *H. pylori* virulence genotypes may be associated with the antibiotic susceptibility of *H. pylori*.[84]

The Canadian North *Helicobacter pylori* (CAN*Help*) Working Group is a research team that links northern Canadian communities, health care providers and regional health authorities with investigators from the University of Alberta to conduct community-driven research in northern Canada. This collaborative group aims to address

concerns raised by northern community leaders in the Northwest Territories and Yukon regarding *H. pylori*-associated health outcomes; the ultimate goal is to inform public health policy pertaining to control of *H. pylori* infection.[321] In northern Canada, *H. pylori* infection is a major health concern due to its high prevalence in this region compared to southern Canada and its link to stomach cancer, which has elevated rates in this region.[322,323] Currently, four CAN*Help* community projects have been established in Aklavik, NT, Old Crow, YT, Fort McPherson, NT, and Tuktoyaktuk, NT. An important aim of the CAN*Help* Working Group research includes improving the clinical management of *H. pylori* infection by identifying factors that limit the effectiveness of anti-*H. pylori* treatment regimens. My aims with the current analysis are to estimate the effect among CAN*Help* project participants of exposure to antibiotics on two health outcomes: 1) the prevalence of antibiotic-resistant *H. pylori* infection; and 2) the risk of anti-*H. pylori* treatment failure.

Methods

Research Setting

For this analysis, I used data collected from participants in CAN*Help* Working Group community *H. pylori* projects in Aklavik, NT, Old Crow, YT, Fort McPherson, NT and Tuktoyaktuk, NT. Each community *H. pylori* project comprises six main components: screening for *H. pylori* infection status with a non-invasive breath test; clinical and epidemiologic questionnaires that collect data on health and socio-environmental exposures; endoscopy with collection of gastric biopsies; treatment; knowledge translation; and policy development. During the initiation of each community project, a project planning committee was formed consisting of community representatives and project staff from the University of Alberta to ensure project components addressed community concerns and were culturally appropriate. For each community project, project staff invited all residents to participate in each project component (with the exception that the Fort McPherson planning committee decided to exclude children <5 years of age from breath-test screening). Participant recruitment occurred following the initiation of each community project (November 2007 in Aklavik,

December 2010 in Old Crow, February 2011 in Tuktoyaktuk, and June 2012 in Fort McPherson). Informed consent was administered to participants ≥17 years of age, with assent obtained from participants aged 7-16 years; parental consent was also obtained for children under 17 years of age. This research was approved by the University of Alberta Health Research Ethics Board, as well as Northwest Territories and Yukon research licensing authorities.

Selection of Participants

To estimate the effect among CAN*Help* project participants of exposure to antibiotics on both the prevalence of antibiotic-resistant *H. pylori* infection and the incidence of anti-*H. pylori* treatment failure, I selected all participants with: 1) data on the antibiotic susceptibility of *H. pylori* isolates cultured from gastric biopsies; and/or 2) a post-treatment breath test result. To have this data, participants must have either: 1) completed endosopy with gastric biopsies, and/or 2) tested positive for *H. pylori* by UBT, histology or culture and participated in the treatment component. Project participants 15 years of age or older, regardless of *H. pylori* status, were encouraged to participate in the endoscopy component of each CAN*Help* community project. At the discretion of the endoscopist, children as young as 10 years old participated in endoscopy if parents actively requested this. All participants identified as *H. pylori*-positive by UBT and/or biopsy-based evidence were invited to participate in the treatment component of their community *H. pylori* project. *H. pylori*-positive participants ≥15 years of age were eligible to enroll in the treatment trial. Exclusion criteria for the treatment trials included: allergies to medications (amoxicillin, metronidazole or clarithromycin); pregnancy or breastfeeding; antibiotic therapy within four weeks of treatment (unless an interim UBT was conducted providing evidence of on-going infection); and severe cardiorespiratory, pulmonary, endocrine, hepatic or renal disease. *H. pylori*-positive participants who were not eligible for inclusion, including children, were offered anti-*H. pylori* treatment outside of the trial. For participants with discordant results on tests, research team gastroenterologists decided whether or not to offer treatment.

Data Collection

Demographic Information

CAN*Help* project participants' demographic and health information were collected by trained project staff during structured questionnaire-based interviews. This analysis used data from participant, household, clinical and post-treatment questionnaires, which ascertained socio-environmental exposures pertaining to individuals and their households, relevant factors concerning individuals' health, and, for those who participated in the treatment component, their experience with the allocated anti-*H. pylori* treatment regimen. Specific variables obtained from questionnaires included age, sex, ethnicity, educational attainment, household income, history of exposure to an anti-*H. pylori* treatment regimen prior to enrolment in a CAN*Help* community project, the anti-*H. pylori* treatment regimen prescribed through a CAN*Help* community project, adherence to the prescribed anti-*H. pylori* treatment regimen, and the occurrence of adverse effects during the course of the prescribed anti-*H. pylori* treatment regimen.

H. pylori *Infection Status*

For most participants, *H. pylori* infection status was classified as positive or negative based initially on screening by the ^{13}C-urea breath test (UBT). The UBT is a non-invasive diagnostic test commonly used for the detection of active *H. pylori* infection in adults and children.[26,27,324–326] Review of the relevant evidence shows that the UBT has a high diagnostic accuracy against a range of biopsy-based gold standards: estimates of both sensitivity and specificity range from 90% to 100%.[326] This test uses urea labelled with ^{13}C to demonstrate the activity of urease secreted by *H. pylori* organisms present in the stomach, an enzyme that breaks down urea into ammonia and carbon dioxide. For this test, project staff collected a baseline breath sample from participants before giving them a ^{13}C-labelled urea solution to drink; a second breath sample was collected 30 minutes after the solution was swallowed. The ^{13}C/^{12}C isotope ratio in collected breath samples was measured by an IRIS® infared breath test analyzer (Kibion/Wagner Analysen Technik, Bremen, Germany). The CAN*Help* Working Group UBT protocol adapts instructions for the collection of breath samples and interpretation of test values from the

IRIS® and ^{13}C-urea manufacturer recommendations (http://www.helikit.com/en/physician-information/), with modifications based on a review of UBT validation studies conducted by Gisbert and Parajes (2004).[326] For children under 5 years of age, the method proposed by Klein *et al.* (1999) was used to adjust for anthropometric differences in CO_2 production that may influence test results.[327] For each breath sample, a delta value was calculated as the difference between the measured $^{13}CO_2/^{12}CO_2$ isotope ratio and the $^{13}CO_2/^{12}CO_2$ ratio in Pee Dee Belemnite limestone, used as a standard international reference.[325,328] To calculate the breath test value, the delta value from the baseline breath sample was subtracted from the delta value from the second breath sample.[325,328] Breath test values from -1.99 to 2.49 were classified as negative; values from 2.50 to 3.99 were classified as borderline; and values ≥4.00 were classified as positive. If the UBT value was classified as borderline or the test value was uncertain (test value ≤-2.00 or the CO_2 concentration was too low in either breath sample for accurate measurement) individuals were asked to repeat the test whenever possible; some borderline and uncertain results persisted on retesting.

Among participants who completed endoscopy, some of whom did not have a UBT result, *H. pylori* infection status was assessed according to culture and pathological examination of gastric biopsies. Experienced physicians performed unsedated gastroscopy after administration of a topical anaesthetic in temporary endoscopy units in community health centres, with 5 biopsies collected for pathological assessment and 2 for culture. Microbiology lab technicians attempted to culture *H. pylori* from the 2 gastric biopsy samples, one each collected from the antrum and the corpus of the stomachs. To confirm the presence of *H. pylori* in bacterial cultures, confirmatory tests conducted by microbiology lab technicians included 3 biochemical enzyme tests (the oxidase, catalase and urease tests) and a 16S rRNA PCR test. This sequence of tests is believed to confer a specificity approaching 100% on the detection of *H. pylori* by culture, while sensitivity was enhanced by the use of optimal culture techniques performed by well-trained, experienced lab technicians.[124] Although biopsy-based diagnostic methods are often regarded as gold standards for detecting *H. pylori* infection, their sensitivity is diminished by the patchy distribution of *H. pylori* in the stomach, which can cause bacterial colonies

to be missed by biopsy sampling.[124] The UBT is not vulnerable to this limitation and is believed to have comparable accuracy to biopsy-based methods.[326] Pathological examination was carried out by an experience pathologist who graded the bacterial density. Among participants with multiple test results, *H. pylori* infection status was classified according to all available information. Individuals with discordant results on UBT, histology and/or culture were classified according to an algorithm developed by the CAN*Help* Working Group; this algorithm classified as *H. pylori*-positive anyone who tested positive by culture regardless of histology or UBT results.

Exposure Ascertainment

Data on general antibiotic exposure were not routinely collected for participants of CAN*Help* community projects, so I constructed a dataset of antibiotic exposure histories of CAN*Help* project participants. To do this, I designed a chart review tool to collect participants' antibiotic exposure histories from medical charts housed in community health centres as part of a summer research project in 2013 (Appendix A1). This chart review collected data on antibiotic prescriptions recorded in medical charts during the 5 years period prior to the enrolment of each participant (all participants were enrolled in community projects during 2007 through 2012). I designed the chart review tool to collect the following information from medical charts: demographic factors; frequency of antibiotic prescriptions; type of antibiotics prescribed; and the reason for each prescription. An antibiotic prescription was defined as a single prescription of a systemic antibiotic regardless of the dose, dosing frequency or duration of the prescription.

Outcome Ascertainment

Antibiotic Susceptibility of H. pylori *Isolates*

The antibiotic susceptibility of *H. pylori* infection was classified for CAN*Help* project participants by testing *H. pylori* cultured from their gastric biopsies for susceptibility to selected antibiotics. The susceptibility of *H. pylori* isolates was assessed for the following antibiotic drugs (antibiotic class): clarithromycin (macrolide); metronidazole (nitroimidazole); amoxicillin (β-lactam); tetracycline (tetracycline);

ciprofloxacin (fluoroquinolone); rifampicin (rifamycin); and nitrofurantoin (nitrofuran). The antibiotic susceptibility of *H. pylori* isolates was classified by Etest® according to the following minimum inhibitory concentrations (MICs): ≥1 μg/mL for clarithromycin; ≥8 μg/mL for metronidazole; ≥1 μg/mL for amoxicillin; ≥4 μg/mL for tetracycline; ≥1 μg/mL for ciprofloxacin; ≥2 μg/mL for rifampicin; and ≥2 μg/mL for nitrofurantoin.

Outcome Ascertainment

Anti-H. pylori *Treatment Failure*

The success of anti-*H. pylori* treatment was ascertained by post-treatment UBT at least 8 weeks after the completion of treatment. Treatment regimens prescribed to CAN*Help* project participants either within or outside of the treatment trial included: standard triple therapy consisting of a PPI (twice daily), amoxicillin (1 gm twice daily) and clarithromycin (500 mg twice daily) for 10 days; sequential therapy consisting of a PPI (twice daily) and amoxicillin (1g twice daily) for days 1-5 of a 10 day regimen and a PPI (twice daily), clarithromycin (500 mg twice daily) and metronidazole (500 mg twice daily) for days 6-10 of a 10 day regimen; and quadruple therapy consisting of a PPI (twice daily), bismuth (2 tablets four times daily), tetracycline (500 mg four times daily), and metronidazole (500 mg three times daily) for 10 days. Treatment failure was defined as a positive result on the post-treatment UBT.

Data Management and Analysis

Data Cleaning & Strategies to Reduce Missing Data

I developed a dataset using STATA 12 including all relevant variables for the current analysis by merging variables generated from antibiotic exposure history data I collected during medical chart reviews with variables from existing CAN*Help* project databases for: demographics; antibiotic susceptibility results; post-treatment UBT results; exposure to anti-*H. pylori* treatment regimens prior to enrolment in a CAN*Help* community project; and anti-*H. pylori* treatment adherence to CAN*Help* prescribed regimens; and side effects that may have occurred during the course of anti-*H. pylori* treatment prescribed through a CAN*Help* community project. Prior to completing any

statistical analysis, I cleaned the dataset by checking all variables for missing or impossible values.

For both analyses, I restricted the dataset to individuals with complete data for all variables of interest, including potential confounding variables. Variables of interest with a large amount of missing data included ethnicity, education, and income variables, as well as the variable for anti-*H. pylori* treatment prior to enrolment in a CAN*Help* community project. In an effort to reduce the amount of missing data for these variables, various strategies were employed (see Appendix A2).

Descriptive Analysis - Antibiotic Dispensation Rates Across CANHelp Community Projects Compared to the Edmonton City Centre

To describe the frequency of antibiotic use across CAN*Help* community projects, I estimated antibiotic dispensation rates expressed as the average number of antibiotic courses dispensed per person per year during the 5-year review period, calculated by dividing the total number of systemic antibiotic courses prescribed for all participants during the 5-year review period by the product of the total number of participants and the sum of the number of years reviewed in the medical charts across all participants. I estimated antibiotic dispensation rates by: community (Aklavik, Old Crow, Fort McPherson, and Tuktoyaktuk); sex; age group ($<$ or $\geq$ the median age of 45 years); and antibiotic class (β-lactams, macrolides, nitroimidazoles, nitrofurans, fluoroquinolones, tetracyclines, and rifamycins). To put the antibiotic dispensation rates observed in CAN*Help* project communities in perspective, I estimated the antibiotic dispensation rate in the Edmonton outpatient population from data available through the Alberta Government Interactive Health Data Application (IHDA), accessible online.[329] The IHDA incorporates data from the following sources: Pharmaceutical Information Network database; Alberta Health Care Insurance Plan Adjusted Mid-Year Population Registry Files; and the Alberta Health and Wellness Postal Code Translation File. I restricted my analysis to data for the Edmonton City Centre, which includes the population residing within the city limits. I calculated the Edmonton City Centre antibiotic dispensation rate by dividing the number of antibiotic courses dispensed by the

sum of the population size during each of the years 2010-2013. I estimated the rate difference comparing the CAN*Help* community project population to the Edmonton population by subtracting the dispensation rate calculated for the Edmonton City Centre from the dispensation rate calculated for all four CAN*Help* community projects combined.

Analysis 1: Estimating the Effect of Exposure to Antibiotics on the Prevalence of Antibiotic-resistant H. pylori *Infection*

I estimated the prevalence odds ratio (OR) as the measure of the effect of antibiotic exposure on the prevalence of antibiotic-resistant *H. pylori* infection. Estimation of the OR is a standard method of effect estimation for cross-sectional studies, which observe disease prevalence and which can be analyzed in a manner analogous to prevalence case-control studies.[330] When a study is conducted to estimate the effect of an exposure on the frequency of a chronic health outcome for which the risk period is unknown, such as the effect of exposure to antibiotics on the occurrence of antibiotic-resistant *H. pylori* infection of unknown time of onset, the incidence rate ratio is a more relevant incidence measure to approximate than the risk ratio (also known as the incidence proportion ratio). The prevalence OR estimates the incidence rate ratio with fewer methodological assumptions than the prevalence ratio,[330] which is the main alternative measure of effect for cross-sectional studies. Additionally, the OR is advantageous because it can be estimated in multivariable analysis with widely available methods including logistic regression.[330]

I used multivariable logistic regression to estimate unadjusted and adjusted ORs and 95% CI for the effect of antibiotic exposure on the prevalence of antibiotic-resistant *H. pylori* infection. I defined the outcome variable (resistance status) as a dichotomous indicator for the presence or absence of resistance in tested isolates to any of the following antibiotics: clarithromycin, metronidazole, amoxicillin, tetracycline, ciprofloxacin, nitrofurantoin, and rifampicin. For this analysis, variables that were examined as potential confounders and/or effect measure modifiers include: age; sex; ethnicity; education; household income; *cagA* genotype; and *vacA* genotype.

Additionally, I investigated the exposure of interest (total antibiotic prescriptions during the 5-year review period) using 3 alternate definitions: 1) number of antibiotic prescriptions; 2) dichotomized antibiotic exposure (one or more antibiotic prescriptions compared to none) by antibiotic class; and 3) dichotomized antibiotic exposure (one or more antibiotic prescriptions compared to none) by specific antibiotic drug.

*Analysis 2: Estimating the Effect of Exposure to Antibiotics on the Risk of Anti-*H. pylori *Treatment Failure*

I estimated risk ratios (RRs) and risk differences (RDs) as measures of the effect of antibiotic exposure on the average risk of anti-*H. pylori* treatment failure. The incidence proportion is a measure of average risk; in my analysis, I defined the average risk (incidence proportion) of treatment failure as the proportion of participants with a positive post-treatment UBT of all individuals who completed a post-treatment UBT. When incidence data are available from a prospective design, the RR and RD are standard measures of association used, due to their interpretability as measures of exposure effects on risk.[330] I included both the RR and RD in my analysis because these measures estimate the relative and absolute effects, respectively, of the exposure on the risk of the outcome.[331] The RR is the ratio of the risk of the outcome of interest in individuals classified in the index exposure group (e.g., exposed) to the risk of this outcome in individuals classified in the reference exposure group (e.g., unexposed). The RD is the difference between the risk of the outcome of interest in individuals classified in the index exposure group and the risk of this outcome in the reference exposure group (e.g., unexposed).[332] The RR is preferable to the OR due to the bias associated with OR estimates when they are used to approximate the relative risk in circumstances where the outcome is not sufficiently rare. When used as an approximation of the relative risk, the numerical value of the OR will exaggerate the magnitude of the estimated association.[332] When the outcome of interest is rare (incidence <10%) the bias in the OR is negligible.[332] However, when the outcome of interest is not rare, the bias in the OR can be substantial.[332] In my research design, the outcome is not rare, given a treatment failure frequency of 40% among CAN*Help* project participants prescribed standard triple therapy who were included in this study.

I initially used a log-binomial regression model to attempt to estimate adjusted RRs and 95% CIs for the effect of antibiotic exposure on the risk of anti-*H. pylori* treatment failure. RRs are commonly estimated with log-binomial regression models when the outcome variable of interest is dichotomous.[333,334] However, the log-binomial regression model often fails to converge and, in such instances, is unable to generate model estimates; additionally it is criticized for generating 95% CIs that are too narrow.[333] When a log-binomial regression model fails to converge, use of the Poisson regression model with robust standard error has been recommended as an alternative.[334] Poisson regression models estimate the incidence rate ratio, which approximates the risk ratio if the incidence is sufficiently low or follow-up is sufficiently brief. A robust standard error will adjust for the incorrectly specified distribution of Poisson regression for modelling a binomially distributed outcome variable.[334] However, Poisson regression may overestimate RRs when used to model binomial data.[6] When log-binomial regression models did not converge in my analysis, I used Poisson regression with a robust standard error to approximate adjusted RRs and 95% CIs from estimated incidence rate ratios for the effect of antibiotic exposure on the incidence of anti-*H. pylori* treatment failure. Additionally, I used binomial regression to estimate unadjusted RDs and 95% CIs for the effect of antibiotic exposure on the average risk of treatment failure.

For this analysis, variables that were examined as potential confounders and/or effect measure modifiers included: age; sex; ethnicity; education; household income; previous exposure to an anti-*H. pylori* treatment regimen; the anti-*H. pylori* treatment regimen prescribed through a CAN*Help* community project; treatment side effects; and treatment adherence. The treatment side effects variable was a dichotomous variable defined as the presence or absence of reported side effects throughout the course of an anti-*H. pylori* treatment regimen prescribed through a CAN*Help* community project. The treatment adherence variable was a dichotomous variable defined by whether the participant completed all prescribed doses of the prescribed treatment regimen. I investigated the exposure of interest using three alternate definitions: 1) number of antibiotics prescribed; 2) dichotomized antibiotic exposure (one or more antibiotics

compared to none) by antibiotic class; and 3) dichotomized antibiotic exposure (one or more antibiotics compared to none) by specific antibiotic drug.

Model Building

I used purposeful selection, as proposed by Hosmer and Lemeshow (2000), to select potential confounding variables and effect measure modifiers to be included in multivariable analysis.[335] For each regression model, I first estimated the unadjusted association between each potential confounding variable and the dependent variable, selecting those with a p-value ≤0.25 for inclusion in the multivariable model. Once potential confounding variables were selected for inclusion in the initial multivariable model, I assessed whether each of these variables was needed in the model. I removed each variable from the multivariable model one at a time and if the coefficient of the exposure variable of interest or its corresponding standard error changed by ≥10%, I put the removed variable back in the model as a control variable. I also used the likelihood-ratio (LR) test to assess whether inclusion of the variable significantly improved the model fit. Potential confounding variables that did not meet the change-in-estimate criterion were included in the model if their inclusion resulted in a LR test p-value <0.05, indicating that inclusion of this variable improved the statistical fit of the model. I also used the LR test to determine whether the addition of scientifically plausible interaction terms improved the statistical fit of the model, adding such terms to the model if their inclusion resulted in a LR test p-value <0.05.

Model Checking and Sensitivity Analysis

For all analyses, I assessed the linear relationship between continuous variables and the outcome using graphic plots of the log odds of the outcome by categories of the continuous variable as well as lowess plots. For continuous variables that did not appear to have a linear relationship when plotted against the outcome of interest, I examined appropriate transformations and used the LR test to decide which had the best fit within the model. Given the nonlinear relationship of age with the treatment failure outcome, I examined the age variable in the analysis investigating the effect of antibiotic exposure on the average risk of treatment failure using three functional forms: continuous; cubic

spline; and categorical. To assess age by cubic spline, I chose the number of knots and their location based on a visual assessment of the data and the knot locations automatically chosen by STATA 12. I used the LR test to assess the fit of the model including each functional form, and selected the form with the best statistical fit. I assessed the degree of collinearity between independent variables by inspecting cross-tabulations and using the Pearson correlation coefficient. I examined all highly correlated covariates (with Pearson correlation coefficients ≥0.7) to decide which set of variables should be included in the final model.[336]

Additionally, I investigated whether a multilevel model should be used to estimate the effect of exposure to antibiotics on the two health outcomes of interest. An underlying assumption of regression models is that outcomes are independent: the outcome of one individual does not influence the outcome of another individual.[337] However, outcomes for individuals within the same community or household may be more similar than outcomes for individuals from different communities or households. Community of residence was assessed in both main analyses as a fixed effect and the inclusion of a random effects parameter for households was assessed with an LR test.

To assess the sensitivity of exposure effect estimates to the categorization of antibiotic exposure, I used more than one categorization of exposure to estimate unadjusted and adjusted measures of effect. I selected category boundaries for the antibiotic exposure variable by inspecting the uncategorized distribution of antibiotic exposure values; for Analysis 1, I also inspected the odds of antibiotic-resistant *H. pylori* infection across narrow antibiotic exposure level categories, and for Analysis 2, I also inspected the average risk of anti-*H. pylori* treatment failure across narrow antibiotic exposure level categories.

Results

Study Population

Across all four CAN*Help* community projects, 315 participants were eligible for inclusion in the current study. For 297 of the 315 eligible participants, I was able to collect antibiotic exposure histories from medical charts with the assistance of a research

assistant. Exposure histories could not be collected for 18 eligible participants for the following reasons: 10 were transient members of the community with no medical chart on file at the community health centre; 2 had recently moved into the community and had no medical records available for the review period of interest; 3 had died and their medical charts were no longer available; 2 had relocated to another community and their medical charts were transferred to another health centre; and 1 was mislabelled in the database used to identify participants for chart review. Two participants with chart review data were excluded during data cleaning because they were incorrectly classified as *H. pylori*-positive by bacterial culture and therefore did not have antibiotic susceptibility results and were not treated with an anti-*H. pylori* regimen.

The study population for the present study included 295 *H. pylori*-positive participants from CAN*Help* community projects in Aklavik, Old Crow, Fort McPherson, and Tuktoyaktuk. The participation of these 295 *H. pylori*-positive individuals in relevant CAN*Help* community project components is presented in Table 3.1. Of these participants, 191 had antibiotic susceptibility results for *H. pylori* isolates cultured from gastric biopsies, 214 completed a post-treatment UBT, and 114 had both the antibiotic susceptibility profile of cultured *H. pylori* isolates and a post-treatment UBT result.

Table 3.1. Number of eligible participants completing relevant project components in Aklavik, Old Crow, Tuktoyaktuk and Fort McPherson.

	Aklavik, NT	**Old Crow, YT**	**Fort McPherson, NT**	**Tuktoyaktuk, NT**	**TOTAL**
TOTAL	**162**	**67**	**52**	**14**	**295**
Endoscopy with Gastric Biopsies	125	56	38	9	228
Antibiotic Susceptibility Results	107	53	28	3	191
Prescribed Treatment	114	64	51	13	242
Post-Treatment UBT	106	50	46	12	214
Post-Treatment Questionnaire	86	45	45	0	176

Antibiotic Dispensation Rate

Among the 295 *H. pylori*-positive individuals included in the present study, the median number of antibiotic prescriptions during the 5-year review period was 3 (range, 0 – 38) and the mean was 4.3. Table 3.2 presents a comparison of the observed antibiotic dispensation rate in CAN*Help* community project participants and the Edmonton City Centre outpatient population by sex, age group (dichotomized based on the median age of the community project participants), and antibiotic class. Overall, a noticeably higher antibiotic dispensation rate was observed in the CAN*Help* community project study population compared to the Edmonton City Centre population: the estimated antibiotic dispensation rate was 32 (95% CI: 27, 37) courses per hundred per year higher in CAN*Help* project participants than in the Edmonton City Centre outpatient population.

Table 3.2. Estimated antibiotic dispensation rate in CAN*Help* community project participants and the City of Edmonton by sex, age and antibiotic class.

	CAN*Help* Communities			**Edmonton City Centre***	
	n	**Rate/ Person/Year**	**95% CI**	**Rate/ Person/Year**	**95% CI**
Total Population		**0.88**	**0.83, 0.93**	**0.555**	**0.554, 0.556**
Sex					
Female	161	1.1	1.0, 1.2	0.662	0.661, 0.664
Male	134	0.64	0.58, 0.71	0.450	0.449, 0.451
Age Group					
<45 years	144	0.86	0.79, 0.93	0.483	0.482, 0.484
≥45 years	150	0.90	0.84, 0.98	0.676	0.674, 0.678
Antibiotic Class					
β-lactams	177	0.30	0.27, 0.33	0.151	0.150, 0.151
Macrolides	99	0.11	0.09, 0.13	0.097	0.097, 0.097
Nitroimidazoles	59	0.06	0.05, 0.07	0.001	0.001, 0.001
Nitrofurans	21	0.04	0.03, 0.05	0.022	0.022, 0.023
Fluoroquinolones	28	0.03	0.02, 0.04	0.078	0.077, 0.078
Tetracyclines	16	0.02	0.01, 0.02	0.043	0.043, 0.043
Rifamycins	1	0.0007	0.000, 0.004	**	-

*Edmonton City Centre population ranged from 830,213 to 903,256 during 2010-2013, including both sexes and all ages.
**Rifamycin antibiotic usage was not specifically reported in the IHDA dataset.

Chapter 3. Estimating the effect of antibiotic exposure on antibiotic-resistant *H. pylori* infection and anti-*H. pylori* treatment failure among participants in CAN*Help* community projects in northern Canada

Prevalence of Antibiotic-resistant *H. pylori* Infection

The prevalence of antibiotic-resistant *H. pylori* infection in the 191 *H. pylori*-positive individuals with *H. pylori* antibiotic susceptibility results is presented in Table 3.3. In this group, the prevalence of resistance to amoxicillin, ciprofloxacin, tetracycline, nitrofurantoin and rifampicin was low (≤4%) while the prevalence of metronidazole resistance and clarithromycin resistance was 36% and 16%, respectively. Resistance to one or more of the antibiotics included in susceptibility testing was observed in 45% (85/191) of *H. pylori*-positive individuals who had *H. pylori* samples tested for antibiotic susceptibility (Table 3.4).

Table 3.3. Prevalence of resistance by antibiotic subtype in 191 *H. pylori*-positive CAN*Help* participants with antibiotic susceptibility results for *H. pylori* isolates cultured from gastric biopsies.

Antibiotic Subtype	**Number Resistant**	**% of 191**	**95% CI**
Metronidazole	69	36	29, 43
Clarithromycin	31	16	11, 22
Amoxicillin	0	0	-
Tetracycline	1	0.5	0.01, 2.8
Ciprofloxacin	8	4	2, 8
Nitrofurantoin	2	1	0.1, 4
Rifampicin	2	1	0.1, 4

Table 3.4. Prevalence of resistance to any antibiotic tested in 191 *H. pylori*-positive CAN*Help* participants with antibiotic susceptibility results for *H. pylori* isolates cultured from gastric biopsies.

	Number Resistant	**% of 191**	**95% CI**
Resistance to just one antibiotic	61	32	25, 39
Resistance to just two antibiotics	20	10	7, 16
Resistance to just three antibiotics	4	2	0.6, 5
Resistance to one or more antibiotics	85	45	37, 52

Incidence of Anti-*H. pylori* Treatment Failure

Table 3.5 presents the observed incidence proportion (average risk) of treatment failure in the group of 214 *H. pylori*-positive individuals who were treated in a CAN*Help* community project and who completed a post-treatment UBT. The average risk of

treatment failure in this group was 27%. It was 11% (6/54) in those treated with quadruple therapy, 26% (24/93) in those treated with sequential therapy, and 40% (21/53) in those treated with standard triple therapy.

Table 3.5. Average risk (incidence proportion) of treatment failure by anti-*H. pylori* treatment regimen in 214 *H. pylori*-positive CAN*Help* participants with a post-treatment UBT result.

Therapy Prescribed	n	Treatment Failure Incidence Proportion	
		%	95% CI
Sequential	93	26	17, 36
Triple	53	40	26, 54
Quadruple	54	11	4, 23
Missing Data*	14	50	23, 77

*Participants with missing data are those who either: 1) were treated through a CAN*Help* community project but data regarding the regimen prescribed was missing; or 2) reported receiving treatment outside the CAN*Help* community project and have a post-treatment UBT result in a CAN*Help* database.

Risk of Treatment Failure in Participants with Susceptibility Results

Table 3.6 presents the observed average risk of treatment failure across prescribed anti-*H. pylori* treatment regimens in this group of *H. pylori*-positive individuals who completed a post-treatment UBT and had antibiotic susceptibility results for cultured *H. pylori* isolates. Across all prescribed anti-*H. pylori* treatment regimens combined, the average risk of treatment failure in those infected with *H. pylori* isolates susceptible to all tested antibiotics was 28% (18/65) and the average risk of treatment failure in those infected with an *H. pylori* isolate resistant to one or more antibiotics was 33% (16/49).

Table 3.6. Average risk (incidence proportion) of anti-*H. pylori* treatment failure in 114 *H. pylori*-positive participants who had a post-treatment UBT and antibiotic susceptibility results, by antibiotic susceptibility status.

Resistance Status	n	Treatment Failure Incidence Proportion (Average Risk)	
		%	95% CI
*Combined Treatment Regimens**			
Susceptible	65	28	17, 40
Resistant to ≥1 antibiotic	49	33	20, 48
Triple Therapy N=26			
Susceptible	18	50	26, 74
Resistant to ≥1 antibiotic	8	50	16, 84
Sequential Therapy N=48			
Susceptible	24	29	13, 51
Resistant to ≥1 antibiotic	24	38	19, 59
Quadruple Therapy N=36			
Susceptible	19	5	0.1, 26
Resistant to ≥1 antibiotic	17	18	4, 43

*Four participants are missing data on the prescribed regimen.

Analysis 1: Estimating the Effect of Exposure to Antibiotics on the Prevalence of Antibiotic-resistant *H. pylori* Infection

Analysis 1: Descriptive Statistics

Through model building by purposeful selection, the following variables, as categorized in Table 3.7 unless otherwise noted, were selected as control variables for Analysis 1: age (continuous), sex, previous treatment to eliminate *H. pylori*, and community. The sample size for this analysis was reduced from 191 to 169 following restriction of the study population to those with complete data on selected variables and removal of participants from Tuktoyaktuk because 3 is too small a category size for analysis requiring adjustment for the community variable. Selected characteristics of the 191 individuals eligible for Analysis 1 and the 169 individuals with complete data (the study sample available for multivariable regression analysis) are presented in Table 3.7.

Chapter 3. Estimating the effect of antibiotic exposure on antibiotic-resistant *H. pylori* infection and anti-*H. pylori* treatment failure among participants in CAN*Help* community projects in northern Canada

Table 3.7. Selected characteristics of 191 eligible participants and 169 participants with complete data for Analysis 1, estimating the effect of antibiotic exposure on the prevalence of antibiotic-resistant *H. pylori* infection.

	191 Eligible Participants			**169 Participants with Complete Data**		
Variables	**n**	**Resistance Prevalence**		**n**	**Resistance Prevalence**	
		%	**95% CI**		**%**	**95% CI**
Age Range in Years*						
<35 Years	71	34	23, 46	66	30	20, 43
35-54 Years	70	47	35, 59	61	44	32, 58
>=55 Years	50	56	41, 70	42	57	41, 72
Sex						
Female	103	51	41, 61	89	48	38, 59
Male	88	36	26, 47	80	35	25, 46
Education						
Less than Grade 12	100	47	37, 57	95	45	35, 56
Grade 12 or Equivalent	33	30	16, 49	33	30	16, 49
Certificate/Diploma/Degree	41	41	26, 58	38	45	29, 62
Missing	17	65	38, 86	3	33	1, 91
Ethnicity						
Gwich'in	86	45	35, 56	86	45	35, 56
Inuvialuit	67	34	23, 47	67	34	23, 47
Other Aboriginal	9	56	21, 86	9	56	21, 86
Non-Aboriginal	4	75	19, 99	4	75	19, 99
Missing	3	33	1, 91	3	33	1, 91
Household Income						
High	64	36	24, 49	63	37	25, 50
Medium	34	38	22, 56	32	34	19, 53
Low & Very Low	39	49	32, 65	39	49	32, 65
Missing	54	56	41, 69	35	51	34, 69
Community						
Aklavik	107	34	25, 43	106	34	25, 44
Old Crow	53	51	37, 65	39	46	30, 63
Fort McPherson	28	68	48, 84	24	71	49, 87
Tuktoyaktuk	3	100	29, 100**	0	-	-
Previous Treatment for *H. pylori*						
None	134	37	29, 46	132	36	28, 45
One or More	38	63	46, 78	37	62	45, 78
Missing	19	58	33, 80	0	-	-

*For the 191 eligible participants, the age range was 11-81 years and the mean age was 43. For the 169 participants with complete data, the age range was 11-80 years and the mean age was 42.

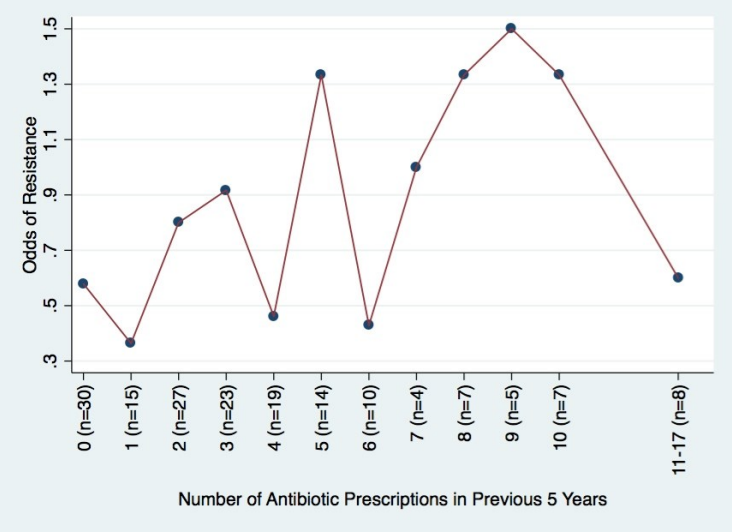
**One-sided, 97.5% CI

Chapter 3. Estimating the effect of antibiotic exposure on antibiotic-resistant *H. pylori* infection and anti-*H. pylori* treatment failure among participants in CAN*Help* community projects in northern Canada

Analysis 1: Prevalence of Outcome by Exposure Group

Examination of the relationship between the number of antibiotic courses prescribed during the 5-year review period and the prevalence odds of antibiotic-resistant *H. pylori* infection did not reveal an exponential or other simple monotonic trend. For this reason, I modeled the exposure as a categorical variable. Plotting the trend in the odds of antibiotic resistance across one-unit increments of exposure (number of antibiotic courses) shows that the odds of resistant infection did not begin to increase until 2 antibiotic prescriptions (Figure 3.1). Because few participants (n=30) had 0 antibiotic prescriptions, I chose to use 0-1 as the reference category to increase precision within the model. While it may be of greater scientific interest to compare those with 0 antibiotic prescriptions to those with higher numbers of antibiotic prescriptions, the data did not facilitate precise estimation of this contrast. In addition to few participants having 0 antibiotic prescriptions during the 5 years prior to enrolment in a CAN*Help* community project, some of these individuals could have had antibiotic exposure just before the start of this review period. Also, participants classified has having 0 prescriptions may have been treated with antibiotics outside of the community health centre. For these reasons, I decided to group 0-1 antibiotic prescriptions together as the reference category, reflecting very low exposure.

Figure 3.1. Odds of antibiotic resistance by the number of antibiotic prescriptions in the previous 5 years in 169 *H. pylori*-positive participants who had samples of *H. pylori* tested for antibiotic susceptibility and complete data on selected model covariates.



Chapter 3. Estimating the effect of antibiotic exposure on antibiotic-resistant *H. pylori* infection and anti-*H. pylori* treatment failure among participants in CAN*Help* community projects in northern Canada

I initially used the following categorization of the number of antibiotic prescriptions in the 5-year period: 0-1; 2-4; 5-7; and 8-17. Due to the small number of participants in the highest exposure category (8-17 antibiotic prescriptions), I collapsed the top two exposure categories (5-7 and 8-17 antibiotic prescriptions), given that the observed trend justified combining the highest two exposure categories and the LR test p-value for including the 3-category version (0.67) was slightly lower than the LR test p-value for including the 4-category version (0.69). Table 3.8 presents the prevalence of resistance across the 4-level and 3-level exposure categorizations.

Table 3.8. Prevalence of antibiotic-resistant *H. pylori* infection by antibiotic exposure level in 169 *H. pylori*-positive participants who had samples of *H. pylori* tested for antibiotic susceptibility and complete data for selected model covariates.

Number of Antibiotic Prescriptions During the 5-year Review Period	**n**	**Resistance Prevalence**	
		%	**95% CI**
Four Categories			
0-1	45	33	20, 49
2-4	69	42	30, 55
5-7	28	46	28, 66
8-17	27	52	32, 71
Three Categories			
0-1	45	33	20, 49
2-4	69	42	30, 55
5-17	55	49	35, 63

Analysis 1: Multivariable Logistic Regression Model

Effect estimates generated from the multivariable logistic regression model estimating the effect of antibiotic exposure on the prevalence of antibiotic-resistant *H. pylori* infection among the *H. pylori*-positive participants included in this analysis are presented in Table 3.9. A product term for age (uncategorized) and sex was observed to improve the fit of the multivariable logistic regression model (LR test value <0.05). Table 3.9, therefore, presents the unadjusted and adjusted prevalence ORs from a model without the interaction term for age and sex (Model 1) and another model with the interaction term for age and sex (Model 2). Unadjusted estimates appear to demonstrate a trend of moderately increasing odds of resistant infection with increasing antibiotic exposure (with the exposure variable categorized as 0-1, 2-4, and 5-17 antibiotic prescriptions),

though with suboptimal precision, given that the wide CIs reflect uncertainty about the magnitude and direction of the association at each exposure level and the score test for this trend has a p-value of 0.11. There also appeared to be increased odds of resistant infection in participants previously treated with an anti-*H. pylori* treatment regimen compared to those with no previous exposure to an anti-*H. pylori* treatment regimen (adjusted OR=2.1; 95% CI: 0.85, 5.3). Higher odds of resistant infection were also observed inparticipants from Fort McPherson compared to those from the other two communities, particularly Aklavik (adjusted OR=4.0; 95% CI: 1.4, 11). Additionally, the odds of resistant infection increased steadily with each one-year increase in age in females but not in males.

Table 3.9. Estimated effects of antibiotic exposure on the prevalence odds of antibiotic-esistant *H. pylori* infection in 169 *H. pylori*-positive participants who had samples of *H. pylori* tested for antibiotic susceptibility and complete data for all selected model covariates: Results of logistic regression analysis.

	Unadjusted		**Model 1***		**Model 2***	
Variable	**OR**	**95% CI**	**OR**	**95% CI**	**OR**	**95% CI**
Antibiotic Exposure						
0-1 Prescriptions	**1.0**	-	**1.0**	-	**1.0**	-
2-4 Prescriptions	**1.5**	0.66, 3.2	**1.5**	0.66, 3.6	**1.5**	0.62, 3.4
5-17 Prescriptions	**1.9**	0.85, 4.3	**1.4**	0.54, 3.8	**1.4**	0.52, 3.8
Age (in years)	**1.02**	1.00, 1.04	**1.02**	1.00, 1.04		
in females**					**1.04**	1.01, 1.07
in males**					**0.996**	0.967, 1.03
Sex						
Females	**1.0**	-	**1.0**	-	**-**	-
Males	**0.58**	0.31, 1.1	**0.58**	0.29, 1.2	**-**	-
Previous Treatment for *H. pylori*						
None	**1.0**	-	**1.0**	-	**1.0**	-
One or More	**2.9**	1.4, 6.1	**2.2**	0.90, 5.3	**2.1**	0.85, 5.3
Community						
Aklavik	**1.0**	-	**1.0**	-	**1.0**	-
Old Crow	**1.7**	0.79, 3.5	**1.5**	0.66, 3.3	**1.5**	0.66, 3.4
Fort McPherson	**4.7**	1.8, 12	**3.9**	1.4, 11	**4.0**	1.4, 11

*Model estimates adjusted for age (continuous), sex, previous treatment for *H. pylori*, and community. Model 2 adds an interaction term for age and sex.
**Stratum-specific estimates were calculated in two separate models.

To assess the sensitivity of the estimates to the categorization of exposure levels, and in particular to defining the reference level as 0-1 or 0, I used an alternate categorization (0, 1-3, 4-17 antibiotic prescriptions) in a model otherwise identical to Model 2. The results (see Appendix A3) show similar results, however, the trend for increasing odds of resistant infection with increasing antibiotic exposure is not as obvious in the model with the alternate categorization (0, 1-3, 4-17 antibiotic prescriptions). Additionally, the LR test p-value of 0.67 for adding the exposure variable categorized with 0-1 as the reference group (0-1, 2-4, 5-17 antibiotic prescriptions) to the model with selected covariates was slightly lower than the LR test value of 0.69 for adding the exposure variable the alternative categorization (0, 1-3, 4-17 antibiotic prescriptions).

Sub-analysis: Estimating the Effect of Exposure to Specific Antibiotic Classes on the Prevalence of Resistant H. pylori *Infection*

I investigated the distribution of antibiotic exposure for each antibiotic class and specific antibiotic drug by the outcome of interest: antibiotic resistance to the corresponding antibiotic drug. The frequency distributions of antibiotic exposure for each antibiotic class and specific antibiotic drug stratified by susceptibility to the corresponding antibiotic drug are presented in Appendix A4. Inspection of the frequency of exposure by antibiotic class and specific antibiotic drug showed there was enough data to estimate effects of exposure to two antibiotic classes, nitroimidazoles and macrolides, on the prevalence of *H. pylori* infection resistant to metronidazole or clarithromycin, respectively.

To estimate the effect of exposure by antibiotic class on the prevalence of *H. pylori* infection resistant to specific antibiotic drugs in the *H. pylori*-positive participants who had samples of *H. pylori* tested for susceptibility to those drugs and complete data on model covariates, I used multivariable logistic regression models to estimate prevalence ORs and 95% CIs adjusted for all variables previously identified by purposeful selection to be included in Model 2 of Analysis 1, which aimed to estimate the effect of antibiotic exposure in general on the prevalence of antibiotic-resistant *H. pylori*

infection. Additionally, I used the change-in-estimate approach to assess whether the inclusion of any additional potential confounding variables improved the fit of the model.

I defined nitroimidazole exposure as a dichotomous variable indicating presence or absence of exposure to nitroimidazole antibiotics (ranging from 1-3 prescriptions) during the 5-year review period. All prescribed nitroimidazole antibiotics were metronidazole. Of the 169 participants included in this analysis, 20% (33/169) were exposed to nitroimidazole antibiotics. Among the 33 individuals exposed to nitroimidazole antibiotics, 55% (18/33) were classified as being infected with metronidazole-resistant *H. pylori* organisms, whereas of the 136 who had no exposure to nitroimidazole antibiotics, 31% (42/136) were infected with metronidazole-resistant *H. pylori* organisms.

Estimates of the effect of exposure to nitroimidazole antibiotics on the prevalence of metronidazole-resistant *H. pylori* infection adjusted for age, sex, previous exposure to an anti-*H. pylori* treatment regimen and community are presented in Table 3.10. After adjustment for selected factors, the odds of infection with metronidazole-resistant *H. pylori* among those prescribed nitroimidazole antibiotics were 2.3 (95% CI: 1.0, 5.3) times the odds in those not prescribed nitroimidazole antibiotics.

I defined macrolide exposure as a dichotomous variable indicating presence or absence of exposure to macrolide antibiotics (ranging from 1-4 prescriptions) during the 5-year review period. Macrolide antibiotics prescribed included clarithromycin, azithromycin, and erythromycin. Of the 169 participants included in this analysis, 34% (57/169) were exposed to macrolide antibiotics. Among the 57 individuals exposed to macrolide antibiotics, 21% (12/57) were classified as being infected with clarithromycin-resistant *H. pylori* organisms, whereas of the 112 individuals who had no exposure to macrolide antibiotics, 12% (13/112) were classified as being infected with clarithromycin-resistant *H. pylori* organisms.

Table 3.10 presents estimates of the effect of exposure to macrolide antibiotics on the prevalence of clarithromycin-resistant *H. pylori* infection adjusted for age, sex, previous exposure to an anti-*H. pylori* treatment regimen, and community, as well as

individual education level, which was identified by the change-in-estimates approach as an additional control variable. The odds of infection with clarithromycin-resistant *H. pylori* organisms among participants exposed to macrolide antibiotics was 2.2 times (95% CI: 0.78, 6.1) the odds in participants who had no exposure to macrolide antibiotics.

Table 3.10. Estimated effects of exposure to specific classes of antibiotics on the prevalence of *H. pylori* infection resistant to specific antibiotic drugs in 169 *H. pylori*-positive participants who had samples of *H. pylori* tested for antibiotic susceptibility and complete data for all selected model covariates: Results of logistic regression analysis.

Variable	n	Unadjusted		Adjusted*	
		OR	95% CI	OR	95% CI
Nitroimidazole Exposure					
0 Prescriptions	136	1.0	-	1.0	-
1-3 Prescriptions	33	2.7	1.2, 5.8	2.3	1.0, 5.3
Macrolide Exposure**					
0 Prescriptions	110	1.0	-	1.0	-
1-4 Prescriptions	56	1.8	0.76, 4.4	2.2	0.78, 6.1

*Estimate for nitroimidazole exposure adjusted for age (continuous), sex, previous treatment for *H. pylori*, and community with all variables except age categorized as in table 3.7; estimate for macrolide exposure adjusted for adjusted for age (continuous), sex, previous treatment for *H. pylori*, community, and education level with all variables except age categorized as in table 3.7.
**3 individuals were excluded from this model due to missing data on education level

Analysis 2: Estimating the Effect of Exposure to Antibiotics on the Risk of Anti-H. pylori *Treatment Failure*

Analysis 2: Descriptive Statistics

The socio-demographic characteristics of the 214 individuals who completed a post-treatment UBT and were, therefore, eligible to be included in Analysis 2, which aims to estimate the effect of exposure to antibiotics on the risk of anti-*H. pylori* treatment failure, are shown in Table 3.11. Through model building by purposeful selection, the following variables were selected as control variables: age (categorical), previous treatment for *H. pylori*, education level, and the anti-*H. pylori* treatment regimen prescribed in the CAN*Help* community project. The sample for this analysis was reduced to 179 individuals following restriction of the study population to participants with

complete data on variables for previous treatment for *H. pylori*, education level, and the anti-*H. pylori* regimen prescribed through CAN*Help* community projects. Selected characteristics of the 179 individuals included in this analysis are also presented in Table 3.11. The follow-up interval for ascertaining anti-*H. pylori* treatment success status by post-treatment UBT ranged from 3 to 114 weeks with a median of 17 weeks (data on the date of treatment, used to calculate the range and median of the post-treatment follow-up interval, was missing for 11 individuals).

Table 3.11. Selected characteristics of 214 eligible individuals and 179 individuals with complete data for all selected variables for Analysis 2 estimating the effect of antibiotic exposure history on the risk of anti-*H. pylori* treatment failure.

	214 Eligible Participants			**179 Participants with Complete Data**		
Variables	**n**	**Risk of Treatment Failure (Incidence Proportion)**		**n**	**Risk of Treatment Failure (Incidence Proportion)**	
		%	**95% CI**		**%**	**95% CI**
Age Range in Years*						
<35 Years	73	36	25, 48	56	34	22, 48
35-54 Years	76	32	21, 43	68	32	22, 45
>=55 Years	65	12	5, 23	55	11	4, 22
Sex						
Female	113	30	22, 39	94	29	20, 39
Male	101	24	16, 33	85	24	15, 34
Education						
Less than Grade 12	106	30	22, 40	92	28	19, 39
Grade 12 or Equivalent	33	42	25, 61	33	42	25, 61
Certificate/Diploma/Degree	57	12	5, 24	54	13	5, 25
Missing	18	28	10, 53	0	-	-
Ethnicity						
Gwich'in	103	24	16, 34	91	23	15, 33
Inuvialuit	80	35	25, 46	68	32	22, 45
Other Aboriginal	17	12	1, 36	14	14	2, 43
Non-Aboriginal	6	33	4, 78	6	33	4, 78
Missing	8	13	0.3, 53	0	-	-
Household Income						
High	71	20	11, 31	65	22	12, 33
Medium	42	29	16, 45	33	33	18, 52
Low & Very Low	53	34	22, 48	46	26	14, 41

Missing	48	29	17, 44	35	29	15, 46
Community						
Aklavik	106	35	26, 45	90	34	25, 45
Old Crow	50	22	12, 36	36	22	10, 39
Fort McPherson	46	13	5, 26	42	12	4, 26
Tuktoyaktuk	12	33	10, 65	11	27	6, 61
Previous Treatment for *H. pylori*						
None	154	24	18, 32	141	23	16, 31
One or More	40	38	23, 54	38	39	24, 57
Missing	20	30	12, 54	0	-	-
Anti-*H. pylori* Treatment Prescribed by CAN*Help* Gastroenterologist						
Sequential	93	26	17, 36	83	25	16, 36
Triple	53	40	26, 54	50	40	26, 55
Quadruple	54	11	4, 23	46	13	5, 26
Treated Outside Research Project	7	71	29, 96	0	-	-
Missing	7	29	4, 71	0	-	-
Treatment Adherence						
Completed All Prescribed Medications	109	23	15, 32	95	24	16, 34
Didn't Complete All Prescribed Medications	34	35	20, 54	29	34	18, 54
Missing	71	30	19, 42	55	25	15, 39
Treatment Side Effects						
Yes	112	27	19, 36	99	28	20, 38
No	54	26	15, 40	46	26	14, 41
Missing	48	29	17, 44	34	21	9, 38

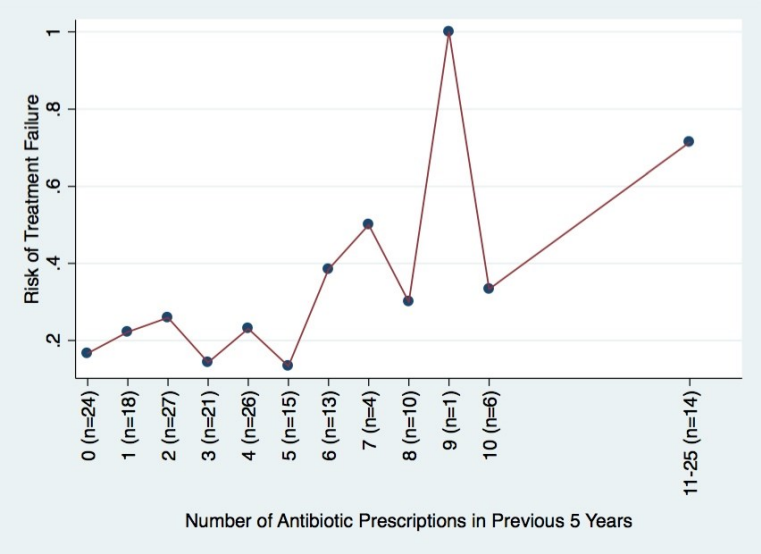
*For the 214 eligible participants, the age range was 4-78 years and the mean age was 43. For the 179 participants with complete data, the age range was 12-78 years and the mean age was 44.

Analysis 2: Risk of Treatment Failure by Exposure Level

Examination of the relationship between the number of antibiotic courses prescribed during the 5-year review period and the average risk of anti-*H. pylori* treatment failure, did not reveal a linear or other simple monotonic trend. For this reason, I did not model the exposure as a continuous variable. Figure 3.2 presents the average risk of treatment failure by the number of antibiotic prescriptions in the 5-year study period in the 179 participants included in this analysis.

Chapter 3. Estimating the effect of antibiotic exposure on antibiotic-resistant *H. pylori* infection and anti-*H. pylori* treatment failure among participants in CAN*Help* community projects in northern Canada

Figure 3.2. Risk of treatment failure by the number of antibiotic prescriptions in the previous 5 years in 179 participants with a post-treatment UBT and complete data for selected model covariates.



In a manner similar to Analysis 1, I defined antibiotic exposure for Analysis 2 using three categories: 0-1, 2-5, and 6-25 antibiotic prescriptions in the 5-year period. For reasons explained in presenting results of Analysis 1, the reference level grouped 0 prescriptions and 1 prescription, rather than being restricted to 0. Initially, I investigated antibiotic exposure with the following category boundaries: 0-1, 2-5, 6-8, and 9-25 prescriptions. However, I collapsed the highest category, 9-25 prescriptions, with the second highest category, 6-8 prescriptions, to avoid a small number (n=21) in the highest exposure category. Combining the highest two exposure categories to improve precision was justified because both of the highest two exposure categories had a higher average risk of treatment failure compared to 0-1 and 2-5 prescriptions. A lower p-value of 0.41 was obtained from the LR test for adding the 3-category exposure variable to the regression model with selected covariates than from the LR test for adding the 4-category exposure variable, for which the p-value was 0.53. Table 3.12 presents the average risk of treatment failure by exposure level, for both the 4-category and 3-category versions, in participants included in Analysis 2.

Table 3.12. Risk of anti-*H. pylori* treatment failure by antibiotic exposure level in 179 participants with a post-treatment UBT and complete data for selected model covariates.

Number of Antibiotic Prescriptions During the 5-year review period	**n**	**Risk of Treatment Failure**	
		%	**95% CI**
Four Categories			
0-1	42	19	9, 34
2-5	89	20	12, 30
6-8	27	37	19, 58
9-25	21	52	30, 74
Three Categories			
0-1	42	19	9, 34
2-5	89	20	12, 30
6-25	48	44	29, 59

Analysis 2: Multivariable Poisson Regression Model with Robust Standard Error

Multivariable log-binomial models for estimating the RR failed to converge, so RRs and 95% CIs estimated for Analysis 2 using Poisson regression with robust standard error are presented in Table 3.13. Examination of the relationship between age and treatment failure revealed that it was nonlinear. Assessment of a cubic spline for age in the STATA 12 statistical software package identified 3 groupings of age values; the boundary values for these groupings (knots) were confirmed by visual assessment of a lowess plot for the risk of treatment failure by age in 1-year increments. LR test results for adding the cubic spline to a model with the exposure variable and the other selected covariates resulted in a p-value of 0.47, which was much higher than the LR test p-value of 0.15 for adding age categorized in 3 levels with category boundaries based on the location of knots generated for the cubic spline. Therefore, I modeled age as a categorical variable with 3 levels: <35 years; 35-54 years; and ≥55 years.

Unadjusted RR estimates for the effect of antibiotic exposure on the average risk of anti-*H. pylori* treatment failure in participants included in this analysis suggest a binary effect of increasing antibiotic exposure, as categorized, on the risk of treatment failure, with a similar risk from 0 through 5 prescriptions and more than double the risk from 6 through 25 prescriptions, although the wide confidence intervals reflect uncertainty about

the magnitude of the effect and the broad exposure categories may obscure the trend. Adjusted RRs and 95% CI estimates show a much smaller increase in the risk of treatment failure when comparing the high exposure category (6-25 prescriptions) to the lower categories, although, as with the unadjusted estimates, the wide confidence intervals reflect uncertainty about the magnitude of the effect and the broad categories may obscure the trend.

Additional estimates from the multivariable Poisson regression model with robust standard error are consistent with a greatly reduced risk of treatment failure among individuals ≥55 years of age compared to younger individuals. Also, results of the multivariable analysis estimate a 2.0-fold (95% CI: 1.2, 36) increase in the risk of treatment failure among individuals previously treated with an anti-*H. pylori* treatment regimen relative to individuals with no history of exposure to an anti-*H. pylori* treatment regimen. Also, the estimated risk of treatment failure was substantially higher among individuals treated with triple therapy compared to those treated with either of the other therapies and substantially lower among those treated with quadruple therapy compared to those treated with either of the other therapies.

Table 3.13. Estimated risk ratios for the effect of antibiotic exposure on anti-*H. pylori* treatment failure in 179 participants with a post-treatment UBT and complete data for all selected model covariates: Results of Poisson regression analysis with robust standard error.

	Unadjusted Estimates		**Model 4***	
Variable	**RR****	**95% CI**	**RR****	**95% CI**
Antibiotic Exposure				
0-1 Prescriptions	**1.0**	-	**1.0**	-
2-5 Prescriptions	**1.1**	0.50, 2.2	**0.87**	0.43, 1.8
6-25 Prescriptions	**2.3**	1.1, 4.6	**1.4**	0.68, 2.9
Age				
<35 Years	**1.0**	-	**1.0**	-
35-54 Years	**0.95**	0.58, 1.6	**1.1**	0.71, 1.8
≥55 Years	**0.32**	0.14, 0.75	**0.49**	0.21, 1.1
Previous Treatment for *H. pylori*				
None	**1.0**	-	**1.0**	-
One or More	**1.7**	1.1, 2.9	**2.0**	1.2, 3.6
Education				
<Grade 12	**1.0**	-	**1.0**	-

Grade 12/Equivalent	**1.5**	0.90, 2.5	**1.6**	0.96, 2.6
Diploma/Certificate/Degree	**0.46**	0.21, 0.99	**0.63**	0.29, 1.4
Therapy				
Sequential	**1.0**	-	**1.0**	-
Triple	**1.6**	0.96, 2.6	**1.8**	1.1, 2.9
Quadruple	**0.52**	0.22, 1.2	**0.54**	0.24, 1.2

***Model 4** included age, previous treatment for *H. pylori*, education level, and the anti-*H. pylori* treatment regimen prescribed in a CAN*Help* community project.
**RR approximated from the incidence rate ratio estimated by Poisson regression with robust standard error.

When attempting to estimate adjusted RDs and 95% CIs for the effect of antibiotic exposure on the average risk of anti-*H. pylori* treatment failure in participants included in this analysis, neither a multivariable binomial regression model or a multivariable Poisson regression model with robust standard error and identity link function, which permits estimation of the RD, would converge. However, a binomial regression model converged when control variables were restricted to age and previous treatment for *H. pylori*; therefore, variables for education level and the anti-*H. pylori* treatment prescribed in a CAN*Help* community project were excluded. Estimated RDs and 95% CIs adjusted for age and previous treatment for *H. pylori* are presented in Table 3.14. Unadjusted RD estimates show a binary effect of antibiotic exposure on the risk of anti-*H. pylori* treatment failure; adjusted RD estimates, however, suggest the risk of treatment failure increases with increasing antibiotic exposure, as categorized, although the wide confidence intervals reflect uncertainty about the magnitude of the effect and the broad exposure categories may obscure the trend.

Table 3.14. Estimated risk differences for the effect of antibiotic exposure on anti-*H. pylori* treatment failure in 179 participants with a post-treatment UBT and complete data for all selected model covariates: Results of binomial regression analysis.

Variable	**RD***	**95% CI**
Unadjusted Risk Difference Estimates		
Antibiotic Exposure		
0-1 Prescriptions	0	-
2-5 Prescriptions	0.01	-0.13, 0.16
6-25 Prescriptions	0.25	0.06, 0.43
Constant §	0.19	0.07, 0.31
Age		
<35 Years	0	-

35-54 Years	-0.02	-0.18, 0.15
≥55 Years	-0.23	-0.38, -0.08
Constant §	0.34	0.22, 0.46
Previous Treatment for *H. pylori*		
None	0	-
One or More	0.17	-0.00, 0.34
Constant §	0.23	0.16, 0.30
Education		
<Grade12	0	-
Grade 12/Equivalent	0.14	-0.05, 0.33
Diploma/Certificate/Degree	-0.15	-0.28, -0.02
Constant §	0.28	0.19, 0.37
Therapy		
Sequential	0	-
Triple	0.15	-0.02, 0.31
Quadruple	-0.12	-0.26, 0.01
Constant §	0.25	0.16, 0.35
Adjusted Risk Difference Estimate**		
Antibiotic Exposure		
0-1 Prescriptions	0	-
2-5 Prescriptions	0.06	-0.02, 0.14
6-25 Prescriptions	0.23	0.06, 0.41
Constant ϕ	0.24	0.11, 0.38

*RD estimated with binomial regression model [fam(binomial) link(identity)].
**Adjusted for age (categorical) and previous treatment for *H. pylori*.
§ The constant is the estimated average risk of treatment failure in the reference group.
ϕ The constant is the estimated risk of treatment failure among individuals exposed to 0-1 antibiotic prescriptions who were <35 years of age and who have no history of exposure to an anti-*H. pylori* treatment regimen.

*Sub-analysis: Investigating the Effect of Exposure to Macrolide Antibiotics on the Risk of Anti-*H. pylori *Treatment Failure*

I tabulated the frequency among all individuals with a post-treatment UBT of exposure to each antibiotic class and specific antibiotic drug by anti-*H. pylori* treatment failure status for all prescribed anti-*H. pylori* treatment regimens combined and for each regimen separately (Appendix A5). Following assessment of the available data, I decided there was sufficient data for just one sub-analysis of the exposure effect on the risk of

treatment failure: the effect of exposure to macrolide antibiotics on the risk of anti-*H. pylori* treatment failure for all treatment regimens combined.

Among the 179 participants included in this analysis, 34% (61/179) had one or more macrolide antibiotic prescriptions (ranging from 1-5) during the 5-year review period. Macrolide antibiotics prescribed included clarithromycin, azithromycin, and erythromycin. Among participants included in this analysis, the average risk of treatment failure across all prescribed treatment regimens was 23% (27/118) in those with no macrolide exposure and 33% (20/61) in those exposed to macrolides. The estimated unadjusted risk ratio for the effect of having been prescribed macrolides on treatment failure was 1.4 (95% CI: 0.88, 2.3). Adjusting for age, previous treatment for *H. pylori*, education level, and the anti-*H. pylori* treatment regimen prescribed in a CAN*Help* community project, the adjusted risk ratio for the effect of having been prescribed macrolide antibiotics on treatment failure was 1.1 (95% CI: 0.73, 1.8).

The RD for the effect of macrolide exposure on the risk of anti-*H. pylori* treatment failure was estimated with a binomial regression model. The unadjusted risk difference for the effect of having been prescribed macrolide antibiotics on treatment failure was 0.09 (95% CI: -0.04, 0.24). The adjusted risk difference from a multivariable binomial regression model that included age and previous treatment for *H. pylori* was 0.06 (95% CI: -0.07, 0.20). The variables for education and the anti-*H. pylori* treatment regimen prescribed through a CAN*Help* community project were not included because the regression model did not converge when these variables were included.

Discussion

The results of this study suggest that among *H. pylori*-positive residents of Arctic Canadian communities, both the prevalence odds of antibiotic-resistant *H. pylori* infection and the risk of anti-*H. pylori* treatment failure were higher in participants with more frequent antibiotic exposure. Additionally, these results suggest that previous exposure to anti-*H. pylori* treatment is associated with increased prevalence odds of antibiotic-resistant *H. pylori* infection and increased risk of anti-*H. pylori* treatment failure.

In the present study, the dispensation rate for systemic antibiotic prescriptions among eligible participants in CAN*Help* Working Group community projects was 0.88 prescriptions per person per year. A somewhat higher antibiotic prescription rate of 1.5 per person per year was reported among individuals from an Alaskan Native population included in the study conducted by McMahon *et al.* (2003).[104] In contrast, the estimated antibiotic dispensation rate for the Edmonton City Centre was notably lower: 0.56 antibiotic prescriptions per person per year. Similar to the Edmonton City Centre, an antibiotic dispensation rate of 0.44 per person per year was reported by McCaig and Hughes in their study of United States physician practices conducted in 1992.[338] A higher observed use of antibiotics in remote Arctic regions may occur due to: 1) a more limited access to diagnostic technology used to confirm bacterial infections compared to southern regions leading to the dispensation of antibiotics prior to a confirmed diagnosis; 2) an overall higher incidence of infectious diseases in Arctic communities; or 3) differences in prescription practices across regions: health care is primarily distributed by nurses in phone consultation with physicians in remote northern Canadian communities while health care in Edmonton is primarily provided directly by physicians.[339]

Among CAN*Help* project participants, the antibiotic dispensation rate was also observed to vary according to sex, age, and antibiotic class. Across all four CAN*Help* project communities, the antibiotic dispensation rate was notably higher in females compared to males and slightly higher in adults ≥45 years of age compared to younger participants. Variation in antibiotic dispensation rates was also observed by antibiotic class; the highest dispensation rates were observed for β-lactam and macrolide antibiotics. Similar antibiotic dispensation patterns were observed in the Edmonton City Centre population.

Variation in the use of antibiotics for the treatment of common bacterial infections may contribute to variation in antibiotic-resistant *H. pylori* infection across geographic regions.[316,317] For example, *H. pylori* infection resistant to clarithromycin and metronidazole have been associated with younger age groups and female sex, respectively.[53] Evidence suggests that higher prevalence of clarithromycin resistance among *H. pylori*-positive children compared to *H. pylori*-positive adults is due to higher

consumption of macrolide antibiotics in younger age groups for the treatment of respiratory tract infections.[55,73] In a study conducted by Banatvala *et al.* (1994) among participants from the United Kingdom, women were more frequently prescribed nitroimidazole antibiotics than men (41% [9/22] vs. 10% [2/20], respectively; p=0.01) and were more frequently infected with metronidazole-resistant *H. pylori* (54% [13/24] vs. 18% [4/22], respectively; p=0.02).[340] Higher prevalence of metronidazole-resistant *H. pylori* infection in *H. pylori*-positive women relative to men may be due to widespread use of nitroimidazole antibiotics for the treatment of gynaecological infections, such as trichomoniasis and bacterial vaginosis.[53,104,260,269,304–306] Additionally, the use of metronidazole in developing countries for the treatment of parasitic diseases may contribute to higher prevalence of nitroimidazole-resistant *H. pylori* infection among *H. pylori*-positive individuals who reside in or are emigrants from developing countries.[53,104,260,269,304–306] Similarly, higher prevalence of tetracycline-resistant *H. pylori* infection in *H. pylori*-positive women relative to men may be due to more frequent use of tetracycline in women for the treatment of urogential infections.[341] Higher prevalence of fluoroquinolone-resistant *H.* pylori infection in *H. pylori*-positive females and *H. pylori*-positive individuals in younger age groups may be associated with more frequent use of fluoroquinolone antibiotics for the treatment of urogenital and respiratory tract infections, respectively.[341]

In this study, among participants who had samples of *H. pylori* tested for antibiotic susceptibility, the prevalence of resistance to any antibiotic tested was 45%. Prevalence estimates for clarithromycin and metronidazole resistance in tested *H. pylori* isolates from participants was 16% and 36%, respectively; in contrast, estimates of the prevalence of resistance to other investigated antibiotics were low: 0-1% for amoxicillin, tetracycline, nitrofurantoin, and rifampicin and 4% for ciprofloxacin.

For context estimates of the prevalence of antibiotic-resistant *H. pylori* infection generated by this study, can be compared to estimates from the literature. A review conducted by Megraud & Lehours (2007) estimated the prevalence of clarithromycin-resistant *H. pylori* infection to range from 0% to 25% among *H. pylori*-positive individuals worldwide.[124] Estimates for clarithromycin-resistant *H. pylori* infection in

Canada are limited. Fallone *et al.* estimated the prevalence of clarithromycin-resistant *H. pylori* infection to be <4% among *H. pylori*-positive individuals in a systematic review including Canadian studies published prior to 2000.[342] Estimates of the prevalence of metronidazole resistance in investigated *H. pylori* isolates range from 20% to 40% in the United States[76,77,343] and Europe,[53] and from 50% to 80% in developing regions.[344,345] Fallone *et al.* estimated the prevalence of metronidazole-resistant *H. pylori* infection in Canada to range from 18% to 22%.[342] Prevalence estimates for amoxicillin- and tetracycline-resistant *H. pylori* infection remain close to zero worldwide, except for a few geographic regions.[346–348] The prevalence of ciprofloxacin resistance among investigated *H. pylori* isolates has been estimated in studies from France, Belgium and Germany to range from 16% to 17%.[349–351] Just one study estimated the prevalence of antibiotic resistance in isolates from an Indigenous Arctic population: McMahon *et al.* observed the prevalence of clarithromycin-resistant and metronidazole-resistant *H. pylori* infection to be 30% (37/125) and 66% (83/125), respectively, in an Alaska Native population.[104]

In addition to providing evidence that the prevalence odds of antibiotic-resistant *H. pylori* infection increase with increasing antibiotic exposure, the results of this study presented evidence that *H. pylori*-positive residents of Canadian Arctic communities with previous exposure to metronidazole had more than double the prevalence odds of infection with metronidazole-resistant *H. pylori* organisms compared to those without previous metronidazole exposure, and those with previous exposure to macrolide had more than double the odds of infection with clarithromycin-resistant *H. pylori* organisms compared to those without previous macrolide exposure.

Previous studies have presented evidence of an association between previous antibiotic use and antibiotic-resistant *H. pylori* infection.[73,104,268,340,352,353] In the report on the Alaskan Native population by McMahon *et al.,* the results suggested that the prior use of macrolide antibiotics and metronidazole were associated with *H. pylori* infection resistant to these antibiotics.[104] In their study, McMahon *et al.* investigated the association between antibiotic prescriptions during the 10 years prior to a diagnosis of *H. pylori* infection.[104] McMahon *et al.* reported an adjusted OR of 1.6 (95% CI: 1.3, 2.0) for association between previous macrolide use (defined as ≥1 antibiotic course compared to

0 antibiotic courses in the 10-year review period) and clarithromycin-resistant *H. pylori* infection, according to the results of a multivariable logistic regression model that included sex, macrolide use, and metronidazole use. McMahon *et al.* reported a much higher OR of 6.3 (95% CI: 2.5, 16) for the association between metronidazole use (defined as ≥1 antibiotic course compared to 0 antibiotic courses in the 10-year review period) and metronidazole-resistant *H. pylori* infection, from the model that included sex, metronidazole use, and macrolide use. McMahon *et al.* also reported that the relatively distant use of macrolides was associated with macrolide-resistant infection by restricting their study population to individuals who had no macrolide exposure in the past 5 years; they reported additionally that among 9 individuals with macrolide-resistant *H. pylori* infection, 6 (67%) had been prescribed a macrolide antibiotic during the 6-8 years before diagnoses with *H. pylori* infection while in contrast, among 45 individuals who were infected with *H. pylori* organisms susceptible to macrolides, just 7 (16%) had been prescribed a macrolide antibiotic during the 6-8 years before diagnosis.[104] Carothers *et al.* (2007) also reported an association between fluoroquinolone use (the antibiotic class the includes ciprofloxacin and levofloxacin) and levofloxacin-resistant *H. pylori* infection among 125 individuals undergoing endoscopy from the same Alaska Native population investigated in the study conducted by McMahon *et al*.[353] In their study, Carothers *et al.* reported the frequency of levofloxacin-resistant *H. pylori* infection to be higher among individuals who had fluoroquinolone exposure during the 10 years prior to diagnosis with *H. pylori* infection.[353] Among 11 individuals with levofloxacin-resistant *H. pylori* infection, 82% had been exposed to a fluoroquinolone antibiotic during the 10-year review period compared to 17% of the 114 individuals with levofloxacin-susceptible *H. pylori* infection (p-value<0.001).[353] Banatvala *et al*. (1994) investigated prior metronidazole use, as reported by an individual's current general practitioner (GP), and the presence of metronidazole-resistant *H. pylori* infection among individuals residing within the United Kingdom: among 32 individuals who had been previously exposed to nitroimidazoles (metronidazole or tinidazole), 27 (84%) were infected with metronidazole-resistant *H. pylori* organisms compared to 21 (41%) of 51 individuals who had no history of nitroimidazole exposure (p-value<0.0001).[340]

The risk of anti-*H. pylori* treatment failure among *H. pylori*-positive CAN*Help* project participants who completed a post-treatment UBT was 40% (21/53) for standard triple therapy, 26% (24/93) for sequential therapy, and 11% (6/54) for quadruple therapy. Estimates of the risk of treatment failure for standard triple therapy, sequential therapy, and quadruple therapy suggest that quadruple therapy was more effective at eliminating *H. pylori* infection among CAN*Help* project participants than standard triple therapy or sequential therapy. Additionally, among participants treated with quadruple therapy who had complete data for this analysis, the estimated risk of treatment failure was lower among participants infected with an *H. pylori* strain susceptible to all tested antibiotics (5% [1/19]) compared to those infected with an *H. pylori* strain resistant to one or more antibiotics (18% [3/17]).

In the literature, articles often report estimates of the effectiveness of anti-*H. pylori* treatment regimens as the proportion of treated individuals whose infection is eliminated.[35] Therefore, for context, estimates of the risk of anti-*H. pylori* treatment failure in the current study can be interpreted as effectiveness estimates as follows: 60% (32/53) for standard triple therapy; 74% (69/93) for sequential therapy; and 89% (48/54) for quadruple therapy. Estimates of the effectiveness of standard triple therapy from the current study are low compared to estimates published in a meta-analysis conducted by Rodgers and van Zanten of Canadian clinical trials of anti-*H. pylori* therapies; meta-analysis estimates for the effectiveness of triple therapy were 84%; (95% CI: 79%, 90%) for regimens combining a PPI, clarithromycin and amoxicillin, and 92% (95% CI: 76%, 88%) for regimens combining a PPI, clarithromycin and metronidazole.[41] In contrast, the effectiveness of quadruple therapy in the current study is similar to the meta-analysis estimate reported by Rodgers and van Zanten of 97% (95% CI: 80%, 95%).[41] Other meta-analyses have produced similar estimates for the effectiveness of standard triple therapy and quadruple therapy when compared to the estimates reported by Rodgers and van Zanten.[42,43] The effectiveness of sequential therapy was recently compared to the effectiveness of standard triple therapy and quadruple therapy in a meta-analysis conducted by Gatta *et al* (2013).[47] Gatta *et al*. reported the treatment effectiveness of sequential therapy and standard triple therapy to be 80.8% (95% CI: 76%, 85.1%) and

81.3% (95% CI: 79.5%, 84.7%), respectively, when prescribed for 14 days.[47] Additionally, Liou *et al.* (2013) reported the effectiveness of sequential therapy and standard triple therapy in a multicentre randomized trial to be 90.7% (95% CI: 87.4%, 94.0%) and 82.3% (95% CI: 78.0%, 86.6%), respectively, when prescribed for 14 days.[48] However, other reports present estimates of the effectiveness of standard triple therapy to be less than 80%. In large US trials, for example, the effectiveness of standard triple therapy has been observed to be low.[50,51] The inconsistent effectiveness of standard triple therapy across geographic regions is likely related to the prevalence of antibiotic-resistant *H. pylori* infection.[52]

Results from this study showed that participants who were previously prescribed an anti-*H. pylori* treatment regimen had roughly twice the odds of antibiotic-resistant *H. pylori* infection compared to those who had no history of exposure to an anti-*H. pylori* treatment regimen. Similarly, the results estimated a 2-fold higher risk of treatment failure across all prescribed anti-*H. pylori* treatment regimens among individuals who were previously prescribed an anti-*H. pylori* treatment regimen compared to those who had no history of exposure to an anti-*H. pylori* treatment regimen. Similarly, Selgrad *et al.* (2013) observed that the prevalence of *H. pylori* infection resistant to antibiotics commonly used in anti-*H. pylori* treatment regimens (including clarithromycin, metronidazole and levofloxacin) increased with the number of prior unsuccessful anti-*H. pylori* treatment attempts.[103] This study did not yield sufficient data for full analysis of the effects of previous exposure to specific antibiotics.

This analysis did not include an indicator for antibiotic-resistant *H. pylori* infection in multivariable regression aimed at estimating effects on the risk of treatment failure, due to the small number of study participants with antibiotic susceptibility data and a post-treatment UBT result as well as complete data on selected model covariates. However, the current study observed that among individuals with a post-treatment UBT, those infected with *H. pylori* organisms susceptible to all tested antibiotics had a lower average risk (28%) of anti-*H. pylori* treatment failure across all prescribed treatment regimen than those infected with *H. pylori* organisms resistant to one or more of the antibiotics tested (33%). In particular, among individuals with clarithromycin-resistant *H.*

pylori infection or those with clarithromycin-susceptible *H. pylori* infection, the average risk of treatment failure across all prescribed regimens was 42% and 27%, respectively (Data shown in Appendix A6). In contrast, a smaller difference was observed between individuals with metronidazole-resistant *H. pylori* infection and those with metronidazole-susceptible *H. pylori* infection, given estimates of the average risk of treatment failure across all regimens of 28% and 31%, respectively (Data shown in Appendix A6).

The frequency of anti-*H. pylori* treatment failure in relation to the antibiotic susceptibility of *H. pylori* organisms has also been reported in the literature. McMahon *et al.* estimated that within their study population of 125 Alaska Native adults, 84% of clarithromycin-based treatment regimens that failed to eliminate the infection were prescribed to individuals infected with clarithromycin-resistant *H. pylori* infection.[104] Additionally, Poon *et al.* (2009), observed changes in the prevalence of metronidazole-resistant *H. pylori* infection and clarithromycin-resistant *H. pylori* infection in a population of patients who underwent endoscopy for the evaluation of dyspeptic symptoms between 1998-2000 and 2001-2004 in Taiwan following restriction of antibiotic treatment of ambulatory patients to those with upper respiratory tract infections and evidence of bacterial infection.[352] The prevalence of metronidazole-resistant *H. pylori* infection decreased notably during this time period from 42% (35/84) to 25% (34/134) ($p<0.05$).[352] The prevalence of clarithromycin-resistant *H. pylori* infection decreased only slightly, however, from 11% (9/84) to 7% (9/134) (p-value=0.42). Additionally, Poon *et al.* reported that the effectiveness of standard triple therapy with clarithromycin and metronidazole increased from 75% (21/28) to 83% (38/46) ($p=0.62$).[352] In contrast, the effectiveness of triple therapy consisting of clarithromycin and amoxicillin remained similar between the examined time periods: 84% (32/38) and 85% (39/46) ($p=0.82$).[352] However, estimates for the change in the effectiveness of anti-*H. pylori* treatment regimens were based on small samples that are too small for adequate precision.

An important limitation of this analysis is the potential underestimation of antibiotic use among participants. Participant medical charts may not capture all

antibiotic prescriptions, given that participants may receive health care in locations outside of their community health centre. However, given that health care is primarily provided to residents of northern Canadian communities at community health centres, it is likely that most antibiotics prescribed during the review period for this study were captured by review of participants' medical charts.

Misclassification of exposure to antibiotics may have also occurred due to imperfect recording of antibiotic prescriptions in medical charts. Given that antibiotic exposure history data was recorded in medical charts before participants were tested for antibiotic-resistant *H. pylori* infection or treated to eliminate *H. pylori* infection in a community project, it is unlikely that the recording of antibiotic prescriptions differed systematically between individuals with either of the two health outcomes of interest: antibiotic-resistant *H. pylori* infection or anti-*H. pylori* treatment failure. Therefore, any inaccuracies in medical charts leading to misclassification of antibiotic exposure history would likely be nondifferential, with respect to the study outcomes.[354] Because the exposure variable is defined by more than 2 ordinal categories, it is difficult to predict whether any bias resulting from this misclassification is toward or away from the null.[354]

Additional misclassification of exposure may have occurred from the use of antibiotic prescriptions recorded in medical charts as a proxy for actual exposure to antibiotics. Information regarding prescriptions does not provide data on whether or not the individual filled the prescription or took the medication.

Also, data on antibiotic exposure history is limited to the 5-year period prior to enrolment in a CAN*Help* community project. Exposure to antibiotics prior to this period is not captured in this dataset and therefore the association between antibiotics used during more distant time periods and antibiotic-resistant *H. pylori* outcomes in CAN*Help* project participants cannot be investigated.

Misclassification of covariates is an additional concern. In particular, due to imperfect recall, individuals may have been misclassified on having been prescribed an anti-*H. pylori* treatment regimen before enrolment in the community project. Such misclassification would likely be nondifferential with respect to their outcome

classification in the present study given that individuals provided information on previous prescriptions upon enrolment in a community project, before being tested for the antibiotic-susceptibility of *H. pylori* infection or treated with an anti-*H. pylori* treatment regimen in the community project. Also, considering the widespread concern about *H. pylori* infection among participants in community projects and the complexity and duration of anti-*H. pylori* treatments, it is likely that the majority of individuals prescribed an anti-*H. pylori* treatment would provide accurate responses regarding their previous exposure to an anti-*H. pylori* treatment regimen.

Selection bias would be a concern for the present analysis if the frequency of participation varied by antibiotic exposure history to a different degree among participants with antibiotic-resistant *H. pylori* infection and those with antibiotic-susceptible *H. pylori* infection, or if the frequency of participation varied by antibiotic exposure history to a different degree among individuals who did or did not have a positive UBT result following treatment with an anti-*H. pylori* treatment regimen. For example, selection bias may have occurred if individuals with higher numbers of antibiotic prescriptions within the 5-year period before enrolment in the community project were more likely to participate in endoscopy if they had antibiotic-resistant *H. pylori* infection than if they had antibiotic-susceptible *H. pylori* infection. However, it is unlikely that an individual's outcome in this study would influence participation because participants and project staff did not have any knowledge of the susceptibility of an individuals' *H. pylori* infection prior to participation in the community project. It is possible, however, that a risk factor for resistant infection, such as previous treatment for *H. pylori* infection, influenced participation to a different degree for participants with higher and lower numbers of antibiotic prescriptions within the 5-year period.

Selection bias can also result from missing data, if the completeness of data is not random with respect to study variables. To assess whether data was missing at random across socio-demographic factors, the distributions of socio-demographic factors is presented for: the study population with complete data for Analysis 1 (n=169); the study population with complete data for Analysis 2 (n=179); and all *H. pylori*-positive individuals enrolled across CAN*Help* community projects (n=559) (Appendix A7).

Similar distributions of socio-demographic factors were observed in the subsets of participants with complete data and among all *H. pylori*-positive individuals enrolled in CAN*Help* community projects except for community of residence. Both study populations had a higher proportion of individuals from Aklavik and a lower proportion of individuals from Tuktoyaktuk compared to all *H. pylori*-positive community project participants. Also, the Analysis 1 study population had a lower proportion of individuals from Fort McPherson compared to all *H. pylori*-positive community project participants. Both study populations had a slightly higher proportion of individuals who reported being previously prescribed an anti-*H. pylori* treatment regimen compared to all *H. pylori*-positive community project participants, which may have occurred because individuals who accessed health care for gastric symptoms that resulted in the prescription of an anti-*H. pylori* treatment regimen may have also been more likely than asymptomatic *H. pylori*-positive individuals to participate in endoscopy and treatment components of community projects. However, given that participants were asked to recall previous exposure to an anti-*H. pylori* treatment regimens before the study outcomes were ascertained, this recall is unlikely to have varied across outcome categories of interest and adjustment for previous anti-*H. pylori* treatment in regression models likely controlled for this source of bias.[355] When comparing the subsets of individuals with complete data for Analysis 1 and for Analysis 2 with all *H. pylori*-positive individuals with antibiotic resistance data for cultured *H. pylori* isolates (n=191) or a post-treatment UBT (n=214), the distribution of relevant socio-demographic variables is similar.

Another potential source of bias in the current study is the misclassification of the *H. pylori* status of study participants. In the current study, all participants, regardless of their infection status, were invited to undergo endoscopy and the antibiotic susceptibility of *H. pylori* isolates was classified following attempts to culture *H. pylori* from gastric biopsy samples. Also, participants in this study were offered treatment if they were classified as *H. pylori*-positive whether or not they had endoscopy with gastric biopsies. While culture-based methods approach 100% specificity if they incorporate the standard series of confirmatory tests, their sensitivity is typically lower; the UBT can lead to a small percentage of false negative and false positive results.[124, 326] Of note, the sensitivity

of culture and the UBT is reduced when the bacterial load is low.[124, 326] The level of agreement of results among participants who had multiple tests indicates the potential amount of misclassification. Among the 295 individuals included in this study (who had either antibiotic susceptibility results for *H. pylori* cultured from gastric biopsies or a post-treatment UBT result), 203 (69%) had both an initial UBT result and data on *H. pylori* status by bacterial culture. Among these 203 individuals, 10% (20/203) initially tested negative for *H. pylori* infection by UBT but were positive by culture and 7% (15/203) initially tested positive for *H. pylori* by UBT but were negative by culture.

In this study, failure to detect *H. pylori* on culture of biopsies from *H. pylori*-positive participants would lead to a false negative classification and exclusion from antibiotic susceptibility testing. This misclassification could lead to selection bias in Analysis 1 if the probability of a false-negative classification depended on the exposure level (the number of antibiotic prescriptions in the previous 5 years) to a degree that differed in *H. pylori*-positive participants with and without antibiotic resistance. Given that detection by culture depends on having a sufficient bacterial load, bias could be introduced if the frequency of low bacterial loads among individuals with higher levels of previous exposure to antibiotics differs by antibiotic susceptibility status. This scenario would lead to an increased probability of false-negatives among those with susceptible bacterial strains and more frequent previous exposure to antibiotics, resulting in an under-representation of this group in the study population, relative to highly exposed participants with resistant infections. This would increase the probability of overestimating the effect of previous exposure to antibiotics on the prevalence of resistant *H. pylori* infection.

Individuals who were initially falsely classified as *H. pylori*-negative would not have been offered treatment. This would have introduced selection bias if differential misclassification influenced which participants received treatment and were, therefore, included in Analysis 2. For example, differential misclassification would have occurred if false-negative classification were more frequent among participants with more frequent previous antibiotic exposure resulting in bacterial loads too low to be detected, and if this occured more often among people with a low risk of treatment failure independent of

exposure than among people with a high risk of treatment failure independent of exposure. If this were the case, the exclusion from treatment of participants falsely classified as negative would lead to underestimation of previous antibiotic exposure among participants with a low risk of treatment failure included in Analysis 2 and overestimation of the effect of previous antibiotic exposure on the risk of treatment failure. Conversely, false-positive classification may have been more frequent among participants with less frequent previous antibiotic exposure. Given that individuals falsely classified as positive would have negative post-treatment UBT results irrespective of their antibiotic exposure history, this would result in underestimation of the risk of treatment failure overall, and if false positives had a lower prevalence of high previous exposure to antibiotics, the effect of previous antibiotic exposure on the risk of treatment failure would be overestimated.

The results of this study contribute to prior evidence reported in the literature that support an association between antibiotic use for the treatment of general bacterial infections and the occurrence of antibiotic-resistant *H. pylori* infection. An important strength of this research is the contributions of investigators from a variety of disciplines, including gastroenterology and microbiology, who provided expertise in the collection of gastric biopsies and the culture and antibiotic susceptibility testing of *H. pylori* isolated from gastric biopsies. This expertise reduces the probability of misclassifying *H. pylori* organisms with respect to their antibiotic susceptibility status or post-treatment status. Also, the comprehensive collection of data from community project participants allowed for the adjustment of potential confounding variables. The community-driven research approach facilitated broad participation of community residents, as well as the accuracy of collected data by interviewer-administered questionnaires. Further, the use of a population-based dataset from community-driven research allows for the estimation of effects in a community setting, which enhances the public health value of this research.

Chapter 4. Describing participant's perspectives regarding antibiotic use and factors that influence regimen adherence among participants in the Aklavik *H. pylori* Project: a qualitative analysis

Introduction

The occurrence of antibiotic resistance in bacteria is a major public health challenge worldwide. Antibiotic-resistant bacteria are a major threat to health because they limit the effectiveness of available therapeutic options. In recent years, organizations including the World Health Organization, the United States Centers for Disease Control and Prevention, the European Centre for Disease Prevention and Control and the National Collaborating Centre for Infectious Diseases in Canada have published reports highlighting the importance of controlling antibiotic-resistant bacteria.[356–359]A major factor associated with the development of antibiotic resistance is the improper use of antibiotics.[360] Use of antibiotics prior to a confirmed diagnosis or improper adherence to a prescribed antibiotic regimen, including prematurely stopping antibiotic treatment or missing scheduled doses, can promote the selection of antibiotic-resistant bacteria due to exposure to antibiotics at sub-optimal concentrations.[361] Previous studies from around the world show that some people often use antibiotics in non-prescribed ways and provide evidence of an association between improper antibiotic use and antibiotic resistance.[362–375] In a study of 5379 interviewees who completed phone surveys in nine countries including the United Kingdom, France, Belgium, Italy, Spain, Turkey, Thailand, Morocco, and Colombia, the proportion of individuals who reported non-adherence to a prescribed antibiotic regimen ranged from 10% to 47% and the proportion who reported saving un-used antibiotics ranged from 4% to 41%.[376] Commonly reported reasons for non-adherence to prescribed antibiotic regimens include feeling better, belief that the medication is no longer required, or the development of adverse effects.[377] Therefore, educating individuals regarding the importance of completing an antibiotic treatment as prescribed is a key element for improving the control of antibiotic resistance.[362,378]

Helicobacter pylori is a bacterium that colonizes the stomach and/or duodenum.[1] Chronic *H. pylori* infection is a risk factor for peptic ulcer disease and gastric cancer.

Chapter 4. Describing participant's perspectives regarding antibiotic use and factors that influence regimen adherence among participants in the Aklavik H. pylori Project: a qualitative analysis

Treatment to eliminate *H. pylori* requires a multi-drug regimen consisting of a proton-pump inhibitor with 2-3 antibiotics for a duration of 10-14 days.[25,27] However, in areas where *H. pylori* infection is common, currently recommended treatment regimens often fail.[99] Major factors associated with the failure of anti-*H. pylori* treatment regimens are antibiotic-resistant *H. pylori* infection[52,58,59] and poor adherence to the prescribed treatment regimen.[89] Poor adherence to anti-*H. pylori* treatment regimens results in sub-optimal antibiotic concentrations at the site of infection.[89] A regression analysis conducted by Graham *et al.* (1992) estimated the treatment effectiveness of an anti-*H. pylori* treatment regimen consisting of bismuth and two antibiotics (tetracycline and metronidazole) at 96% among individuals who completed 60% or more of the prescribed regimen and 69% for individuals who completed less than 60% of the prescribed regimen.[90] A major factor associated with poor adherence to anti-*H. pylori* treatment regimens is the occurrence of adverse effects, which have a frequency estimated to range from approximately 5% to 20%.[91] Adverse effects commonly associated with anti-*H. pylori* treatment regimens include: nausea; vomiting; diarrhea; and a metallic taste in the mouth.[379]

Northern Canadian Aboriginal communities are disproportionately affected by *H. pylori* infection, with prevalence estimated to range from 51% to 95% compared to southern Canada where the prevalence is estimated to range from 30% to 40%.[322,323] The Canadian North *Helicobacter pylori* (CAN*Help*) Working Group conducts community-driven research among northern Aboriginal communities in the Northwest Territories and Yukon to investigate *H. pylori*-associated health outcomes and inform public health policy pertaining to their control. The CAN*Help* Working Group launched the first community project, the Aklavik *H. pylori* Project, in 2007 in Aklavik, NT and later expanded to include the Old Crow *H. pylori* Project in Old Crow, YT, the Inuvialuit Settlement Region (ISR) *H. pylori* Project in Tuktoyaktuk, NT and the Fort McPherson *H. pylori* Project in Fort McPherson, NT. One goal of this research is to improve the effectiveness of anti-*H. pylori* treatment regimens by identifying obstacles to successful treatment. Given the importance of treatment adherence, the aim of this qualitative

analysis of semi-structured interviews is to describe Aklavik *H. pylori* Project participants' perspectives on the value and potential harms of using antibiotics to treat infections in general, and *H. pylori* infection in particular, and on factors that influence adherence to prescribed antibiotic regimens. The results of this analysis will be used to inform treatment, knowledge exchange, and health policy components of the CAN*Help* Working Group's ongoing research in northern Canadian Aboriginal communities.

Methods

To collect data pertaining to perspectives on the value and potential harms of using antibiotics to treat infection and factors that influence adherence to prescribed antibiotic regimens, I developed a semi-structured questionnaire (Appendix B) during spring 2015 with guidance from the CAN*Help* Working Group Ethnographic Fieldwork Lead, a medical anthropologist, and community consultants who were members of the Aklavik *H. pylori* Project planning committee. In July 2015, using the semi-structured questionnaire, I interviewed Aklavik *H. pylori* Project participants in Aklavik, NT. To maximize participation, I used several methods of recruitment, including: flyers; local radio announcements; word of mouth; and making myself available to the public in a temporary CAN*Help* project office in the community health centre where I would inform patients who approached the office while waiting to visit a nurse about my interest in interviewing community members for my thesis research. I used convenience sampling to select individuals for this study. Convenience sampling is a method that selects participants for inclusion in a study based on easy accessibility and availability at the time of recruitment; it can be useful for studying socio-cultural attributes shared within a group of people because it allows for elicitation of commonly-held ideas, beliefs, attitudes, and behavioural norms.[380] I used convenience sampling in this study due to time restraints and the limited population present within the community during the summer months. Additionally, I used a chain of reference approach by asking volunteering participants to identify additional Aklavik *H. pylori* Project participants who were in

town at the time and who they believed would be interested in participating in the study.[380]

In total, I conducted 10 one-on-one interviews during July 7th to 14th, 2015 with Aklavik *H. pylori* Project participants who expressed interest in participating. I used the principle of theoretical saturation to guide the number of interviews conducted: I conducted interviews and transcribed data simultaneously until similar responses were collected and no new information was identified.[380] At the time the interviews were conducted, the average age of the participants was 55 years (range, 30 to 69 years) with an equal number of participants being male and female. During each interview, I referred to adverse events associated with antibiotic treatment as 'side effects' because this term was used more widely by respondents. I recorded interviews with a handheld digital audio recorder (Tascam DR-05) after obtaining each participant's oral consent. I conducted interviews at the community health centre or, at the request of the individual, at another community location. Following each recording, I transcribed interviews in Microsoft Word. Table 4.1 presents the transcription conventions I used for recorded interviews.

Table 4.1. Conventions used for transcribed interviews in Microsoft Word.

Transcription Convention	**Description**
(?)	Speech too obscure to transcribe
[	Overlapping talk begins
]	Overlapping talk ends
Beca-	Cut off, interruption of a sound
he says.	Emphasis
CAPITAL LETTERS	Louder speech, raised voice
?	Rising intonation
..	Short pause
…	Long pause
gesture	Unheard body movement

I used thematic analysis to identify patterns of local beliefs and behaviours regarding the use of antibiotics to treat infection and factors that influence adherence. Thematic analysis has been defined by Braun and Clark (2006) as "a method for

identifying, analysing and reporting patterns (themes) within data".[381] For this analysis, I read each transcript in its entirety to gain familiarity with the data and identify initial categories. I modified categories as they were applied to the entire dataset to best represent consistent thoughts and ideas expressed among participants. Finally, I identified themes that best described the relationship among categories.

Results

Three main themes emerged from the analysis of the transcribed interviews (Table 4.2): 1) lay concepts of antibiotics and antibiotic resistance; 2) concerns about medication; and 3) perseverance.

Table 4.2. Identified themes and categories among transcribed interviews.

Theme	**Categories**
Lay Concepts of Antibiotics & Antibiotic Resistance	Antibiotic uses
	Identification of antibiotics
	Antibiotic resistance
Concerns About Medication	Knowledge of side effects
	Concern about side effects
	Reasons for previous antibiotic treatment non-adherence
	Side effects that would alter the course of treatment
	Sharing antibiotics
Perseverance	Experience with anti-*H. pylori* treatment regimens
	Side effects with anti-*H. pylori* treatment regimens
	Adherence with anti-*H. pylori* treatment regimens

<u>Lay Concepts of Antibiotics & Antibiotic Resistance</u>

A theme identified among participants' responses was lay concepts of antibiotics and antibiotic resistance. Lay concepts of antibiotics and antibiotic resistance included

participant responses coded within the following 3 categories: antibiotic uses; identification of antibiotics; and antibiotic resistance. Among the respondents, knowledge of antibiotics appeared to be associated with experience with antibiotic treatment. Knowledge of antibiotics and antibiotic resistance was most limited among participants who stated that they had had no previous exposure to antibiotics (three out of ten respondents).

Participants' perspectives on indications for antibiotic use and antibiotic resistance varied. When asked what people use antibiotics for, nine of the ten participants mentioned indications they believed antibiotics were used for, while one individual said they did not know. Regarding antibiotic uses, participants mentioned: infections (skin, throat, ear, chest, internal, external); cuts; "whatever effects my body"; "for healing"; "for fixing their problems"; TB (tuberculosis); colds; "to treat pain"; headache; and allergic reaction. When asked to identify any antibiotic by name, three of the nine individuals who mentioned an indication for antibiotic use identified an antibiotic by name. Antibiotics that were identified by these three participants included penicillin, cloxacillin, and amoxicillin.

Knowledge about antibiotic resistance varied across participants. When participants were asked if they had ever heard of antibiotic resistance, six initially stated that they had never heard of antibiotic resistance. When these participants were asked further if they had ever heard about an antibiotic treatment not curing an infection because the infection was resistant to the drugs, three participants responded that yes, they remembered hearing about such events but did not remember any specific details. Among the three participants who maintained that they had never heard of antibiotic resistance, two were among those who stated they had no previous exposure to antibiotics. However, when asked what people had heard about antibiotic resistance, one participant stated:

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> Male Participant 1, age 56: "Um… if.. if you, don't take the pills you're, the.. whatever your trying to.. ah, get rid of, the bacteria can become resistant to it and infection can become worse."

Similarly, another participant stated:

> Female Participant 1, age 51: "[T]hat's why they did the study here in Aklavik 'cause um.. a lot of people, I guess, had that *H. pylori* infection and, and the meds that they were taking was being, was kinda being resistant to it and that's why they wanted to come and start doing um.. um.. finding out what kind of meds would <u>really</u> work for the treatment because ah.. a lot of the meds, e-even me when I was taking them wasn't um.. ah.. was resistant by the bug and then they wanted to find out.. what would work for us in the long run."

When asked if there was ever a time that they did not finish taking all of the antibiotics prescribed to them, one individual stated:

> Female Participant 2, age 56: "Whenever they give me, I take it right 'til it's finished so that way the infection wouldn't return? Like, [Yeah.] they always say to, always finish the antibiotics that you were given?"

Additionally, when asked to recall nurses' instructions regarding previously prescribed antibiotic treatment, one participant said:

> Female Participant 3, age 51: "Make sure I take the whole 10 days of it otherwise.. ah.. it won't, it will clear up the infection after 6 or 7 days but you have to finish the course because if you get another infection it might not work cause your body is resis- or how you say?, resistant [Resistant?] to it? Yeah. So it won't work for you after, 2 or 3 times they give you the penicillin. That's what I, that's what I, doct-, or nurse told me. So I try to take the whole 10 days."

Participant responses suggest that some individuals within the community understand the importance of completing the entirety of a prescribed antibiotic treatment to ensure the medication is successful at eliminating the infection. However, participant responses suggest that some community members perceive antibiotic resistance as being a property of their body rather than a property of the bacteria. For example, another individual, when asked what they had heard about antibiotic resistance, stated:

> Female Participant 3, age 51: "Just that it won't work for you after two or three times cause your body gets immune to it. Yeah, immune, that's what I could see. Yeah. [Ok.] Not resistant, immune, ah? Like when your body gets use to those pills? And it-, it doesn't work after a while."

The above statement suggests that some community members do not clearly distinguish antibiotic resistance in bacterial infections from immunity.

Overall, participants who stated that they had not been previously prescribed antibiotics could not identify an antibiotic by name and either did not know what type of ailments antibiotics are used to treat or mentioned ailments that would occur due to a bacterial infection in addition to mentioning ailments that would not occur due to a bacterial infection. Alternatively, the participants who were able to identify an antibiotic by name also identified appropriate indications for antibiotic use and were among the participants who stated that they were previously prescribed antibiotics. However, among the three participants who stated they had no previous exposure to antibiotics, one later identified themselves as having been previously treated for *H. pylori* infection. This observation suggests that there may be varying perspectives among community members regarding which medications are classified as antibiotics.

<u>Concerns About Medication</u>

Another theme identified among interviewed participants was concerns about medication. Concerns about medication included participant responses coded within the

following 5 categories: knowledge of side effects; concern about side effects; reason for previous antibiotic treatment non-adherence; side effects that would alter the course of treatment; and sharing antibiotics. When asked what potential side effects, to their knowledge, could occur during antibiotic treatment, responses included: nausea; vomiting; sweating; tiredness; headaches; itchy skin; skin rashes; and allergic reactions. However, four of the ten respondents were unable to provide a response to this question; these four included the three who stated that they had not been previously prescribed antibiotics. A limited understanding of antibiotic side effects appears to be influenced by limited experiences taking antibiotics. For example, one of the participants who previously stated they had no previous exposure to antibiotics responded when asked what side effects could occur during a prescribed course of antibiotics as follows:

> Male Participant 2, age 69: "Uh… like I said, I never take pills. [No?] So it doesn't affect me even if I have to take pills."

When participants were asked if they would be worried about the occurrence of side effects during antibiotic treatment, five stated that they would not be worried. Among those who stated that they would be concerned about the occurrence of side effects, three participants mentioned asking a health care professional what to expect in regards to treatment side effects or looking up potential side effects for prescribed medications online. When asked if they would be worried about the occurrence of side effects during a prescribed course of antibiotic treatment, one participant stated:

> Female Participant 2, age 56: "I usually check online to see if there is any side effects or anything once they're prescribed to me. So now that we have, um.. we can Google, and.. find out all the.. um, see what side effects we could get from the medication? Yeah."

Additional responses from participants, when asked if they would be worried about the occurrence of side effects during antibiotic treatment, included: only if the doctor told

them that they would get really sick during the treatment duration; or that it would depend on how long they were taking the treatment for.

Among the seven interviewed participants who stated that they had previously been treated with antibiotics, three participants stated that they had not completed a prescribed antibiotic treatment. When asked why they could not finish the prescribed antibiotics, participants' responses included: feeling poorly, forgetfulness; and taste. When asked what kind of side effects occurring during a course of antibiotic treatment would prevent the completion of their treatment, seven individuals provided a response including: "something serious enough to stop"; fatigue; upset stomach; nausea; constipation; and skin rash. Nausea and vomiting were identified as significant side effects:

> Male Participant 3, age 64: "Ho, that's pretty hard. It just depends on what.. I guess."
>
> Interviewer: "What you're treating?"
>
> Male Participant 3, age 64: "About the treatment, yup, but, if I get like nausea or.. dizzy spells, running to the washroom all the time after I eat and that, probably.. try tough it out, but yeah. A few others. It just depends on how bad the side effects is."

Another participant stated:

> Male Participant 1, age 56: "Um.. only if I threw up or couldn't function. Or if I became violently ill."

Additionally, when asked to recall instructions provided by a nurse for a previous antibiotic treatment, another participant stated:

> Female Participant 2, age 56: "Uh, if I have any side effects to let them know if, if this is, if I get side effects from them, [Ok.] they usually tell me, if I do have side effects, is to, to stop them or bring them back, or see them again, [Mmhmm.] so that way they can prescribe other, [Ok.] other that were not, that we are, not allergic too."

Two participants were unable to provide a response when asked what side effects, if they occurred during antibiotic treatment, would lead them to stop treatment early.

Participants were also asked if they had ever shared unused antibiotic pills with other family members or friends or if family members or friends have ever shared un-used antibiotic pills with them. All participants responded, to both of these questions, that they had never shared antibiotics or been offered leftover antibiotic pills from a previous treatment during a time of illness. One participant stated:

> Female Participant 4, age 60: "I'd throw them away if I'm finished with it like, cause I like, don't want my family to use it. [Mmhmm.] That's how I always know. Any kind of pills I don't share with my friends or family."

Similarly, another participant stated:

> Male Participant 1, age 56: "Never heard of it. I've never heard of people sharing pills."

Overall, participant responses suggest a careful use of antibiotic medications in the community and the widespread concern for the occurrence of side effects during antibiotic therapy.

<u>Perseverance</u>

A theme identified among participant responses was perseverance. Perseverance to complete prescribed anti-*H. pylori* treatment regimens as prescribed was a theme that included participant responses coded within the following three categories: experience with anti-*H. pylori* treatment regimens; side effects with anti-*H. pylori* treatment

regimens; and adherence with anti-*H. pylori* treatment regimens. Seven of the ten participants stated that they had previously been prescribed an anti-*H. pylori* treatment regimen. Among participants previously prescribed an anti-*H. pylori* treatment regimen, five mentioned experiencing side effects including: nausea; diarrhea; upset stomach; abdominal pain; sweatiness; fatigue; metallic taste; and complaints of the medication being hard on their stomach. One participant, when asked whether they experienced side effects during anti-*H. pylori* treatment, stated:

> Male Participant 3, age 64: "I was sweating, I would say sweating bullets, so it was rough. Nausea, so I wanted to throw up, with nothing in my stomach and.. that I eat I'm kinda like, oh my gosh.. I hope it don't do a 360 on me, and I wait for a while and I eat. Normal eating, breakfast, dinner, supper and then I get up, and well, off to the washroom, and it's like water when it came out of me."

Another individual, when asked whether they experienced side effects during anti-*H. pylori* treatment, stated:

> Male Participant 1, age 56: "Other than feeling not top notch, you know, you feel 75% of what you normally feel like.. a little bit sluggish but other than that is was ok. I didn't get really sick with it.. I know some people did but I didn't get sick."

Among the seven participants who stated that they had previously been prescribed an anti-*H. pylori* treatment regimen to eliminate their infection, five stated that they finished their treatment as prescribed. However, two out of the five who stated that they had completed their treatment as prescribed complained that the treatment was too long or that they didn't like taking their medication. When describing experiences with the prescribed anti-*H. pylori* treatment regimen, one participant stated:

> Female Participant 1, age 51: "Um.. *clears throat* mostly.. referring to my HPAC, um.. I couldn't finish it because it was, um.. it made me too

> nauseated, it made me too sick so I didn't finish, um.. maybe one of them. Um.. and if I had any other infection, internally or externally, that one I finish because.. it would just contain one pill and it was easy to do. But with the HPAC you were looking at maybe six to seven pills maybe.. um... morning, dinner and night? So that was like, quite a bit of pills to take in one day and I think that's why I couldn't finish them 'cause they were too much for me."

Three of the participants who identified themselves as having been previously prescribed an anti-*H. pylori* treatment regimen emphasized the importance of completing the antibiotic treatment. One participant, when asked whether they had completed the full anti-*H. pylori* treatment, stated:

> Female Participant 2, age 56: "I knew I had to take it 'cause of the infection that was within me that I had to finished the medication, so whatever they give me I try to finish the whole… whatever they prescribed me with."

One participant even suggested increased monitoring of adherence and patient education regarding treatment adherence. When asked if they had any problems taking all of the medication prescribed to them in their anti-*H. pylori* treatment regimen, he stated:

> Male Participant 1, age 56: "No, it's ah.. given to you in a blister pack it should be sitting in front of your.. counter every day so you know to take it and generally you know that you got ah, 7, 10 day period, 14 day period to take it, it's not a big deal. Don't really need a reminder, mind you, it'd been nice if they would have did that. To have a call or a phone around to make sure that.. people took them."

The same participant went on later to state:

> Male Participant 1, age 56: "Cause I know a lot of people.. that's a part that if you give them a chance they'll break that cycle of taking those pills..

> cause it makes them feel sick they will. Yeah. If you don't give them the chance.. then.. you know, then they just re-ensure they take these pills you'll feel sick and after a while you'll feel better. It would've been good. There needs to be a little bit more explanation how.. like how antibiotics work."

Participant responses regarding antibiotic treatment to eliminate *H. pylori* infection demonstrated knowledge of the importance of completing the full treatment as prescribed and a general perseverance to complete their medication despite the occurrence of side effects. However, views on why the treatment does not always work varied. Some participants mentioned that treatment failure was associated with not completing the full treatment but there were varying perspectives regarding how antibiotic resistance develops. Some participants described treatment to be unsuccessful if the body becomes immune or resistant to the antibiotics.

Discussion

According to an extensive search of the literature, this is the first report of an investigation of individuals' perspectives on the value and potential harms of using antibiotics to treat infections and factors that influence adherence to prescribed antibiotic regimens among members of a northern Canadian Aboriginal community. In general, participants included in the current study who demonstrated knowledge of antibiotics and treatment adherence were those who identified themselves as having prior experience with prescription antibiotics. Knowledge regarding antibiotic names and side effects appeared to be associated with personal experience or the experience of close friends and family. However, the response of one participant suggests that there may be some community members who are unaware of which prescribed medications are classified as antibiotics, given that this participant said they had never been prescribed antibiotics but later said they had been prescribed treatment to eliminate *H. pylori* infection.

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In the present study, ideas expressed by participants generally corresponded well with how the scientific community regards the importance of adhering to prescribed antibiotic treatment regimens. Despite varying knowledge levels regarding the use of antibiotics to treat bacterial infection, participants' responses in the present study reflected for the most part careful and prudent use of antibiotics. Participants identified the occurrence of side effects and forgetfulness as the primary reasons for missing doses of a prescribed course of antibiotics or discontinuing it altogether. This can be compared to the study reported by Hawkings *et al*., in which the major factors contributing to antibiotic treatment non-adherence identified by participants were: forgetfulness; a belief that the medications were no longer required; and daily constraints including work, school, child day care, and social priorities.[377] Aklavik *H. pylori* Project participants included in this study also described a strong sense of perseverance with respect to completing their prescribed anti-*H. pylori* treatment regimens despite the long treatment duration and the occurrence of side effects. Many of the participants who identified themselves as having been prescribed an anti-*H. pylori* treatment regimen demonstrated an understanding of the importance of completing the prescribed regimen to successfully eliminate their infection and expressed the desire to avoid having to re-take their medication as a motivation for perseverance.

Some participant responses suggest, however, that the ideas of some individuals diverged from the scientific community's understanding of the development of resistance. For example, some individuals seemed to understand and define common medical concepts such as "immunity" differently than the scientific community does and confuse this concept with resistance. Immunity is generally defined as resistance of a host organism, or individual, to a pathogen or foreign substance.[35] It should be noted that antibiotic resistance of bacterial organisms and human immunity to infectious agents are similar in that both involve a lack of susceptibility to the effects of an external agent. However, antibiotic resistance of a bacterial organism involves a lack of susceptibility of the bacterium to an antibiotic whereas human immunity to infection involves protection of humans against the harmful effects of an infectious agent.[68] When discussing antibiotic

resistance, one participant mentioned the body becoming immune to the drugs following repeated treatment. This response suggests that some community members may understand treatment failure to be the result of the person's own body becoming resistant to the medication effects over time, while health care providers view treatment failure as a result of the infectious agent becoming resistant to the medications.

Recent literature provides evidence that lay understandings equating antibiotic resistance with immunity, in a manner inconsistent with how the scientific community understands these concepts, may be common in various populations worldwide, and is thus not unique to residents of Aklavik. In a European study conducted by Brookes-Howell *et al*. (2011), the authors reported that most participants associated antibiotic use with resistance but these authors characterized many respondents as interpreting 'resistance' to be a property of the body 'becoming used to' or 'immune' to the prescribed antibiotics rather than a property of the bacteria.[371] When investigating the attitudes of the public regarding bacterial resistance, Hawkings *et al*. also observed among 46 individuals in South Whales, UK that individuals expressed concerns about repeated use of antibiotics having a negative impact on their body due to changes in the body's response to an antibiotic: their bodies 'got used to' antibiotics.[372]

Also, some community members appear to perceive indications for antibiotic use differently than the scientific community: some respondents mentioned ailments not associated with bacterial infections as indications for antibiotic therapy. Previous studies investigating perspectives on and knowledge of antibiotic use in various populations worldwide have also observed variation in lay understandings of antibiotic indications. Belongia *et al.* conducted a population-based survey of 680 individuals aimed at investigating knowledge of the indications for antibiotic use among adults and parents of children 5 years of age or younger in Wisconsin and Minnesota, USA in 1999; these authors reported a limited understanding of the appropriate use of antibiotics.[368] In their study, Belongia *et al.* reported that approximately a third of participants expressed the understanding that antibiotics were required for the treatment of symptoms, such as nasal

discharge, common for viral infections.[368] In a report on their 2008 study, which used semi-structured interviews among 46 individuals from South Whales, UK, Hawkings *et al*. described interviewees as being uncertain about how antibiotics work to eliminate bacterial infections and about indications for antibiotic use.[377] Additionally, variation in lay understandings of antibiotic use and antibiotic resistance has been observed to vary by culture, or more specifically, by ethnicity, in many settings worldwide, including the United States[366–368] and Europe.[365] For example, Corbett *et al.* (2005) hypothesized that differences in lay understandings of antibiotic use and antibiotic resistance between non-Hispanic white individuals and Hispanic individuals in Colorado, USA, which persisted after adjustment for educational attainment, may have occurred due to differing exposure to health information in the media.[366]

Participants included in this study were selected through the use of a convenience sample and were, therefore, not a random sample of Aklavik *H. pylori* Project participants. Also, the restricted age range of the study population limits the perspectives to those of adults. Thus, data obtained for this study do not allow for the estimation of relevant quantitative measures such as the proportion of community members who can identify clinically appropriate uses of antibiotics. However, a convenience sample does reveal a sampling of individual views, which can be used to design future investigations of local knowledge and perceptions surrounding the themes identified here.[380]

Overall, there is widespread knowledge among community members of the importance of completing prescribed antibiotic treatments to eliminate infection. Additionally, participant responses suggest a widespread knowledge of the association between the development of repeated treatment exposure and diminished treatment effectiveness. However, understandings of some participants appear to differ from understandings of the scientific community regarding indications for antibiotic use and the development of antibiotic resistance. It seems likely that conversations between community members and health practitioners regarding the importance of adhering to anti-*H. pylori* treatment regimens has been an effective strategy for communicating

factors that impact treatment effectiveness. Participants identified side effects as a widespread concern regarding antibiotic treatment and a major factor that negatively impacts treatment adherence. Respondents also identified forgetfulness as another factor that negatively impacted their adherence to antibiotic regimens. Strategies that support community members to manage side effects and remember to take their medication may be useful for improving adherence to complex treatment regimens such as those used to eliminate *H. pylori* infection.

Chapter 5

Conclusion

Awareness of the risk antibiotic resistance in bacteria imposes on public health has grown in recent years.[356–359] Antibiotic-resistant bacteria threaten public health because they limit the effectiveness of available therapeutic options. Antibiotic-resistance in *H. pylori* infection is widely recognized as a major risk factor for reduced effectiveness of anti-*H. pylori* treatment regimens.[52] Given the large global disease burden from *H. pylori* infection and the current lack of an effective vaccine or other public health measures for its prevention, the reduced effectiveness of anti-*H. pylori* treatment regimens is an important public health issue.

My literature review on the prevalence of antibiotic-resistant *H. pylori* infection presents evidence of the widespread occurrence of antibiotic-resistant *H. pylori* infection across geographic regions. The results of my literature review provide a comprehensive description of the prevalence of antibiotic-resistant *H. pylori* by geographic region and by specific antibiotic drug. The information synthesized is valuable to clinical practice because it can be used to inform effective prescription practices for the elimination of *H. pylori* infection across geographic regions. My results also highlight a gap in the current literature, which lacks current estimates of the prevalence of antibiotic-resistant *H. pylori* infection in North American countries. Canadian clinical guidelines suggest that knowledge regarding local antibiotic resistance patterns and the local effectiveness of anti-*H. pylori* treatment regimens is needed to optimize the effectiveness of prescribed regimens, and therefore, up-to-date estimates would be beneficial to inform local prescription practices.[54]

Results of my quantitative analysis contribute to the current body of literature that includes several reports that provide consistent evidence of an association between previous antibiotic exposure for the treatment of unrelated bacterial infections and the prevalence of antibiotic-resistant *H. pylori* infection.[73,104,268,340,352,353] My results also contribute to the current body of literature that includes several reports that provide evidence of an association between previous exposure to unsuccessful anti-*H. pylori*

treatment regimens and both the prevalence of antibiotic-resistant *H. pylori* infection and the risk of anti-*H. pylori* treatment failure.[79–81,103] These results highlight the importance of achieving a high level of effectiveness with initially prescribed anti-*H. pylori* treatment regimens to prevent the occurrence of antibiotic-resistant *H. pylori* infection and subsequent treatment failure. However, the literature lacks prospective studies that compare the antibiotic susceptibility of *H. pylori* infection before and after exposure to antibiotics for the treatment of unrelated bacterial infections. Similarly, my literature review did not identify articles that presented results of a prospective study investigating the antibiotic susceptibility of *H. pylori* infection before and after exposure to an unsuccessful anti-*H. pylori* treatment regimen. Results of such studies would further support a causal direction for the hypothesized association between antibiotic exposure and antibiotic-resistant *H. pylori* infection or anti-*H. pylori* treatment failure.

Given that adherence to prescribed regimens is a major risk factor for the development of antibiotic-resistant *H. pylori* infection, my thesis also contributes to the literature by describing perspectives of residents of a northern Canadian Aboriginal community on factors that influenced adherence to prescribed antibiotic regimens for eliminating *H. pylori* infection. For the most part, my northern Canadian informants demonstrated that they knew the importance of completing prescribed antibiotic treatment to eliminate infection, but pointed to side effects and forgetfulness as reasons for not adhering to prescribed anti-*H. pylori* treatment regimens. Understanding such perspectives is useful for informing strategies to improve antibiotic treatment adherence, which is particularly important for northern Aboriginal communities, given evidence revealed by my thesis analysis of higher antibiotic dispensation rates among CANHelp community project participants compared to residents of Edmonton, Alberta, a major urban centre in southern Canada. Of note, estimates of the prevalence of antibiotic-resistant *H. pylori* infection among CAN*Help* project participants are generally similar to estimates from southern Canadian populations published prior to 2000, which may not reflect the current prevalence if resistance has increased,[342] thus factors other than antibiotic resistance, including treatment adherence, should be considered when designing strategies to improve treatment effectiveness. Overall, the results of my thesis research are valuable to public health because they contribute to a body of evidence that

can be used to inform public health guidelines aimed at improving the control of infectious diseases, and in particular, reducing health risks associated with antibiotic resistance.

References

1. Ford, A. C. & Axon, A. T. R. Epidemiology of Helicobacter pylori infection and public health implications. *Helicobacter.* **15 Suppl 1,** 1–6 (2010).
2. Marshall, B. & Warren. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. *Lancet.* **1,** 1311–5 (1984).
3. Warren, R. & Marshall, B. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. *Lancet.* **1,** 1273–5 (1983).
4. International Agency for Research on Cancer. *Monographs on the Evaluation of Carcinogenic Risks to Humans (Vol. 60): Infection with Helicobacter pylori*. 177–240 (1994).
5. Sierra, M., Hastings, E., Fagan-Garcia, K., Colquhoun, A. & Goodman, K. in *Helicobacter Pylori: A Worldwide Perspective 2014.* 25–79 (2014).
6. Fischbach, L. A., Goodman, K. J., Feldman, M. & Aragaki, C. Sources of variation of Helicobacter pylori treatment success in adults worldwide: a meta-analysis. *Int. J. Epidemiol.* **31,** 128–39 (2002).
7. Goodman, K. J. & Correa, P. The Transmission of Helicobacter pylori. A Critical Review of the Evidence. *Int. J. Epidemiol.* **24,** 875–887 (1995).
8. Everhart, J. E. Recent developments in the epidemiology of Helicobacter pylori. *Gastroenterol. Clin. North Am.* **29,** 559–578 (2000).
9. Brown, L. M. Helicobacter pylori: epidemiology and routes of transmission. *Epidemiol. Rev.* **22,** 283–97 (2000).
10. van Blankenstein, M., van Vuuren, A. J., Looman, C. W. N., Ouwendijk, M. & Kuipers, E. J. The prevalence of Helicobacter pylori infection in the Netherlands. *Scand. J. Gastroenterol.* **48,** 794–800 (2013).
11. Khurana, R. *et al.* Meta-analysis: Helicobacter pylori eradication treatment efficacy in children. *Aliment. Pharmacol. Ther.* **25,** 523–36 (2007).
12. Banatvala, N. *et al.* The cohort effect and Helicobacter pylori. *J. Infect. Dis.* **168,** 219–21 (1993).
13. Khalifa, M. M., Sharaf, R. R. & Aziz, R. K. Helicobacter pylori: a poor man's gut pathogen? *Gut Pathog.* **2,** 2 (2010).

14. Thomson, A. B. R. *et al.* The prevalence of clinically significant endoscopic findings in primary care patients with uninvestigated dyspepsia: the Canadian Adult Dyspepsia Empiric Treatment – Prompt Endoscopy (CADET–PE) study. *Aliment. Pharmacol. Ther.* **17,** 1481–91 (2003).
15. Naja, F., Kreiger, N. & Sullivan, T. Helicobacter pylori infection in Ontario: prevalence and risk factors. *Can. J. Gastroenterol.* **21,** 501–6 (2007).
16. Veldhuyzen van Zanten, S. J., Pollak, P. T., Best, L. M., Bezanson, G. S. & Marrie, T. Increasing prevalence of Helicobacter pylori infection with age: continuous risk of infection in adults rather than cohort effect. *J. Infect. Dis.* **169,** 434–7 (1994).
17. Pérez- Pérez, G. I. *et al.* Country- specific constancy by age in cagA+ proportion of Helicobacter pylori infections. *Int. J. Cancer* **72,** 453–6 (1997).
18. Bernstein, C. N. *et al.* Seroprevalence of Helicobacter pylori, incidence of gastric cancer, and peptic ulcer-associated hospitalizations in a Canadian Indian population. *Dig. Dis. Sci.* **44,** 668–74 (1999).
19. McKeown, I. *et al.* Helicobacter pylori in the Canadian arctic: seroprevalence and detection in community water samples. *Am. J. Gastroenterol.* **94,** 1823–9 (1999).
20. Sinha, S. K., Martin, B., Sargent, M., McConnell, J. P. & Bernstein, C. N. Age at acquisition of Helicobacter pylori in a pediatric Canadian First Nations population. *Helicobacter* **7,** 76–85 (2002).
21. Takenaka, R. *et al.* Helicobacter pylori eradication reduced the incidence of gastric cancer, especially of the intestinal type. *Aliment. Pharmacol. Ther.* **25,** 805–12 (2007).
22. Hosking, S. W. *et al.* Duodenal ulcer healing by eradication of Helicobacter pylori without anti-acid treatment: randomised controlled trial. *Lancet Lond. Engl.* **343,** 508–10 (1994).
23. Sugiyama, T. *et al.* Sensitivity of biopsy site in evaluating regression of gastric atrophy after Helicobacter pylori eradication treatment. *Aliment. Pharmacol. Ther.* **16 Suppl 2,** 187–190 (2002).
24. Vannella, L. *et al.* Reversal of atrophic body gastritis after H. pylori eradication at long-term follow-up. *Dig. Liver Dis. Off. J. Ital. Soc. Gastroenterol. Ital. Assoc. Study Liver* **43,** 295–299 (2011).

25. Hunt, R. H., Fallone, C. A. & Thomson, A. B. Canadian Helicobacter pylori Consensus Conference update: infections in adults. Canadian Helicobacter Study Group. *Can. J. Gastroenterol.* **13,** 213–7 (1999).
26. Malfertheiner, P. *et al.* Management of Helicobacter pylori infection--the Maastricht IV/ Florence Consensus Report. *Gut.* **61,** 646–64 (2012).
27. Hunt, R. *et al.* Canadian Helicobacter Study Group Consensus Conference: Update on the management of Helicobacter pylori--an evidence-based evaluation of six topics relevant to clinical outcomes in patients evaluated for H pylori infection. *Can. J. Gastroenterol.* **18,** 547–54 (2004).
28. Vakil, N. & Vaira, D. Treatment for H. pylori infection: new challenges with antimicrobial resistance. *J. Clin. Gastroenterol.* **47,** 383–8 (2013).
29. van Zanten, S. J. *et al.* Secretion of intravenously administered antibiotics in gastric juice: implications for management of Helicobacter pylori. *J. Clin. Pathol.* **45,** 225–227 (1992).
30. Debets-Ossenkopp, Y. J., Namavar, F. & MacLaren, D. M. Effect of an acidic environment on the susceptibility of Helicobacter pylori to trospectomycin and other antimicrobial agents. *Eur. J. Clin. Microbiol. Infect. Dis.* **14,** 353–355 (1995).
31. Millar, M. R. & Pike, J. Bactericidal activity of antimicrobial agents against slowly growing Helicobacter pylori. *Antimicrob. Agents Chemother.* **36,** 185–187 (1992).
32. Damianos, A. J. & McGarrity, T. J. Treatment strategies for Helicobacter pylori infection. *Am. Fam. Physician* **55,** 2765–2774, 2784–2786 (1997).
33. Liang, X. *et al.* Efficacy of Bismuth-Containing Quadruple Therapies for Clarithromycin-, Metronidazole-, and Fluoroquinolone-Resistant Helicobacter pylori Infections in a Prospective Study. *Clin. Gastroenterol. Hepatol.* **11,** 802–807.e1 (2013).
34. Lambert, J. R. & Midolo, P. The actions of bismuth in the treatment of Helicobacter pylori infection. *Aliment. Pharmacol. Ther.* **11 Suppl 1,** 27–33 (1997).
35. Porta, M. S. *A Dictionary of Epidemiology*. (Oxford University Press, 2014).
36. Malfertheiner, P. *et al.* Current concepts in the management of Helicobacter pylori infection: the Maastricht III Consensus Report. *Gut.* **56,** 772–81 (2007).

37. Hunt, R. & Thomson, A. B. Canadian Helicobacter pylori consensus conference. Canadian Association of Gastroenterology. *Can. J. Gastroenterol.* **12,** 31–41 (1998).
38. Fock, K. M. *et al.* Asia-Pacific consensus guidelines on gastric cancer prevention. *J. Gastroenterol. Hepatol.* **23,** 351–65 (2008).
39. Gisbert, J. P. 'Rescue' regimens after Helicobacter pylori treatment failure. *World J. Gastroenterol.* **14,** 5385–5402 (2008).
40. Hsu, P.-I., Wu, D.-C., Wu, J.-Y. & Graham, D. Y. Modified sequential Helicobacter pylori therapy: proton pump inhibitor and amoxicillin for 14 days with clarithromycin and metronidazole added as a quadruple (hybrid) therapy for the final 7 days. *Helicobacter.* **16,** 139–145 (2011).
41. Rodgers, C. & van Zanten, S. V. A meta-analysis of the success rate of Helicobacter pylori therapy in Canada. *Can. J. Gastroenterol.* **21,** 295–300 (2007).
42. Gené, E., Calvet, X., Azagra, R. & Gisbert, J. P. Triple vs. quadruple therapy for treating Helicobacter pylori infection: a meta-analysis. *Aliment. Pharmacol. Ther.* **17,** 1137–43 (2003).
43. Luther, J. *et al.* Empiric quadruple vs. triple therapy for primary treatment of Helicobacter pylori infection: Systematic review and meta-analysis of efficacy and tolerability. *Am. J. Gastroenterol.* **105,** 65–73 (2010).
44. Peedikayil, M. C., Alsohaibani, F. I. & Alkhenizan, A. H. Levofloxacin-based first-line therapy versus standard first-line therapy for Helicobacter pylori eradication: meta-analysis of randomized controlled trials. *PloS One.* **9,** e85620 (2014).
45. Xiao, S.-P., Gu, M. & Zhang, G.-X. Is levofloxacin-based triple therapy an alternative for first-line eradication of Helicobacter pylori? A systematic review and meta-analysis. *Scand. J. Gastroenterol.* **49,** 528–38 (2014).
46. Wang, B. *et al.* Review: Efficacy and Safety of Hybrid Therapy for Helicobacter pylori Infection: A Systematic Review and Meta-analysis. *Helicobacter.* (2015). doi:10.1111/hel.12180
47. Gatta, L., Vakil, N., Vaira, D. & Scarpignato, C. Global eradication rates for Helicobacter pylori infection: systematic review and meta-analysis of sequential therapy. *BMJ.* **347,** f4587 (2013).

48. Liou, J.-M. *et al.* Sequential versus triple therapy for the first-line treatment of Helicobacter pylori: a multicentre, open-label, randomised trial. *Lancet Lond. Engl.* **381,** 205–13 (2013).
49. Graham, D. Y. & Fischbach, L. Helicobacter pylori treatment in the era of increasing antibiotic resistance. *Gut.* **59,** 1143–53 (2010).
50. Laine, L., Frantz, J. E., Baker, A. & Neil, G. A. A United States multicentre trial of dual and proton pump inhibitor-based triple therapies for Helicobacter pylori. *Aliment. Pharmacol. Ther.* **11,** 913–7 (1997).
51. Bochenek, W. J. *et al.* Eradication of Helicobacter pylori by 7-day triple-therapy regimens combining pantoprazole with clarithromycin, metronidazole, or amoxicillin in patients with peptic ulcer disease: results of two double-blind, randomized studies. *Helicobacter.* **8,** 626–42 (2003).
52. Fischbach L. & Evans E.L. Meta-analysis: The effect of antibiotic resistance status on the efficacy of triple and quadruple first-line therapies for Helicobacter pylori. *Aliment. Pharmacol. Ther.* **26,** 343–57 (2007).
53. Megraud, F. H pylori antibiotic resistance: prevalence, importance, and advances in testing. *Gut.* **53,** 1374–84 (2004).
54. Fallone, C. A. *et al.* The Toronto Consensus for the Treatment of Helicobacter pylori Infection in Adults. *Gastroenterol.* **151,** 51–69 (2016).
55. Agudo, S., Perez-Perez, G., Alarcon, T. & Lopez-Brea, M. High prevalence of clarithromycin-resistant Helicobacter pylori strains and risk factors associated with resistance in Madrid, Spain. *J. Clin. Microbiol.* **48,** 3703–7 (2010).
56. Broutet, N. *et al.* Risk factors for failure of Helicobacter pylori therapy--results of an individual data analysis of 2751 patients. *Aliment. Pharmacol. Ther.* **17,** 99–109 (2003).
57. Houben, M. H. *et al.* A systematic review of Helicobacter pylori eradication therapy--the impact of antimicrobial resistance on eradication rates. *Aliment. Pharmacol. Ther.* **13,** 1047–55 (1999).
58. Dore M.P., Leandro G., Realdi G., Sepulveda A.R. & Graham D.Y. Effect of pretreatment antibiotic resistance to metronidazole and clarithromycin on outcome

of Helicobacter pylori therapy: A meta-analytical approach. *Dig. Dis. Sci.* **45,** 68–76 (2000).

59. van der Wouden, E. J., Thijs, J. C., van Zwet, A. A., Sluiter, W. J. & Kleibeuker, J. H. The influence of in vitro nitroimidazole resistance on the efficacy of nitroimidazole-containing anti-Helicobacter pylori regimens: a meta-analysis. *Am. J. Gastroenterol.* **94,** 1751–9 (1999).
60. Dore, M. P., Kwon, D. H., Sepulveda, A. R., Graham, D. Y. & Realdi, G. Stable amoxicillin resistance in Helicobacter pylori. *Helicobacter.* **6,** 79 (2001).
61. Realdi, G. *et al.* Pretreatment antibiotic resistance in Helicobacter pylori infection: results of three randomized controlled studies. *Helicobacter.* **4,** 106–12 (1999).
62. Megraud, F. & Doermann, H. P. Clinical relevance of resistant strains of Helicobacter pylori: a review of current data. *Gut.* **43 Suppl 1,** S61–5 (1998).
63. Ecclissato, C. *et al.* Increased primary resistance to recommended antibiotics negatively affects Helicobacter pylori eradication. *Helicobacter.* **7,** 53–9 (2002).
64. Andersson D.I. The ways in which bacteria resist antibiotics. *Int. J. Risk Saf. Med.* **17,** 111–6 (2005).
65. Andersson, D. I. & Levin, B. R. The biological cost of antibiotic resistance. *Curr. Opin. Microbiol.* **2,** 489–493 (1999).
66. Björkman, J. & Andersson, D. I. The cost of antibiotic resistance from a bacterial perspective. *Drug Resist. Updat. Rev. Comment. Antimicrob. Anticancer Chemother.* **3,** 237–245 (2000).
67. Bjorkholm B. *et al.* Mutation frequency and biological cost of antibiotic resistance in Helicobacter pylori. *Proc. Natl. Acad. Sci. U. S. A.* **98,** 14607–12 (2001).
68. Graham, D. Y. Antibiotic resistance in Helicobacter pylori: implications for therapy. *Gastroenterol.* **115,** 1272–7 (1998).
69. Woodford N. & Ellington M.J. The emergence of antibiotic resistance by mutation. *Clin. Microbiol. Infec.* **13,** 5-18 (2007).
70. Horst, J. P., Wu, T. H. & Marinus, M. G. Escherichia coli mutator genes. *Trends Microbiol.* **7,** 29–36 (1999).
71. Davison, H. C., Low, J. C. & Woolhouse, M. E. What is antibiotic resistance and how can we measure it? *Trends Microbiol.* **8,** 554–559 (2000).

72. Boyanova, L. & Mitov, I. Geographic map and evolution of primary Helicobacter pylori resistance to antibacterial agents. *Expert Rev. Anti Infect. Ther.* **8,** 59–70 (2010).
73. Perez Aldana L. *et al.* The relationship between consumption of antimicrobial agents and the prevalence of primary Helicobacter pylori resistance. *Helicobacter.* **7,** 306–9 (2002).
74. Debets-Ossenkopp, Y. J. *et al.* Prevalence of Helicobacter pylori resistance to metronidazole, clarithromycin, amoxycillin, tetracycline and trovafloxacin in The Netherlands. *J. Antimicrob. Chemother.* **43,** 511–5 (1999).
75. Cars, O., Mölstad, S. & Melander, A. Variation in antibiotic use in the European Union. *Lancet.* **357,** 1851–3 (2001).
76. Meyer, J. M. *et al.* Risk factors for Helicobacter pylori resistance in the United States: the surveillance of H. pylori antimicrobial resistance partnership (SHARP) study, 1993-1999. *Ann. Intern. Med.* **136,** 13–24 (2002).
77. Osato, M. S. *et al.* Pattern of primary resistance of Helicobacter pylori to metronidazole or clarithromycin in the United States. *Arch. Intern. Med.* **161,** 1217–20 (2001).
78. Fraser, A. G., Moore, L., Hackett, M. & Hollis, B. Helicobacter pylori treatment and antibiotic susceptibility: results of a five-year audit. *Aust. N. Z. J. Med.* **29,** 512–6 (1999).
79. Romano M. *et al.* Failure of first-line eradication treatment significantly increases prevalence of antimicrobial-resistant Helicobacter pylori clinical isolates. *J. Clin. Pathol.* **61,** 1112–5 (2008).
80. Chang W.-L. *et al.* Resistance to metronidazole, clarithromycin and levofloxacin of Helicobacter pylori before and after clarithromycin-based therapy in Taiwan. *J. Gastroenterol. Hepatol.* **24,** 1230–5 (2009).
81. Heep, M., Kist, M., Strobel, S., Beck, D. & Lehn, N. Secondary resistance among 554 isolates of Helicobacter pylori after failure of therapy. *Eur. J. Clin. Microbiol. Infect. Dis.* **19,** 538–41 (2000).
82. Lu, H., Yamaoka, Y. & Graham, D. Y. Helicobacter pylori virulence factors: facts and fantasies. *Curr. Opin. Gastroenterol.* **21,** 653–9 (2005).

83. Stein, M., Rappuoli, R. & Covacci, A. in *Helicobacter pylori: Physiology and Genetics* (eds. Mobley, H. L., Mendz, G. L. & Hazell, S. L.) (ASM Press, 2001).
84. Taneike, I. *et al.* Analysis of drug resistance and virulence-factor genotype of Irish Helicobacter pylori strains: is there any relationship between resistance to metronidazole and cagA status?. *Aliment. Pharmacol. Ther.* **30,** 784–90 (2009).
85. van Doorn, L. J. *et al.* Importance of Helicobacter pylori cagA and vacA status for the efficacy of antibiotic treatment. *Gut.* **46,** 321–6 (2000).
86. Broutet, N. *et al.* cagA Status and eradication treatment outcome of anti-Helicobacter pylori triple therapies in patients with nonulcer dyspepsia. *J. Clin. Microbiol.* **39,** 1319–22 (2001).
87. Saruç, M. *et al.* The effect of CagA status on response to Helicobacter pylori eradication therapy in Western Turkey. *Braz. J. Med. Biol. Res.* **34,** 1435–9 (2001).
88. Treiber, G. *et al.* Clinical outcome and influencing factors of a new short-term quadruple therapy for Helicobacter pylori eradication: a randomized controlled trial (MACLOR study). *Arch. Intern. Med.* **162,** 153–60 (2002).
89. Ierardi, E., Giorgio, F., Losurdo, G., Di Leo, A. & Principi, M. How antibiotic resistances could change Helicobacter pylori treatment: A matter of geography?. *World J. Gastroenterol.* **19,** 8168–80 (2013).
90. Graham, D. Y. *et al.* Factors influencing the eradication of Helicobacter pylori with triple therapy. *Gastroenterol.* **102,** 493–6 (1992).
91. Chey, W. D., Wong, B. C. Y. & Practice Parameters Committee of the American College of Gastroenterology. American College of Gastroenterology guideline on the management of Helicobacter pylori infection. *Am. J. Gastroenterol.* **102,** 1808–25 (2007).
92. Graham D.Y., Lee Y. & Wu M. Rational helicobacter pylori therapy: Evidence-based medicine rather than medicine-based evidence. *Clin. Gastroenterol. Hepatol.* **12,** 177-86.e3 (2014).
93. Al-Eidan, F. A., McElnay, J. C., Scott, M. G. & McConnell, J. B. Management of Helicobacter pylori eradication--the influence of structured counselling and follow-up. *Br. J. Clin. Pharmacol.* **53,** 163–171 (2002).

94. Statistics Canada. 2006 Census of Population, Statistics Canada Catalogue no. 94-581-XCB2006001. *Statistics Canada* (2006).
95. An international association between Helicobacter pylori infection and gastric cancer. The EUROGAST Study Group. *Lancet Lond. Engl.* **341,** 1359–62 (1993).
96. Correa, P., Ruiz, B. & Hunter, F. Clinical Trials as Etiologic Research Tools in Helicobacter-Associated Gastritis. *Scand. J. Gastroenterol.* **26,** 15–9 (1991).
97. Rugge, M. *et al.* Pathology of the gastric antrum and body associated with Helicobacter pylori infection in non-ulcerous patients: is the bacterium a promoter of intestinal metaplasia? *Histopathol.* **22,** 9–15 (1993).
98. Parsonnet, J. Helicobacter pylori and gastric cancer. *Gastroenterol. Clin. North Am.* **22,** 89–104 (1993).
99. Rimbara, E., Fischbach, L. A. & Graham, D. Y. Optimal therapy for Helicobacter pylori infections. *Nat. Rev. Gastroenterol. Hepatol.* **8,** 79–88 (2011).
100. O'Connor, A., Vaira, D., Gisbert, J. P. & O'Morain, C. Treatment of Helicobacter pylori infection 2014. *Helicobacter.* **19 Suppl 1,** 38–45 (2014).
101. Georgopoulos, S. D., Papastergiou, V. & Karatapanis, S. Current options for the treatment of Helicobacter pylori. *Expert Opin. Pharmacother.* **14,** 211–23 (2013).
102. De Francesco, V. *et al.* Worldwide H. pylori antibiotic resistance: a systematic review. *J. Gastrointestin. Liver Dis.* **19,** 409–14 (2010).
103. Selgrad, M. *et al.* Antibiotic susceptibility of Helicobacter pylori in central Germany and its relationship with the number of eradication therapies. *Eur. J. Gastroenterol. Hepatol.* **25,** 1257–60 (2013).
104. McMahon, B. J. *et al.* The relationship among previous antimicrobial use, antimicrobial resistance, and treatment outcomes for Helicobacter pylori infections. *Ann. Intern. Med.* **139,** 463–9 (2003).
105. Megraud, F. Resistance of Helicobacter pylori to antibiotics and its impact on treatment options. *Drug Resist. Updat. Rev. Comment. Antimicrob. Anticancer Chemother.* **4,** 178–86 (2001).
106. Gerrits, M. M., van Vliet, A. H. M., Kuipers, E. J. & Kusters, J. G. Helicobacter pylori and antimicrobial resistance: molecular mechanisms and clinical implications. *Lancet Infect. Dis.* **6,** 699–709 (2006).

107. Kawakami, Y. *et al.* Successful development of air-dried microplates (HP-Plates) for susceptibility testing against Helicobacter pylori isolates. *Microbiol. Immunol.* **41,** 703–8 (1997).
108. Jorgensen, J. H. & Ferraro, M. J. Antimicrobial Susceptibility Testing: A Review of General Principles and Contemporary Practices. *Clin. Infect. Dis.* **49,** 1749–55 (2009).
109. Mégraud, F. *et al.* Antimicrobial susceptibility testing of Helicobacter pylori in a large multicenter trial: the MACH 2 study. *Antimicrob. Agents Chemother.* **43,** 2747–52 (1999).
110. van der Wouden, E. J., de Jong, A., Thijs, J. C., Kleibeuker, J. H. & van Zwet, A. A. Subpopulations of Helicobacter pylori are responsible for discrepancies in the outcome of nitroimidazole susceptibility testing. *Antimicrob. Agents Chemother.* **43,** 1484–6 (1999).
111. Piccolomini, R., Di Bonaventura, G., Catamo, G., Carbone, F. & Neri, M. Comparative evaluation of the E test, agar dilution, and broth microdilution for testing susceptibilities of Helicobacter pylori strains to 20 antimicrobial agents. *J. Clin. Microbiol.* **35,** 1842–6 (1997).
112. Chaves, S., Gadanho, M., Tenreiro, R. & Cabrita, J. Assessment of metronidazole susceptibility in Helicobacter pylori: statistical validation and error rate analysis of breakpoints determined by the disk diffusion test. *J. Clin. Microbiol.* **37,** 1628–31 (1999).
113. Glupczynski, Y., Labbé, M., Hansen, W., Crokaert, F. & Yourassowsky, E. Evaluation of the E test for quantitative antimicrobial susceptibility testing of Helicobacter pylori. *J. Clin. Microbiol.* **29,** 2072–5 (1991).
114. Hirschl, A. M., Hirschl, M. M. & Rotter, M. L. Comparison of three methods for the determination of the sensitivity of Helicobactor pylori to metronidazole. *J. Antimicrob. Chemother.* **32,** 45–9 (1993).
115. von Recklinghausen, G. & Ansorg, R. Metronidazole susceptibility testing of Helicobacter pylori with the PDM epsilometer test (E test). *Zentralbl. Bakteriol.* **282,** 83–5 (1995).

116. Midolo P.D., Bell J.M., Lambert J.R., Turnidge J.D. & Lindsay Grayson M. Antimicrobial resistance testing of Helicobacter pylori: A comparison of Etest and disk diffusion methods. *Pathology.* **29,** 411–4 (1997).
117. DeCross, A. J. *et al.* Metronidazole susceptibility testing for Helicobacter pylori: comparison of disk, broth, and agar dilution methods and their clinical relevance. *J. Clin. Microbiol.* **31,** 1971–4 (1993).
118. Weiss, K., Laverdière, M. & Restieri, C. Comparison of activity of 10 antibiotics against clinical strains of Helicobacter pylori by three different techniques. *Can. J. Gastroenterol.* **12,** 181–5 (1998).
119. Xia, H. X., Daw, M. A., Beattie, S., Keane, C. T. & O'Morain, C. A. Prevalence of metronidazole-resistant Helicobacter pylori in dyspeptic patients. *Ir. J. Med. Sci.* **162,** 91–4 (1993).
120. Xia, H., Keane, C. T., Beattie, S. & O'Morain, C. A. Standardization of disk diffusion test and its clinical significance for susceptibility testing of metronidazole against Helicobacter pylori. *Antimicrob. Agents Chemother.* **38,** 2357–61 (1994).
121. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial susceptibility testing. Approved standard M7-A5. Informational supplement M100-S10. (2000).
122. Glupczynski Y. *et al.* Comparison of the E test and agar dilution method for antimicrobial suceptibility testing of Helicobacter pylori. *Eur. J. Clin. Microbiol. Infect. Dis.* **21,** 549–52 (2002).
123. Osato, M. S., Reddy, R., Reddy, S. G., Penland, R. L. & Graham, D. Y. Comparison of the Etest and the NCCLS-approved agar dilution method to detect metronidazole and clarithromycin resistant Helicobacter pylori. *Int. J. Antimicrob. Agents* **17,** 39–44 (2001).
124. Megraud, F. & Lehours, P. Helicobacter pylori detection and antimicrobial susceptibility testing. *Clin. Microbiol. Rev.* **20,** 280–322 (2007).
125. Cerqueira, L. *et al.* Validation of a fluorescence in situ hybridization method using peptide nucleic acid probes for detection of Helicobacter pylori clarithromycin resistance in gastric biopsy specimens. *J. Clin. Microbiol.* **51,** 1887–93 (2013).

126. Schmitt, B. H., Regner, M., Mangold, K. A., Thomson, R. B. & Kaul, K. L. PCR detection of clarithromycin-susceptible and -resistant Helicobacter pylori from formalin-fixed, paraffin-embedded gastric biopsies. *Mod. Pathol.* **26,** 1222–7 (2013).
127. Rimbara, E., Sasatsu, M. & Graham, D. Y. PCR detection of Helicobacter pylori in clinical samples. *Methods Mol. Biol.* **943,** 279–87 (2013).
128. Miendje Deyi, V. Y. *et al.* Practical use of GenoType® HelicoDR, a molecular test for Helicobacter pylori detection and susceptibility testing. *Diagn. Microbiol. Infect. Dis.* **70,** 557–60 (2011).
129. Versalovic, J. *et al.* Point mutations in the 23S rRNA gene of Helicobacter pylori associated with different levels of clarithromycin resistance. *J. Antimicrob. Chemother.* **40,** 283–6 (1997).
130. Fontana, C. *et al.* Detection of clarithromycin-resistant Helicobacter pylori in stool samples. *J. Clin. Microbiol.* **41,** 3636–40 (2003).
131. Furuta, T. *et al.* Modified allele-specific primer-polymerase chain reaction method for analysis of susceptibility of Helicobacter pylori strains to clarithromycin. *J. Gastroenterol. Hepatol.* **22,** 1810–5 (2007).
132. Nakamura, A. *et al.* Determination of mutations of the 23S rRNA gene of Helicobacter pylori by allele specific primer-polymerase chain reaction method. *J. Gastroenterol. Hepatol.* **22,** 1057–63 (2007).
133. Nishizawa, T. *et al.* Rapid detection of point mutations conferring resistance to fluoroquinolone in gyrA of Helicobacter pylori by allele-specific PCR. *J. Clin. Microbiol.* **45,** 303–5 (2007).
134. Trebesius, K. *et al.* Rapid and specific detection of Helicobacter pylori macrolide resistance in gastric tissue by fluorescent in situ hybridisation. *Gut.* **46,** 608–14 (2000).
135. Sommer M.O.A. & Dantas G. Antibiotics and the resistant microbiome. *Curr. Opin. Microbiol.* **14,** 556–63 (2011).
136. Stroup, D. F. *et al.* Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. *JAMA.* **283,** 2008–12 (2000).

137. Tenover, F. C. Mechanisms of antimicrobial resistance in bacteria. *Am. J. Infect. Control* **34,** S3–10; discussion S64–73 (2006).
138. Gumbo, T. in *Goodman & Gilman's The Pharmacological Basis of Therapeutics, 12e* (eds. Brunton, L. L., Chabner, B. A. & Knollmann, B. C.) (The McGraw-Hill Companies, 2011).
139. Petri, W. A. in *Goodman & Gilman's The Pharmacological Basis of Therapeutics, 12e* (eds. Brunton, L. L., Chabner, B. A. & Knollmann, B. C.) (The McGraw-Hill Companies, 2011).
140. McManus, M. C. Mechanisms of bacterial resistance to antimicrobial agents. *Am. J. Health. Syst. Pharm.* **54,** 1420–33 (1997).
141. Drlica, K. & Zhao, X. DNA gyrase, topoisomerase IV, and the 4-quinolones. *Microbiol. Mol. Biol. Rev.* **61,** 377–92 (1997).
142. Franklin, T. J., Snow, G. A. & Franklin, T. J. *Biochemistry and molecular biology of antimicrobial drug action.* (New York : Springer, c2005., 2005).
143. Wehrli, W. Rifampin: Mechanisms of Action and Resistance. *Rev. Infect. Dis.* **5,** S407–11 (1983).
144. Petri, W. A. in *Goodman & Gilman's The Pharmacological Basis of Therapeutics, 12e* (eds. Brunton, L. L., Chabner, B. A. & Knollmann, B. C.) (The McGraw-Hill Companies, 2011).
145. Bbosa, G. S., Mwebaza, N., Odda, J., Kyegombe, D. B. & Ntale, M. Antibiotics/antibacterial drug use, their marketing and promotion during the post-antibiotic golden age and their role in emergence of bacterial resistance. *Health (N. Y.)* **6,** 410–25 (2014).
146. Lewis, K. Platforms for antibiotic discovery. *Nat. Rev. Drug Discov.* **12,** 371–87 (2013).
147. Brown, D. Antibiotic resistance breakers: can repurposed drugs fill the antibiotic discovery void? *Nat. Rev. Drug Discov.* **14,** 821–32 (2015).
148. Walsh, C. *Antibiotics: Actions, Origins, Resistance.* (ASM Press, 2003).
149. Megraud, F. Helicobacter pylori resistance to antibiotics: prevalence, mechanism, detection. What's new?. *Can. J. Gastroenterol.* **17 Suppl B,** 49B–52B (2003).

150. Hua, J.-S., Zheng, P.-Y., Fong, T. K., Khin, M. M. & Bow, H. O. Helicobacter pylori acquistion of metronidazole resistance by natural transformation in vitro. *World J. Gastroenterol.* **4,** 385–7 (1998).
151. Yue, Yue, Wang, Song & Gao. CagA status & genetic characterization of metronidazole resistant strains of H. pylori from a region at high risk of gastric cancer. *Pak. J. Med. Sci.* **30,** 804–8 (2014).
152. MacDougall, C. & Chambers, H. F. in *Goodman & Gilman's The Pharmacological Basis of Therapeutics, 12e* (eds. Brunton, L. L., Chabner, B. A. & Knollmann, B. C.) (The McGraw-Hill Companies, 2011).
153. Vester, B. & Douthwaite, S. Macrolide resistance conferred by base substitutions in 23S rRNA. *Antimicrob. Agents Chemother.* **45,** 1–12 (2001).
154. Seck, A. *et al.* Primary antibiotic resistance and associated mechanisms in Helicobacter pylori isolates from Senegalese patients. *Ann. Clin. Microbiol. Antimicrob.* **12,** (2013).
155. Weisblum, B. Erythromycin resistance by ribosome modification. *Antimicrob. Agents Chemother.* **39,** 577–85 (1995).
156. Francesco, V. D. *et al.* Mechanisms of Helicobacter pylori antibiotic resistance: An updated appraisal. *World J. Gastrointest. Pathophysiol.* **2,** 35–41 (2011).
157. Burucoa, C., Landron, C., Garnier, M. & Fauchère, J.-L. T2182C mutation is not associated with clarithromycin resistance in Helicobacter pylori. *Antimicrob. Agents Chemother.* **49,** 868; author reply 868–70 (2005).
158. Rimbara, E. *et al.* Mutations in the 23S rRNA gene of clarithromycin-resistant Helicobacter pylori from Japan. *Int. J. Antimicrob. Agents* **30,** 250–4 (2007).
159. Binh T.T. *et al.* Discovery of novel mutations for clarithromycin resistance in Helicobacter pylori by using next-generation sequencing. *Journal of Antimicrobial Chemotherapy* (2014).
160. Phillips, M. A. & Stanley, S. L. in *Goodman & Gilman's: The Pharmacological Basis of Therapeutics, 12e* (eds. Brunton, L. L., Chabner, B. A. & Knollmann, B. C.) (McGraw-Hill Education, 2011).

161. Ings, R. M., McFadzean, J. A. & Ormerod, W. E. The mode of action of metronidazole in Trichomonas vaginalis and other micro-organisms. *Biochem. Pharmacol.* **23,** 1421–9 (1974).
162. Jorgensen, M. A., Manos, J., Mendz, G. L. & Hazell, S. L. The mode of action of metronidazole in Helicobacter pylori: futile cycling or reduction? *J. Antimicrob. Chemother.* **41,** 67–75 (1998).
163. Goodwin, A. *et al.* Metronidazole resistance in Helicobacter pylori is due to null mutations in a gene (rdxA) that encodes an oxygen-insensitive NADPH nitroreductase. *Mol. Microbiol.* **28,** 383–93 (1998).
164. Debets-Ossenkopp, Y. J. *et al.* Insertion of mini-IS605 and deletion of adjacent sequences in the nitroreductase (rdxA) gene cause metronidazole resistance in Helicobacter pylori NCTC11637. *Antimicrob. Agents Chemother.* **43,** 2657–62 (1999).
165. Tankovic, J. *et al.* Frequent association between alteration of the rdxA gene and metronidazole resistance in French and North African isolates of Helicobacter pylori. *Antimicrob. Agents Chemother.* **44,** 608–13 (2000).
166. Jorgensen, M. A., Trend, M. A., Hazell, S. L. & Mendz, G. L. Potential involvement of several nitroreductases in metronidazole resistance in Helicobacter pylori. *Arch. Biochem. Biophys.* **392,** 180–91 (2001).
167. Bereswill, S., Krainick, C., Stähler, F., Herrmann, L. & Kist, M. Analysis of the rdxA gene in high-level metronidazole-resistant clinical isolates confirms a limited use of rdxA mutations as a marker for prediction of metronidazole resistance in Helicobacter pylori. *FEMS Immunol. Med. Microbiol.* **36,** 193–8 (2003).
168. Chisholm, S. A. & Owen, R. J. Mutations in Helicobacter pylori rdxA gene sequences may not contribute to metronidazole resistance. *J. Antimicrob. Chemother.* **51,** 995–9 (2003).
169. Jenks P.J., Ferrero R.L. & Labigne A. The role of the rdxA gene in the evolution of metronidazole resistance in Helicobacter pylori. *J. Antimicrob. Chemother.* **43,** 753–8 (1999).
170. Kwon, D. H. *et al.* Analysis of rdxA and involvement of additional genes encoding NAD(P)H flavin oxidoreductase (FrxA) and ferredoxin-like protein (FdxB) in

metronidazole resistance of Helicobacter pylori. *Antimicrob. Agents Chemother.* **44,** 2133–42 (2000).

171. Kwon, D. H., Kato, M., El-Zaatari, F. A., Osato, M. S. & Graham, D. Y. Frame-shift mutations in NAD(P)H flavin oxidoreductase encoding gene (frxA) from metronidazole resistant Helicobacter pylori ATCC43504 and its involvement in metronidazole resistance. *FEMS Microbiol. Lett.* **188,** 197–202 (2000).
172. Kwon, D. H. *et al.* DNA sequence analysis of rdxA and frxA from 12 pairs of metronidazole-sensitive and -resistant clinical Helicobacter pylori isolates. *Antimicrob. Agents Chemother.* **45,** 2609–15 (2001).
173. Jeong, J. Y. *et al.* Sequential inactivation of rdxA (HP0954) and frxA (HP0642) nitroreductase genes causes moderate and high-level metronidazole resistance in Helicobacter pylori. *J. Bacteriol.* **182,** 5082–90 (2000).
174. Marais, A., Bilardi, C., Cantet, F., Mendz, G. L. & Mégraud, F. Characterization of the genes rdxA and frxA involved in metronidazole resistance in Helicobacter pylori. *Res. Microbiol.* **154,** 137–44 (2003).
175. Albert, T. J. *et al.* Mutation discovery in bacterial genomes: metronidazole resistance in Helicobacter pylori. *Nat. Methods* **2,** 951–3 (2005).
176. Tsugawa H. *et al.* Enhanced bacterial efflux system is the first step to the development of metronidazole resistance in Helicobacter pylori. *Biochem. Biophys. Res. Commun.* **404,** 656-60 (2011).
177. Bush, K. New beta-lactamases in gram-negative bacteria: diversity and impact on the selection of antimicrobial therapy. *Clin. Infect. Dis.* **32,** 1085–9 (2001).
178. Dore, M. P. *et al.* Amoxycillin tolerance in Helicobacter pylori. *J. Antimicrob. Chemother.* **43,** 47–54 (1999).
179. van Zwet, A. A. *et al.* Stable amoxicillin resistance in Helicobacter pylori. *Lancet.* **352,** 1595 (1998).
180. Dore, M. P., Graham, D. Y. & Sepulveda, A. R. Different penicillin-binding protein profiles in amoxicillin-resistant Helicobacter pylori. *Helicobacter.* **4,** 154–61 (1999).

181. Kwon, D. H. *et al.* High-level beta-lactam resistance associated with acquired multidrug resistance in Helicobacter pylori. *Antimicrob. Agents Chemother.* **47,** 2169–78 (2003).
182. Gerrits M.M. *et al.* Alterations in penicillin-binding protein 1A confer resistance to beta-lactam antibiotics in Helicobacter pylori. *Antimicrob. Agents Chemother.* **46,** 2229–33 (2002).
183. Gerrits, M. M. *et al.* Multiple mutations in or adjacent to the conserved penicillin-binding protein motifs of the penicillin-binding protein 1A confer amoxicillin resistance to Helicobacter pylori. *Helicobacter.* **11,** 181–7 (2006).
184. DeLoney, C. R. & Schiller, N. L. Characterization of an In vitro-selected amoxicillin-resistant strain of Helicobacter pylori. *Antimicrob. Agents Chemother.* **44,** 3368–73 (2000).
185. Tseng Y.-S. *et al.* Amoxicillin resistance with beta-lactamase production in Helicobacter pylori. *Eur. J. Clin. Invest.* **39,** 807–12 (2009).
186. Cao, P., McClain, M. S., Forsyth, M. H. & Cover, T. L. Extracellular release of antigenic proteins by Helicobacter pylori. *Infect. Immun.* **66,** 2984–6 (1998).
187. Mittl, P. R., Lüthy, L., Hunziker, P. & Grütter, M. G. The cysteine-rich protein A from Helicobacter pylori is a beta-lactamase. *J. Biol. Chem.* **275,** 17693–9 (2000).
188. Jones K.R., Cha J.-H. & Merrell D.S. Who's winning the war? Molecular mechanisms of antibiotic resistance in Helicobacter pylori. *Curr. Drug Ther.* **3,** 190–203 (2008).
189. Chopra, I. & Roberts, M. Tetracycline Antibiotics: Mode of Action, Applications, Molecular Biology, and Epidemiology of Bacterial Resistance. *Microbiol. Mol. Biol. Rev.* **65,** 232–60 (2001).
190. Gerrits, M. M., de Zoete, M. R., Arents, N. L. A., Kuipers, E. J. & Kusters, J. G. 16S rRNA mutation-mediated tetracycline resistance in Helicobacter pylori. *Antimicrob. Agents Chemother.* **46,** 2996–3000 (2002).
191. Dailidiene, D. *et al.* Emergence of tetracycline resistance in Helicobacter pylori: multiple mutational changes in 16S ribosomal DNA and other genetic loci. *Antimicrob. Agents Chemother.* **46,** 3940–6 (2002).

192. Trieber, C. A. & Taylor, D. E. Mutations in the 16S rRNA genes of Helicobacter pylori mediate resistance to tetracycline. *J. Bacteriol.* **184,** 2131–40 (2002).
193. Wu, J. Y. *et al.* Tetracycline-Resistant Clinical Helicobacter pylori Isolates with and without Mutations in 16S rRNA-Encoding Genes. *Antimicrob. Agents Chemother.* **49,** 578–83 (2005).
194. Kutschke, A. & de Jonge, B. L. M. Compound efflux in Helicobacter pylori. *Antimicrob. Agents Chemother.* **49,** 3009–10 (2005).
195. Moore, R. A., Beckthold, B., Wong, S., Kureishi, A. & Bryan, L. E. Nucleotide sequence of the gyrA gene and characterization of ciprofloxacin-resistant mutants of Helicobacter pylori. *Antimicrob. Agents Chemother.* **39,** 107–11 (1995).
196. Piddock, L. J. V. Clinically relevant chromosomally encoded multidrug resistance efflux pumps in bacteria. *Clin. Microbiol. Rev.* **19,** 382–402 (2006).
197. Megraud, F. Helicobacter pylori and antibiotic resistance. *Gut.* **56,** 1502 (2007).
198. Crumplin, G. C., Kenwright, M. & Hirst, T. Investigations into the mechanism of action of the antibacterial agent norfloxacin. *J. Antimicrob. Chemother.* **13 Suppl B,** 9–23 (1984).
199. Bina, J. E. *et al.* Helicobacter pylori uptake and efflux: basis for intrinsic susceptibility to antibiotics in vitro. *Antimicrob. Agents Chemother.* **44,** 248–54 (2000).
200. Gumbo, T. in *Goodman & Gilman's: The Pharmacological Basis of Therapeutics, 12e* (eds. Brunton, L. L., Chabner, B. A. & Knollmann, B. C.) (McGraw-Hill Education, 2011).
201. Heep, M., Beck, D., Bayerdorffer, E. & Lehn, N. Rifampin and rifabutin resistance mechanism in Helicobacter pylori. *Antimicrob. Agents Chemother.* **43,** 1497–9 (1999).
202. Heep, M. *et al.* Mutations at four distinct regions of the rpoB gene can reduce the susceptibility of Helicobacter pylori to rifamycins. *Antimicrob. Agents Chemother.* **44,** 1713–5 (2000).
203. Heep, M., Rieger, U., Beck, D. & Lehn, N. Mutations in the beginning of the rpoB gene can induce resistance to rifamycins in both Helicobacter pylori and Mycobacterium tuberculosis. *Antimicrob. Agents Chemother.* **44,** 1075–7 (2000).

204. Glocker E., Bogdan C. & Kist M. Characterization of rifampicin-resistant clinical Helicobacter pylori isolates from Germany. *J. Antimicrob. Chemother.* **59,** 874–9 (2007).
205. Wang, G., Wilson, T. J., Jiang, Q. & Taylor, D. E. Spontaneous mutations that confer antibiotic resistance in Helicobacter pylori. *Antimicrob. Agents Chemother.* **45,** 727–33 (2001).
206. Jenks, P. J., Ferrero, R. L., Tankovic, J., Thiberge, J. M. & Labigne, A. Evaluation of nitrofurantoin combination therapy of metronidazole-sensitive and -resistant Helicobacter pylori infections in mice. *Antimicrob. Agents Chemother.* **44,** 2623–9 (2000).
207. Whiteway, J. *et al.* Oxygen-insensitive nitroreductases: analysis of the roles of nfsA and nfsB in development of resistance to 5-nitrofuran derivatives in Escherichia coli. *J. Bacteriol.* **180,** 5529–39 (1998).
208. Kwon, D. H. *et al.* Furazolidone- and nitrofurantoin-resistant Helicobacter pylori: prevalence and role of genes involved in metronidazole resistance. *Antimicrob. Agents Chemother.* **45,** 306–8 (2001).
209. Sisson, G. *et al.* Enzymes associated with reductive activation and action of nitazoxanide, nitrofurans, and metronidazole in Helicobacter pylori. *Antimicrob. Agents Chemother.* **46,** 2116–23 (2002).
210. Yakoob, J. *et al.* Low rate of recurrence of Helicobacter pylori infection in spite of high clarithromycin resistance in Pakistan. *BMC Gastroenterol.* **13,** 33 (2013).
211. Rasheed, F. *et al.* Analysis of clinical isolates of Helicobacter pylori in Pakistan reveals high degrees of pathogenicity and high frequencies of antibiotic resistance. *Helicobacter.* **19,** 387–99 (2014).
212. Alfaresi, M. S. & Elkoush, A. A. Characterization of clarithromycin resistance in isolates of Helicobacter pylori from the UAE. *Indian J. Gastroenterol. Off. J. Indian Soc. Gastroenterol.* **29,** 116–20 (2010).
213. Peretz, A. *et al.* Resistance of Helicobacter pylori to tetracycline, amoxicillin, clarithromycin and metronidazole in Israeli children and adults. *J. Antibiot.* **67,** 555–7 (2014).

214. Alfizah, H., Norazah, A., Hamizah, R. & Ramelah, M. Resistotype of Helicobacter pylori isolates: the impact on eradication outcome. *J. Med. Microbiol.* **63,** 703–9 (2014).
215. Okamura, T. *et al.* Antimicrobial resistance and characteristics of eradication therapy of Helicobacter pylori in Japan: a multi-generational comparison. *Helicobacter.* **19,** 214–20 (2014).
216. Gao, W. *et al.* The evolution of Helicobacter pylori antibiotics resistance over 10 years in Beijing, China. *Helicobacter.* **15,** 460–6 (2010).
217. Binh, T. T. *et al.* The incidence of primary antibiotic resistance of Helicobacter pylori in Vietnam. *J. Clin. Gastroenterol.* **47,** 233–8 (2013).
218. Siavoshi, F., Saniee, P., Latifi-Navid, S., Massarrat, S. & Sheykholeslami, A. Increase in resistance rates of H. pylori isolates to metronidazole and tetracycline--comparison of three 3-year studies. *Arch. Iran. Med.* **13,** 177–87 (2010).
219. Tomatari F.H., Mobarez A.M., Amini M., Hosseini D. & Bezmin Abadi A.T. Helicobacter pylori resistance to metronidazole and clarithromycin in dyspeptic patients in Iran. *IRCMJ.* **12,** 409–12 (2010).
220. Sirous M. *et al.* Prevalence of antimicrobial resistance in helicobacter pylori isolates from Iran. *Afr. J. Biotechnol.* **9,** 5962–5 (2010).
221. Milani, M. *et al.* The status of antimicrobial resistance of Helicobacter pylori in Eastern Azerbaijan, Iran: comparative study according to demographics. *J. Infect. Chemother.* **18,** 848–52 (2012).
222. Mirzaei N. *et al.* Prevalence of resistance of Helicobacter pylori strains to selected antibiotics in Isfahan, Iran. *Jundishapur J Microbiol* **6,** e6342 (2013).
223. Rajper, S. *et al.* Macrolide and fluoroquinolone resistance in Helicobacter pylori isolates: an experience at a tertiary care centre in Pakistan. *JPMA.* **62,** 1140–4 (2012).
224. Seo J.-H. *et al.* Changing pattern of antibiotic resistance of Helicobacter pylori in children during 20 years in Jinju, South Korea. *Pediatr. Int.* **55,** 332–6 (2013).
225. Zevit, N., Levy, I., Shmuely, H., Samra, Z. & Yahav, J. Antibiotic resistance of Helicobacter pylori in Israeli children. *Scand. J. Gastroenterol.* **45,** 550–5 (2010).

226. Nguyen, T. V. H. *et al.* Eradication of Helicobacter pylori in Children in Vietnam in Relation to Antibiotic Resistance. *Helicobacter.* **17,** 319–25 (2012).
227. Vilaichone, R. *et al.* Antibiotics resistance rate of Helicobacter pylori in Bhutan. *World J. Gastroenterol.* **19,** 5508–12 (2013).
228. Goh, K.-L. & Navaratnam, P. High Helicobacter pylori resistance to metronidazole but zero or low resistance to clarithromycin, levofloxacin, and other antibiotics in Malaysia. *Helicobacter.* **16,** 241–5 (2011).
229. Talebi Bezmin Abadi, A., Mobarez, A. M., Taghvaei, T. & Wolfram, L. Antibiotic resistance of Helicobacter pylori in Mazandaran, North of Iran. *Helicobacter.* **15,** 505–9 (2010).
230. Sun, Q.-J. *et al.* Resistance of Helicobacter pylori to antibiotics from 2000 to 2009 in Shanghai. *World J. Gastroenterol.* **16,** 5118–21 (2010).
231. Su, P. *et al.* Antibiotic resistance of Helicobacter pylori isolated in the Southeast Coastal Region of China. *Helicobacter.* **18,** 274–9 (2013).
232. Seo J.-H. *et al.* Comparison of the antibiotic resistance of Helicobacter pylori isolated in Jinju over a 15-year period. *J. Bacteriol. Virol.* **42,** 305–12 (2012).
233. An, B. *et al.* Antibiotic resistance in Helicobacter pylori strains and its effect on H. pylori eradication rates in a single center in Korea. *Ann. Lab. Med.* **33,** 415–9 (2013).
234. Yamade, M. *et al.* Resistance of Helicobacter pylori to quinolones and clarithromycin assessed by genetic testing in Japan. *J. Gastroenterol. Hepatol.* **26,** 1457–61 (2011).
235. Song, Z. *et al.* Prospective multi-region study on primary antibiotic resistance of Helicobacter pylori strains isolated from Chinese patients. *Dig. Liver Dis.* **46,** 1077–81 (2014).
236. Vilaichone, R.-K., Gumnarai, P., Ratanachu-Ek, T. & Mahachai, V. Nationwide survey of Helicobacter pylori antibiotic resistance in Thailand. *Diagn. Microbiol. Infect. Dis.* **77,** 346–9 (2013).
237. Saracino, I. M. *et al.* High prevalence of primary antibiotic resistance in Helicobacter pylori isolates in Italy. *J. Gastrointestin. Liver Dis.* **21,** 363–5 (2012).

238. Abadi, A. T. B., Taghvaei, T., Mobarez, A. M., Carpenter, B. M. & Merrell, D. S. Frequency of antibiotic resistance in Helicobacter pylori strains isolated from the northern population of Iran. *J. Microbiol.* **49,** 987–93 (2011).
239. Talebi Bezmin Abadi, A., Ghasemzadeh, A., Taghvaei, T. & Mobarez, A. M. Primary resistance of Helicobacter pylori to levofloxacin and moxifloxacine in Iran. *Intern. Emerg. Med.* **7,** 447–52 (2012).
240. Zendedel A., Moradimoghadam F., Almasi V. & Zivarifar H. Antibiotic resistance of Helicobacter pylori in Mashhad, Iran. *J. Pak. Med. Assoc.* **63,** 336–9 (2013).
241. Pandya, H. B., Agravat, H. H., Patel, J. S. & Sodagar, N. R. K. Emerging antimicrobial resistance pattern of Helicobacter pylori in central Gujarat. *Indian J. Med. Microbiol.* **32,** 408–13 (2014).
242. Liu, G. *et al.* Primary antibiotic resistance of Helicobacter pylori isolated from Beijing children. *Helicobacter.* **16,** 356–62 (2011).
243. Kato, S. & Fujimura, S. Primary antimicrobial resistance of Helicobacter pylori in children during the past 9 years. *Pediatr. Int.* **52,** 187–90 (2010).
244. Hwang, T. J. *et al.* Change in antibiotic resistance of Helicobacter pylori strains and the effect of A2143G point mutation of 23S rRNA on the eradication of H. pylori in a single center of Korea. *J. Clin. Gastroenterol.* **44,** 536–43 (2010).
245. Chung, J.-W. *et al.* Resistance of Helicobacter pylori strains to antibiotics in Korea with a focus on fluoroquinolone resistance. *J. Gastroenterol. Hepatol.* **27,** 493–7 (2012).
246. Lee, J. W. *et al.* Prevalence of primary and secondary antimicrobial resistance of Helicobacter pylori in Korea from 2003 through 2012. *Helicobacter.* **18,** 206–14 (2013).
247. Farshad, S. *et al.* Antimicrobial susceptibility of Helicobacter pylori strains isolated from patients in Shiraz, Southern Iran. *World J. Gastroenterol.* **16,** 5746–51 (2010).
248. Shokrzadeh, L. *et al.* Antibiotic susceptibility profile of Helicobacter pylori isolated from the dyspepsia patients in Tehran, Iran. *Saudi J. Gastroenterol.* **17,** 261–4 (2011).

249. Sadeghifard, N. *et al.* Survey in Iran of clarithromycin resistance in Helicobacter pylori isolates by PCR-RFLP. *Southeast Asian J. Trop. Med. Public Health* **44,** 89–95 (2013).
250. Ahmad, N., Zakaria, W. R. & Mohamed, R. Analysis of antibiotic susceptibility patterns of Helicobacter pylori isolates from Malaysia. *Helicobacter.* **16,** 47–51 (2011).
251. Teh, X. *et al.* Functional and molecular surveillance of Helicobacter pylori antibiotic resistance in Kuala Lumpur. *PloS One.* **9,** e101481 (2014).
252. Ho, S.-L., Tan, E. L., Sam, C. K. & Goh, K.-L. Clarithromycin resistance and point mutations in the 23S rRNA gene in Helicobacter pylori isolates from Malaysia. *J. Dig. Dis.* **11,** 101–5 (2010).
253. Reva, I. *et al.* Virulence genotypes and drug resistance of Helicobacter pylori from Vladivostok, Russia: another feature in the Far East. *Microbiol. Immunol.* **56,** 198–202 (2012).
254. Raymond, J., Lamarque, D., Kalach, N., Chaussade, S. & Burucoa, C. High level of antimicrobial resistance in French Helicobacter pylori isolates. *Helicobacter.* **15,** 21–7 (2010).
255. Kalem F., Ozdemir M., Basaranoglu M., Toy H. & Baysal B. Helicobacter pylori isolates recovered from antral gastric biopsies of patients with dyspeptic symptoms: Antimicrobial resistance of metronidazole, clarithromycin and amoxicillin. *Anatol. J. Clin. Investig.* **6,** 37–40 (2012).
256. Prechtl, J. *et al.* Monitoring of antibiotic resistance rates of Helicobacter pylori in Austrian children, 2002-2009. *Pediatr. Infect. Dis. J.* **31,** 312–4 (2012).
257. Cuadrado-Lavin, A. *et al.* Antimicrobial susceptibility of Helicobacter pylori to six antibiotics currently used in Spain. *J. Antimicrob. Chemother.* **67,** 170–3 (2012).
258. Agudo, S., Perez-Perez, G., Alarcon, T. & Lopez-Brea, M. Rapid detection of clarithromycin resistant Helicobacter pylori strains in Spanish patients by polymerase chain reaction-restriction fragment length polymorphism. *Rev. Esp. Quimioter.* **24,** 32–6 (2011).
259. Karczewska, E., Wojtas-Bonior, I., Sito, E., Zwolinska-Wcislo, M. & Budak, A. Primary and secondary clarithromycin, metronidazole, amoxicillin and levofloxacin

resistance to Helicobacter pylori in southern Poland. *Pharmacol. Rep.* **63,** 799–807 (2011).

260. O'Connor, A. *et al.* Helicobacter pylori resistance to metronidazole and clarithromycin in Ireland. *Eur. J. Gastroenterol. Hepatol.* **22,** 1123–7 (2010).
261. Almeida, N. *et al.* Helicobacter pylori antimicrobial resistance rates in the central region of Portugal. *Clin. Microbiol. Infect.* **20,** 1127–33 (2014).
262. Boyanova, L. *et al.* Helicobacter pylori susceptibility to fosfomycin, rifampin, and 5 usual antibiotics for H. pylori eradication. *Diagn. Microbiol. Infect. Dis.* **79,** 358–61 (2014).
263. Wueppenhorst, N., Stueger, H.-P., Kist, M. & Glocker, E.-O. High secondary resistance to quinolones in German Helicobacter pylori clinical isolates. *J. Antimicrob. Chemother.* **68,** 1562–6 (2013).
264. Selgrad, M. *et al.* Different antibiotic susceptibility between antrum and corpus of the stomach, a possible reason for treatment failure of Helicobacter pylori infection. *World J. Gastroenterol.* **20,** 16245–51 (2014).
265. Wüppenhorst, N. *et al.* Prospective multicentre study on antimicrobial resistance of Helicobacter pylori in Germany. *J. Antimicrob. Chemother.* **69,** 3127–33 (2014).
266. Karczewska E. *et al.* Variability in prevalence of helicobacter pylori strains resistant to clarithromycin and levofloxacin in Southern Poland. *Gastroenterol. Res. Pract.* (2012). doi:10.1155/2012/418010
267. Biernat M.M. *et al.* Antimicrobial susceptibility of helicobacter pylori isolates from lower silesia, poland. *Arch. Med. Sci.* **10,** 505–9 (2014).
268. Megraud, F. *et al.* Helicobacter pylori resistance to antibiotics in Europe and its relationship to antibiotic consumption. *Gut.* **62,** 34–42 (2013).
269. Kostamo, P., Veijola, L., Oksanen, A., Sarna, S. & Rautelin, H. Recent trends in primary antimicrobial resistance of Helicobacter pylori in Finland. *Int. J. Antimicrob. Agents* **37,** 22–5 (2011).
270. De Francesco, V. *et al.* Primary clarithromycin resistance in Helicobacter pylori: the Multicentric Italian Clarithromycin Resistance Observational (MICRO) study. *J. Gastrointestin. Liver Dis.* **20,** 235–9 (2011).

271. Miendje Deyi, V. Y. *et al.* Multicenter survey of routine determinations of resistance of Helicobacter pylori to antimicrobials over the last 20 years (1990 to 2009) in Belgium. *J. Clin. Microbiol.* **49,** 2200–9 (2011).
272. Gosciniak, G. *et al.* The antimicrobial susceptibility of Helicobacter pylori strains isolated from children and adults with primary infection in the Lower Silesia Region, Poland. *Pol. J. Microbiol.* **63,** 57–61 (2014).
273. Boyanova, L. *et al.* Two-decade trends in primary Helicobacter pylori resistance to antibiotics in Bulgaria. *Diagn. Microbiol. Infect. Dis.* **67,** 319–26 (2010).
274. Kupcinskas, L. *et al.* Evolution of Helicobacter pylori susceptibility to antibiotics during a 10-year period in Lithuania. *APMIS.* **121,** 431–6 (2013).
275. Larsen, A. L., Ragnhildstveit, E., Moayeri, B., Eliassen, L. & Melby, K. K. Resistance rates of metronidazole and other antibacterials in Helicobacter pylori from previously untreated patients in Norway. *APMIS Acta Pathol. Microbiol. Immunol. Scand.* **121,** 353–8 (2013).
276. Karamanolis, G. P. *et al.* The evolution of Helicobacter pylori antibiotics resistance over 10 years in Greece. *Digestion.* **90,** 229–31 (2014).
277. Tonkic, A. *et al.* Time trends of primary antibiotic resistance of Helicobacter pylori isolates in Southern Croatia. *J. Chemother.* **24,** 182–4 (2012).
278. Secka, O. *et al.* Antimicrobial susceptibility and resistance patterns among Helicobacter pylori strains from The Gambia, West Africa. *Antimicrob. Agents Chemother.* **57,** 1231–7 (2013).
279. Ben Mansour, K. *et al.* Primary resistance to clarithromycin, metronidazole and amoxicillin of Helicobacter pylori isolated from Tunisian patients with peptic ulcers and gastritis: a prospective multicentre study. *Ann. Clin. Microbiol. Antimicrob.* **9,** 22 (2010).
280. Tanih, N. F. *et al.* Marked susceptibility of South African Helicobacter pylori strains to ciprofloxacin and amoxicillin: clinical implications. *S. Afr. Med. J.* **100,** 49–52 (2010).
281. Tanih N.F. & Ndip R.N. Molecular detection of antibiotic resistance in South African isolates of Helicobacter pylori. *Gastroenterol. Res. Pract.* (2013). doi:10.1155/2013/259457

282. Kimang'a, A. N., Revathi, G., Kariuki, S., Sayed, S. & Devani, S. Helicobacter pylori: prevalence and antibiotic susceptibility among Kenyans. *S. Afr. Med. J.* **100,** 53–7 (2010).
283. Picoli, S. U. *et al.* Resistance to amoxicillin, clarithromycin and ciprofloxacin of Helicobacter pylori isolated from Southern Brazil patients. *Rev. Inst. Med. Trop. Sao Paulo* **56,** 197–200 (2014).
284. Ogata, S. K., Godoy, A. P. O., da Silva Patricio, F. R. & Kawakami, E. High Helicobacter pylori resistance to metronidazole and clarithromycin in Brazilian children and adolescents. *J. Pediatr. Gastroenterol. Nutr.* **56,** 645–8 (2013).
285. Suzuki, R. B., Lopes, R. A. B., da Camara Lopes, G. A., Hung Ho, T. & Speranca, M. A. Low Helicobacter pylori primary resistance to clarithromycin in gastric biopsy specimens from dyspeptic patients of a city in the interior of Sao Paulo, Brazil. *BMC Gastroenterol.* **13,** 164 (2013).
286. Eisig, J. N. *et al.* Helicobacter pylori antibiotic resistance in Brazil: clarithromycin is still a good option. *Arq. Gastroenterol.* **48,** 261–4 (2011).
287. Garcia, G. T. *et al.* High prevalence of clarithromycin resistance and cagA, vacA, iceA2, and babA2 genotypes of Helicobacter pylori in Brazilian children. *J. Clin. Microbiol.* **48,** 4266–8 (2010).
288. Llanes, R. *et al.* Phenotypic and genetic characterization of antimicrobial profiles of Helicobacter pylori strains in Cuba. *J. Health Popul. Nutr.* **28,** 124–9 (2010).
289. Ayala, G. *et al.* Resistance to antibiotics and characterization of Helicobacter pylori strains isolated from antrum and body from adults in Mexico. *Microb. Drug Resist.* **17,** 149–55 (2011).
290. Tveit, A. H. *et al.* Alaska sentinel surveillance study of Helicobacter pylori isolates from Alaska Native persons from 2000 to 2008. *J. Clin. Microbiol.* **49,** 3638–43 (2011).
291. Hsiang, J. *et al.* Increasing primary antibiotic resistance and ethnic differences in eradication rates of Helicobacter pylori infection in New Zealand--a new look at an old enemy. *N. Z. Med. J.* **126,** 64–76 (2013).

292. Alfizah, H., Rukman, A. H., Norazah, A., Hamizah, R. & Ramelah, M. Ethnicity association of Helicobacter pylori virulence genotype and metronidazole susceptibility. *World J. Gastroenterol.* **19,** 1283–91 (2013).
293. Karabiber, H., Selimoglu, M. A., Otlu, B., Yildirim, O. & Ozer, A. Virulence factors and antibiotic resistance in children with Helicobacter pylori gastritis. *J. Pediatr. Gastroenterol. Nutr.* **58,** 608–12 (2014).
294. Vecsei, A. *et al.* Time trends of Helicobacter pylori resistance to antibiotics in children living in Vienna, Austria. *Helicobacter.* **15,** 214–20 (2010).
295. Hojsak, I. *et al.* Antibiotic resistance of Helicobacter pylori in pediatric patients -- 10 years' experience. *Eur. J. Pediatr.* **171,** 1325–30 (2012).
296. Oleastro, M. *et al.* Primary antibiotic resistance of Helicobacter pylori strains isolated from Portuguese children: a prospective multicentre study over a 10 year period. *J. Antimicrob. Chemother.* **66,** 2308–11 (2011).
297. O'Connor, A. *et al.* Helicobacter pylori resistance rates for levofloxacin, tetracycline and rifabutin among Irish isolates at a reference centre. *Ir. J. Med. Sci.* **182,** 693–5 (2013).
298. Toledo, H. & Lopez-Solis, R. Tetracycline resistance in Chilean clinical isolates of Helicobacter pylori. *J. Antimicrob. Chemother.* **65,** 470–3 (2010).
299. Karczewska, E. *et al.* Levofloxacin resistance of Helicobacter pylori strains isolated from patients in southern Poland, between 2006-2012. *Acta Pol. Pharm.* **71,** 477–83 (2014).
300. Keshavarz Azizi Raftar, S. *et al.* Helicobacter pylori resistance to ciprofloxacin in Iran. *Int. J. Antimicrob. Agents* **43,** 573–4 (2014).
301. Cabrita, J. *et al.* Features and trends in Helicobacter pylori antibiotic resistance in Lisbon area, Portugal (1990-1999). *J. Antimicrob. Chemother.* **46,** 1029–31 (2000).
302. Pilotto, A. *et al.* Prevalence of Helicobacter pylori resistance to antibiotics in Northeast Italy: a multicentre study. GISU. Interdisciplinary Group for the Study of Ulcer. *Dig. Liver Dis.* **32,** 763–8 (2000).
303. Van Boeckel, T. P. *et al.* Global antibiotic consumption 2000 to 2010: an analysis of national pharmaceutical sales data. *Lancet Infect. Dis.* **14,** 742–50 (2014).

304. Koivisto, T. T. *et al.* Primary Helicobacter pylori resistance to metronidazole and clarithromycin in the Finnish population. *Aliment. Pharmacol. Ther.* **19,** 1009–17 (2004).
305. Nahar, S. *et al.* Antimicrobial susceptibility of Helicobacter pylori strains isolated in Bangladesh. *J. Clin. Microbiol.* **42,** 4856–8 (2004).
306. Zullo, A. *et al.* Primary antibiotic resistance in Helicobacter pylori strains isolated in northern and central Italy. *Aliment. Pharmacol. Ther.* **25,** 1429–34 (2007).
307. Katelaris, P. H. Helicobacter pylori: antibiotic resistance and treatment options. *J. Gastroenterol. Hepatol.* **24,** 1155–7 (2009).
308. Gisbert, J. P. Second-line rescue therapy of helicobacter pylori infection. *Ther. Adv. Gastroenterol.* **2,** 331–56 (2009).
309. Kim, J. J., Kim, J. G. & Kwon, D. H. Mixed-infection of antibiotic susceptible and resistant Helicobacter pylori isolates in a single patient and underestimation of antimicrobial susceptibility testing. *Helicobacter.* **8,** 202–6 (2003).
310. Arents N.L.A. *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.* **20,** 418–20 (2001).
311. Lee, Y. C. *et al.* Isogenic Variation of Helicobacter pylori Strain Resulting in Heteroresistant Antibacterial Phenotypes in a Single Host In Vivo. *Helicobacter.* **10,** 240–8 (2005).
312. Matteo, M. J. *et al.* DNA sequence analysis of rdxA and frxA from paired metronidazole-sensitive and -resistant Helicobacter pylori isolates obtained from patients with heteroresistance. *Int. J. Antimicrob. Agents.* **27,** 152–8 (2006).
313. van der Ende, A. *et al.* Clarithromycin-susceptible and -resistant Helicobacter pylori isolates with identical randomly amplified polymorphic DNA-PCR genotypes cultured from single gastric biopsy specimens prior to antibiotic therapy. *J. Clin. Microbiol.* **39,** 2648–51 (2001).
314. Gomollón, F. *et al.* Third line treatment for Helicobacter pylori: a prospective, culture-guided study in peptic ulcer patients. *Aliment. Pharmacol. Ther.* **14,** 1335–8 (2000).

315. Venerito, M., Krieger, T., Ecker, T., Leandro, G. & Malfertheiner, P. Meta-analysis of bismuth quadruple therapy versus clarithromycin triple therapy for empiric primary treatment of Helicobacter pylori infection. *Digestion.* **88,** 33–45 (2013).
316. Kato, M. *et al.* Regional differences in metronidazole resistance and increasing clarithromycin resistance among Helicobacter pylori isolates from Japan. *Antimicrob. Agents Chemother.* **44,** 2214–6 (2000).
317. Elviss, N. C., Owen, R. J., Xerry, J., Walker, A. M. & Davies, K. Helicobacter pylori antibiotic resistance patterns and genotypes in adult dyspeptic patients from a regional population in North Wales. *J. Antimicrob. Chemother.* **54,** 435–40 (2004).
318. Megraud, F. & Marshall, B. J. How to treat Helicobacter pylori. First-line, second-line, and future therapies. *Gastroenterol. Clin. North Am.* **29,** 759–73 (2000).
319. Suzuki, T. *et al.* Systematic review and meta-analysis: importance of CagA status for successful eradication of Helicobacter pylori infection. *Aliment. Pharmacol. Ther.* **24,** 273–80 (2006).
320. Godoy, A. P. O. *et al.* Analysis of antimicrobial susceptibility and virulence factors in Helicobacter pylori clinical isolates. *BMC Gastroenterol.* **3,** 20 (2003).
321. Cheung, J. *et al.* Helicobacter pylori infection in Canada's arctic: Searching for the solutions. *Can. J. Gastroenterol.* **22,** 912–6 (2008).
322. Goodman, K. J., Jacobson, K. & van Zanten, S. V. Helicobacter pylori infection in Canadian and related Arctic Aboriginal populations. *Can. J. Gastroenterol.* **22,** 289–95 (2008).
323. Jones, N. *et al.* Helicobacter pylori in First Nations and recent immigrant populations in Canada. *Can. J. Gastroenterol.* **26,** 97–103 (2012).
324. Bourke, B. *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.* **19,** 399–408 (2005).
325. Graham, D. Y. *et al.* Campylobacter pylori detected noninvasively by the 13C-urea breath test. *Lancet Lond. Engl.* **1,** 1174–7 (1987).
326. Gisbert, J. P. & Pajares, J. M. Review article: 13C-urea breath test in the diagnosis of Helicobacter pylori infection -- a critical review. *Aliment. Pharmacol. Ther.* **20,** 1001–17 (2004).

327. Klein, P. D. *et al.* Normalizing results of 13C-urea breath testing for CO2 production rates in children. *J. Pediatr. Gastroenterol. Nutr.* **29,** 297–301 (1999).
328. Savarino, V., Vigneri, S. & Celle, G. The 13C urea breath test in the diagnosis ofHelicobacter pylori infection. *Gut.* **45,** I18–22 (1999).
329. Government of Alberta. Interactive Health Data Application. (2016)
330. Pearce, N. Effect measures in prevalence studies. *Environ. Health Perspect.* **112,** 1047–50 (2004).
331. Rothman, K. in *Epidemiology: An Introduction* 38–68 (Oxford University Press, 2012).
332. Szklo, M. & Nieto, F. J. in *Epidemiology: Beyond the Basics* 79–106 (Jones & Bartlett Learning, 2014).
333. McNutt, L.-A., Wu, C., Xue, X. & Hafner, J. P. Estimating the relative risk in cohort studies and clinical trials of common outcomes. *Am. J. Epidemiol.* **157,** 940–3 (2003).
334. Zou, G. A Modified Poisson Regression Approach to Prospective Studies with Binary Data. *Am. J. Epidemiol.* **159,** 702–6 (2004).
335. Hosmer, D. W. & Lemeshow, S. *Applied logistic regression. [electronic resource]*. (New York : Wiley, c2000., 2000).
336. Vittinghoff, E., Glidden, D. V., Shiboski, S. C. & McCulloch, P. C. E. in *Regression Methods in Biostatistics* 7–26 (Springer US, 2012).
337. Kleinbaum, D. & Klein, M. *Logistic Regression: A Self-Learning Text. Statistics for Biology and Health*. (Springer, 2002).
338. McCaig, L. F. & Hughes, J. M. Trends in antimicrobial drug prescribing among office-based physicians in the United States. *JAMA.* **273,** 214–9 (1995).
339. Parkinson, A. J., Bruce, M. G. & Zulz, T. International Circumpolar Surveillance, an Arctic network for the surveillance of infectious diseases. *Emerg. Infect. Dis.* **14,** 18–24 (2008).
340. Banatvala, N. *et al.* High prevalence of Helicobacter pylori metronidazole resistance in migrants to east London: relation with previous nitroimidazole exposure and gastroduodenal disease. *Gut.* **35,** 1562–6 (1994).

341. Boyanova, L. *et al.* Numerous risk factors for Helicobacter pylori antibiotic resistance revealed by extended anamnesis: a Bulgarian study. *J. Med. Microbiol.* **61,** 85–93 (2012).
342. Fallone, C. A. Epidemiology of the antibiotic resistance of Helicobacter pylori in Canada. *Can. J. Gastroenterol.* **14,** 879–82 (2000).
343. Duck, W. M. *et al.* Antimicrobial resistance incidence and risk factors among Helicobacter pylori-infected persons, United States. *Emerg. Infect. Dis.* **10,** 1088–94 (2004).
344. Torres, J. *et al.* Increasing multidrug resistance in Helicobacter pylori strains isolated from children and adults in Mexico. *J. Clin. Microbiol.* **39,** 2677–80 (2001).
345. Wheeldon, T.-U. *et al.* The importance of the level of metronidazole resistance for the success of Helicobacter pylori eradication. *Aliment. Pharmacol. Ther.* **19,** 1315–21 (2004).
346. Hu, C.-T. *et al.* Resistance rate to antibiotics of Helicobacter pylori isolates in eastern Taiwan. *J. Gastroenterol. Hepatol.* **22,** 720–3 (2007).
347. Mendonca, S. *et al.* Prevalence of Helicobacter pylori resistance to metronidazole, clarithromycin, amoxicillin, tetracycline, and furazolidone in Brazil. *Helicobacter.* **5,** 79–83 (2000).
348. Kim, J. J. *et al.* Analysis of metronidazole, clarithromycin and tetracycline resistance of Helicobacter pylori isolates from Korea. *J. Antimicrob. Chemother.* **47,** 459–61 (2001).
349. Cattoir V. *et al.* Update on fluoroquinolone resistance in Helicobacter pylori: new mutations leading to resistance and first description of a gyrA polymorphism associated with hypersusceptibility. *Int. J. Antimicrob. Agents* **29,** 389–96 (2007).
350. Bogaerts P., Berhin C., Nizet H. & Glupczynski Y. Prevalence and mechanisms of resistance to fluoroquinolones in Helicobacter pylori strains from patients living in Belgium. *Helicobacter.* **11,** 441–5 (2006).
351. Glocker E., Stueger H.-P. & Kist M. Quinolone resistance in Helicobacter pylori isolates in Germany. *Antimicrob. Agents Chemother.* **51,** 346–9 (2007).

352. Poon, S.-K. *et al.* Prevalence of antimicrobial resistance in Helicobacter pylori isolates in Taiwan in relation to consumption of antimicrobial agents. *Int. J. Antimicrob. Agents* **34,** 162–5 (2009).
353. Carothers J.J. *et al.* The relationship between previous fluoroquinolone use and levofloxacin resistance in Helicobacter pylori infection. *Clin. Infect. Dis.* **44,** e5–8 (2007).
354. Rothman, K. in *Epidemiology: An Introduction* 124–47 (Oxford University Press, 2012).
355. Greenland, S. & Lash, T. L. in *Modern Epidemiology* 345–80 (Lippincott Williams & Wilkins, 2008).
356. Grant, J., Saxinger, L. & Patrick D. Surveillance of Antimicrobial Resistance and Antimicrobial Utilization in Canada: Prepared for the National Collaborating Centre for Infection Diseases. (2014).
357. Centers for Disease Control and Prevention. Antibiotic Resistance Threats in the United States, 2013. (2013).
358. World Health Organization. Antibiotic resistance - a treat to global health security. (2014).
359. European Centre for Disease Prevention and Control. Summary of the latest data on antibiotic resistance in the European Union. (2013).
360. Kardas, P., Devine, S., Golembesky, A. & Roberts, C. A systematic review and meta-analysis of misuse of antibiotic therapies in the community. *Int. J. Antimicrob. Agents.* **26,** 106–13 (2005).
361. Andersson, D. I. & Hughes, D. Antibiotic resistance and its cost: is it possible to reverse resistance? *Nat. Rev. Microbiol.* **8,** 260–71 (2010).
362. Norris, P. *et al.* Public Beliefs about Antibiotics, Infection and Resistance: A Qualitative Study. *Antibiotics.* **2,** 465–76 (2013).
363. Branthwaite, A. & Pechère, J. C. Pan-European survey of patients' attitudes to antibiotics and antibiotic use. *J. Int. Med. Res.* **24,** 229–38 (1996).
364. Chandy, S. J. *et al.* Antibiotic use and resistance: perceptions and ethical challenges among doctors, pharmacists and the public in Vellore, South India. *J. Med. Ethics.* **10,** 20–7 (2013).

365. Grigoryan, L. *et al.* Attitudes, beliefs and knowledge concerning antibiotic use and self-medication: a comparative European study. *Pharmacoepidemiol. Drug Saf.* **16,** 1234–43 (2007).
366. Corbett, K. K. *et al.* Appropriate antibiotic use: variation in knowledge and awareness by Hispanic ethnicity and language. *Prev. Med.* **40,** 162–9 (2005).
367. Cummings, K. C., Rosenberg, J. & Vugia, D. J. Beliefs about Appropriate Antibacterial Therapy, California. *Emerg. Infect. Dis.* **11,** 1138–41 (2005).
368. Belongia, E. A., Naimi, T. S., Gale, C. M. & Besser, R. E. Antibiotic use and upper respiratory infections: a survey of knowledge, attitudes, and experience in Wisconsin and Minnesota. *Prev. Med.* **34,** 346–52 (2002).
369. Vanden Eng, J. *et al.* Consumer Attitudes and Use of Antibiotics. *Emerg. Infect. Dis.* **9,** 1128–35 (2003).
370. Wilson, A. A., Crane, L. A., Barrett, P. H. & Gonzales, R. Public beliefs and use of antibiotics for acute respiratory illness. *J. Gen. Intern. Med.* **14,** 658–62 (1999).
371. Brookes-Howell, L. *et al.* 'The body gets used to them': patients' interpretations of antibiotic resistance and the implications for containment strategies. *J. Gen. Intern. Med.* **27,** 766–72 (2012).
372. Hawkings, N. J., Wood, F. & Butler, C. C. Public attitudes towards bacterial resistance: a qualitative study. *J. Antimicrob. Chemother.* **59,** 1155–60 (2007).
373. Cals, J. W. L. *et al.* Public beliefs on antibiotics and respiratory tract infections: an internet-based questionnaire study. *Br. J. Gen. Pract.* **57,** 942–7 (2007).
374. Wun, Y. T., Lam, T. P., Lam, K. F., Ho, P. L. & Yung, W. H. R. The public's perspectives on antibiotic resistance and abuse among Chinese in Hong Kong. *Pharmacoepidemiol. Drug Saf.* **22,** 241–9 (2013).
375. McNulty, C. A. M., Nichols, T., Boyle, P. J., Woodhead, M. & Davey, P. The English antibiotic awareness campaigns: did they change the public's knowledge of and attitudes to antibiotic use? *J. Antimicrob. Chemother.* **65,** 1526–33 (2010).
376. Pechère, J. C. Patients' interviews and misuse of antibiotics. *Clin. Infect. Dis.* **33 Suppl 3,** S170–3 (2001).
377. Hawkings, N. J., Butler, C. C. & Wood, F. Antibiotics in the community: a typology of user behaviours. *Patient Educ. Couns.* **73,** 146–52 (2008).

378. Radosevic, N. *et al.* Attitudes towards antimicrobial drugs among general population in Croatia, Fyrom, Greece, Hungary, Serbia and Slovenia. *Pharmacoepidemiol. Drug Saf.* **18,** 691–6 (2009).
379. Dang, Y., Reinhardt, J. D., Zhou, X. & Zhang, G. The effect of probiotics supplementation on Helicobacter pylori eradication rates and side effects during eradication therapy: a meta-analysis. *PloS One.* **9,** e111030 (2014).
380. Bernard, H. R. *Research Methods in Anthropology: Qualitative and Quantitative Approaches*. (Lanham, MD : AltaMira, c2011., 2011).
381. Braun, V. & Clarke, V. Using thematic analysis in psychology. *Qual. Res. Psychol.* **3,** 77–101 (2006).

Appendix A1

Date: Day __ __ Month __ __ Year __ __ __ __

Antibiotic Chart Review Form – *H. pylori* project

ID number: __ __ __ -__ __

Community: _____

Participant Name: _____ Performed by: _____

Date of Birth: Day __ __ Month __ __ Year __ __ __ __ Sex: ❑ Male ❑ Female

1. Period of Review: Date of Clinical Survey (dd/mm/yyyy)_____ 5 years earlier (dd/mm/yyyy): _____

Antibiotics prescribed over the past 5 years:

2.	Date (dd/mm/yyyy)	Metronidazole (Flagyl)	Clarithromycin (Biaxin)	Amoxicillin	Tetracycline	Ciprofloxacin	Nitrofurantoin	Rifampicin	Levofloxacin	Other (antibiotic prescribed at the same time):	Reason for Prescription	Duration	Dose	Prescribed at Local Health Centre?		If no, where?
														Yes	No	
a.		❑	❑	❑	❑	❑	❑	❑	❑	❑_____				❑	❑	
b.		❑	❑	❑	❑	❑	❑	❑	❑	❑_____				❑	❑	
c.		❑	❑	❑	❑	❑	❑	❑	❑	❑_____				❑	❑	
d.		❑	❑	❑	❑	❑	❑	❑	❑	❑_____				❑	❑	
e.		❑	❑	❑	❑	❑	❑	❑	❑	❑_____				❑	❑	
f.		❑	❑	❑	❑	❑	❑	❑	❑	❑_____				❑	❑	
g.		❑	❑	❑	❑	❑	❑	❑	❑	❑_____				❑	❑	
h.		❑	❑	❑	❑	❑	❑	❑	❑	❑_____				❑	❑	
i.		❑	❑	❑	❑	❑	❑	❑	❑	❑_____				❑	❑	

Note: An answer of "None" does not indicate that the answer is not true of the participants medical history, but that it was not apparent in the medical chart from their community health centre/nursing station. Information from their medical visits to regional hospitals are not likely to be present in these charts.

Appendix A1

Date: Day __ __ Month __ __ Year __ __ __ __ **Antibiotic Chart Review Form – *H. pylori* project** ID number: __ __ __ -__ __

Community: _____

j.		❑	❑	❑	❑	❑	❑	❑	❑	❑_____				❑	❑	
k.		❑	❑	❑	❑	❑	❑	❑	❑	❑_____				❑	❑	
l.		❑	❑	❑	❑	❑	❑	❑	❑	❑_____				❑	❑	
❑ NONE																

3.	**HP-PAC** or **H-PAC** prescribed over the past 5 years:			Prescribed at Local Health Centre?		If no, where?
	Date (dd/mm/yyyy)	Details specified about regimen (ex. antibiotics prescribed, dose, ect.):	Duration or Number of Packs Prescribed:	Yes	No	
a.				❑	❑	
b.				❑	❑	
c.				❑	❑	
d.				❑	❑	
e.				❑	❑	
❑ NONE						

Note: An answer of "None" does not indicate that the answer is not true of the participants medical history, but that it was not apparent in the medical chart from their community health centre/nursing station. Information from their medical visits to regional hospitals are not likely to be present in these charts.

Appendix A1

Date: Day __ __ Month __ __ Year __ __ __ __

Antibiotic Chart Review Form – *H. pylori* project

ID number: __ __ __ -__ __

Community: _____

4.	Other Antibiotics prescribed over the past 5 years:					Prescribed at Local Health Centre?		If no, where?
	Date (dd/mm/yyyy)	Antibiotic(s) Prescribed:	Reason for Prescription:	Duration	Dose	Yes	No	
a.						❑	❑	
b.						❑	❑	
c.						❑	❑	
d.						❑	❑	
e.						❑	❑	
f.						❑	❑	
g.						❑	❑	
h.						❑	❑	
i.						❑	❑	
j.						❑	❑	
k.						❑	❑	
l.						❑	❑	
❑ NONE								

Note: An answer of “None” does not indicate that the answer is not true of the participants medical history, but that it was not apparent in the medical chart from their community health centre/nursing station. Information from their medical visits to regional hospitals are not likely to be present in these charts.

Appendix A1

Date: Day __ __ Month __ __ Year __ __ __ __ **Antibiotic Chart Review Form – *H. pylori* project** ID number: __ __ __ -__ __

Community: _____

5.	Known Drug Allergies	Date Reported
a.		
b.		
c.		
d.		
e.		
❑ NONE		

Note: An answer of "None" does not indicate that the answer is not true of the participants medical history, but that it was not apparent in the medical chart from their community health centre/nursing station. Information from their medical visits to regional hospitals are not likely to be present in these charts.

Appendix A2

Strategies Used to Reduce Missing Data

Ethnicity data was collected by the interviewer-administered participant questionnaire completed during enrolment in a CAN*Help* community project. If the participant questionnaire was not completed, ethnicity was ascertained from data collected from CAN*Help* project medical chart reviews. In the event that ethnicity data was missing in the CAN*Help* chart review database, an individual's ethnicity was inferred from the known ethnicity of family members when possible.

Individual income data was collected by the interviewer-administered participant questionnaire at the time of enrolment in a CAN*Help* community project and household income data was collected by the interviewer-administered household questionnaire. However, data on individual income was missing more frequently than data on household income across CAN*Help* community projects. Therefore, household income was substituted for individual income as an indicator for socio-economic status (SES) for this analysis. Depending on the age of the individual and the length of time they have been a member of a given household, household income may be a better indicator of an individual's SES for a longer duration of an individual's lifetime compared to their own income level, so household income may be a better SES indicator for this analysis.

Data regarding treatment with antibiotics to eliminate *H. pylori* infection prior to enrolment in a CAN*Help* community project was collected by the interviewer-administered clinical questionnaire completed at the time of enrolment. For individuals who were missing data on previous exposure to an anti-*H. pylori* treatment regimen, missing values were replaced by information collected from the antibiotic chart reviews when available. As a component of the antibiotic chart reviews, I collected data from participant medical charts regarding whether antibiotics were prescribed to eliminate *H. pylori* infection during the 5-year review period.

Appendix A3

Sensitivity Analysis of Exposure Variable Categorization

Table A3.1. Prevalence of antibiotic-resistant *H. pylori* infection by antibiotic exposure level among 169 *H. pylori*-positive individuals who had samples of *H. pylori* tested for antibiotic susceptibility and who had complete data for selected model covariates.

Number of Antibiotic Prescriptions During the 5-year Review Period	**n**	**Resistance Prevalence**	
		%	**95% CI**
Four Categories			
0-1	45	33	20, 49
2-4	69	42	30, 55
5-7	28	46	28, 66
8-17	27	52	32, 71
Three Categories			
0-1	45	33	20, 49
2-4	69	42	30, 55
5-17	55	49	35, 63
Alternative Exposure Variable Categorization (used for sensitivity analysis)			
Four Categories			
0	30	37	20, 56
1-3	65	42	29, 54
4-7	47	40	26, 56
8-17	27	52	32, 71
Three Categories			
0	30	37	20, 56
1-3	65	42	29, 54
4-17	74	45	33, 57

Appendix A3

Table A3.2. Effect of antibiotic exposure on the prevalence odds of antibiotic-resistant *H. pylori* infection among 169 *H. pylori*-positive individuals who had samples of *H. pylori* tested for antibiotic susceptibility: Results of multivariable logistic regression analysis comparing two categorical definitions of the exposure variable of interest.

	Model 2*			**Model 3***	
Variable	**OR**	**95% CI**	**Variable**	**OR**	**95% CI**
Antibiotic Exposure			**Antibiotic Exposure**		
0-1 Prescriptions	**1.0**	-	0 Prescriptions	**1.0**	-
2-4 Prescriptions	**1.5**	0.62, 3.4	1-3 Prescriptions	**1.3**	0.48, 3.4
5-17 Prescriptions	**1.4**	0.52, 3.8	4-17 Prescriptions	**0.92**	0.33, 2.5
Age (in years)			**Age**		
in females	**1.04**	1.01, 1.07	in females	**1.04**	1.01, 1.07
in males	**0.996**	0.967, 1.03	in males	**0.994**	0.965, 1.02
Treatment for *H. pylori* in Previous 5 Years			**Treatment for *H. pylori* in Previous 5 Years**		
None Recorded in Chart	**1.0**	-	None Recorded in Chart	**1.0**	-
One or More Recorded in Chart	**2.1**	0.85, 5.3	One or More Recorded in Chart	**2.6**	1.1, 6.2
Community			**Community**		
Aklavik	**1.0**	-	Aklavik	**1.0**	-
Old Crow	**1.5**	0.66, 3.4	Old Crow	**1.5**	0.66, 3.4
Fort McPherson	**4.0**	1.4, 11	Fort McPherson	**3.8**	1.3, 11

***Models 2 & 3** included age (continuous), sex, previous exposure to an anti-*H. pylori* treatment regimen, community, and a product term for age and sex.

A lower p-value of 0.67 was obtained from the LR test for adding the 3-category exposure variable categorized such that 0-1 is the reference group (0-1, 2-4, 5-17 antibiotic prescriptions) to the regression model with selected covariates than the LR test for adding the 3-category exposure variable categorized such that 0 is the reference group (0, 1-3, 4-17 antibiotic prescriptions), for which the p-value was 0.69.

Appendix A4

Antibiotic Exposure for each Antibiotic Class and Specific Antibiotic Drug by the Antibiotic Susceptibility of H. pylori Infection to the Corresponding Antibiotic Drug

Table A4.1. Prevalence of metronidazole-resistant *H. pylori* infection by nitroimidazole and metronidazole exposure among 191 *H. pylori*-positive individuals who had samples of *H. pylori* tested for metronidazole susceptibility.

	n	Prevalence of Metronidazole Resistance	
		%	95% CI
Nitroimidazole Exposure*			
1-4 Prescriptions	38	55	38, 71
0 Prescriptions	153	31	24, 39
Metronidazole Exposure			
1-4 Prescriptions	38	55	38, 71
0 Prescriptions	153	31	24, 39

*Nitroimidazole antibiotic class only consists of metronidazole prescriptions.

Table A4.2. Prevalence of clarithromycin-resistant *H. pylori* infection by macrolide and clarithromycin exposure among 191 *H. pylori*-positive individuals who had samples of *H. pylori* tested for clarithromycin susceptibility.

	n	Prevalence of Clarithromycin Resistance	
		%	95% CI
Macrolide Exposure			
1-4 Prescriptions	65	18	10, 30
0 Prescriptions	126	15	9, 23
Clarithromycin Exposure			
1 Prescription	4	0	-
0 Prescriptions	187	17	12, 23

Table A4.3. Prevalence of amoxicillin-resistant *H. pylori* infection by β-lactam and amoxicillin exposure among 191 *H. pylori*-positive individuals who had samples of *H. pylori* tested for amoxicillin susceptibility.

	n	Prevalence of Amoxicillin Resistance	
		%	95% CI
β-lactam Exposure			
1-10 Prescriptions	109	0	-
0 Prescriptions	82	0	-
Amoxicillin Exposure			
1-8 Prescriptions	52	0	-
0 Prescriptions	139	0	-

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Table A4.4. Prevalence of tetracycline-resistant *H. pylori* infection by tetracycline (class) and tetracycline (specific drug) among 191 *H. pylori*-positive individuals who had samples of *H. pylori* tested for tetracycline susceptibility.

	n	Prevalence of Tetracycline Resistance	
		%	95% CI
Tetracycline Exposure (Class)			
1-3 Prescriptions	8	13	0.3, 53
0 Prescriptions	183	0	-
Tetracycline Exposure (Specific Drug)			
0 Prescriptions	191	0.5	0.01, 3

Table A4.5. Prevalence of ciprofloxacin-resistant *H. pylori* infection by fluoroquinolone and ciprofloxacin exposure among 191 *H. pylori*-positive individuals who had samples of *H. pylori* tested for ciprofloxacin susceptibility.

	n	Prevalence of Ciprofloxacin Resistance	
		%	95% CI
Fluoroquinolone Exposure*			
1-3 Prescriptions	19	21	6, 46
0 Prescriptions	172	2	0.6, 6
Ciprofloxacin Exposure			
1-4 Prescriptions	19	21	6, 46
0 Prescriptions	172	2	0.6, 6

*Fluoroquinolone antibiotic class only consists of ciprofloxacin prescriptions.

Table A4.6. Prevalence of nitrofurantoin-resistant *H. pylori* infection by nitrofuran and nitrofurantoin exposure among 191 *H. pylori*-positive individuals who had samples of *H. pylori* tested for nitrofurantoin susceptibility.

	n	Prevalence of Nitrofurantoin Resistance	
		%	95% CI
Nitrofuran Exposure*			
1-6 Prescriptions	13	0	-
0 Prescriptions	178	1	0.1, 4
Nitrofurantoin Exposure			
1-6 Prescriptions	13	0	-
0 Prescriptions	178	1	0.1, 4

*Nitrofuran antibiotic class only consists of nitrofurantoin prescriptions.

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Table BA4.7. Prevalence of rifampicin-resistant *H. pylori* infection by rifamycin and rifampicin exposure among 191 *H. pylori*-positive individuals who had samples of *H. pylori* tested for rifampicin susceptibility.

	n	Prevalence of Rifampicin Resistance	
		%	95% CI
Rifamycin Exposure			
1 Prescriptions	11	0	-
0 Prescriptions	190	1	0.1, 4
Rifampicin Exposure			
1 Prescriptions	1	0	-
0 Prescriptions	190	1	0.1, 4

Appendix A5

Risk of Anti-H. pylori Treatment Failure by Exposure to Antibiotic Class and Specific Antibiotic Drug

Table A5.1. Incidence of sequential therapy failure by exposure to antibiotic class among 93 *H. pylori*-positive individuals treated with sequential therapy who completed a post-treatment UBT.

Exposure by Antibiotic Class	n	Risk of Sequential Therapy Treatment Failure (Incidence Proportion)	
		%	95% CI
Nitroimidazoles			
1-7 Prescriptions	20	40	19, 64
0 Prescriptions	73	22	13, 33
Macrolides			
1-3 Prescriptions	26	19	7, 29
0 Prescriptions	67	28	18, 41
β-lactams			
1-7 Prescriptions	58	28	17, 41
0 Prescriptions	35	23	10, 40
Tetracyclines			
1-3 Prescriptions	5	40	5, 85
0 Prescriptions	88	25	16, 35
Fluoroquinolones			
1-3 Prescriptions	7	57	18, 90
0 Prescriptions	86	23	15, 34
Nitrofurans			
1-6 Prescriptions	6	33	4, 78
0 Prescriptions	87	25	17, 36
Rifamycins			
0 Prescriptions	93	26	17, 36

Table A5.2. Incidence of sequential therapy failure by specific antibiotic drug among 93 *H. pylori*-positive individuals treated with sequential therapy who completed a post-treatment UBT.

Exposure by Specific Antibiotic Drug	n	Risk of Sequential Therapy Treatment Failure (Incidence Proportion)	
		%	95% CI
Metronidazole			
1-7 Prescriptions	20	40	19, 64
0 Prescriptions	73	22	13, 33
Clarithromycin			
0 Prescriptions	93	26	17, 36

Amoxicillin			
1-5 Prescriptions	20	40	19, 64
0 Prescriptions	73	22	13, 33
Tetracycline			
0 Prescriptions	93	26	17, 36
Ciprofloxacin			
1-3 Prescriptions	7	57	18, 90
0 Prescriptions	86	23	15, 34
Levofloxacin			
0 Prescriptions	93	26	17, 36
Nitrofurantoin			
1-6 Prescriptions	6	33	4, 78
0 Prescriptions	87	25	17, 36
Rifampicin			
0 Prescriptions	93	26	17, 36

Table A5.3. Incidence of triple therapy failure by exposure to antibiotic class among 53 *H. pylori*-positive individuals who were treated with standard triple therapy and who completed a post-treatment UBT.

Exposure by Antibiotic Class	**n**	**Risk of Triple Therapy Treatment Failure (Incidence Proportion)**	
		%	**95% CI**
Nitroimidazoles			
1-2 Prescriptions	10	10	0.3, 45
0 Prescriptions	43	47	31, 62
Macrolides			
1-3 Prescriptions	21	48	26, 70
0 Prescriptions	32	34	19, 53
β-lactams			
1-9 Prescriptions	39	41	26, 58
0 Prescriptions	14	36	13, 65
Tetracyclines			
1 Prescriptions	1	0	-
0 Prescriptions	52	40	27, 55
Fluoroquinolones			
1 Prescriptions	3	33	0.8, 91
0 Prescriptions	50	40	26, 55
Nitrofurans			
1-4 Prescriptions	2	50	1, 99
0 Prescriptions	51	39	26, 54
Rifamycins			
0 Prescriptions	53	40	26, 54

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Table A5.4. Incidence of triple therapy failure by exposure to specific antibiotic drugs among 53 *H. pylori*-positive individuals who were treated with standard triple therapy and who completed a post-treatment UBT.

Exposure by Specific Antibiotic Drug	n	Risk of Triple Therapy Treatment Failure (Incidence Proportion)	
		%	95% CI
Metronidazole			
1-2 Prescriptions	10	10	0.3, 45
0 Prescriptions	43	47	31, 62
Clarithromycin			
1 Prescriptions	2	0	-
0 Prescriptions	51	41	28, 56
Amoxicillin			
1-8 Prescriptions	14	43	18, 71
0 Prescriptions	39	38	23, 55
Tetracycline			
0 Prescriptions	53	40	26, 54
Ciprofloxacin			
1 Prescriptions	3	33	0.8, 91
0 Prescriptions	50	40	26, 55
Levofloxacin			
0 Prescriptions	53	40	26, 54
Nitrofurantoin			
1-3 Prescriptions	2	50	0.1, 99
0 Prescriptions	51	39	26, 54
Rifampicin			
0 Prescriptions	53	40	26, 54

Table A5.5. Incidence of quadruple therapy failure by exposure to antibiotic class among 54 *H. pylori*-positive individuals who were treated with bismuth-based quadruple therapy and who completed a post-treatment UBT.

Exposure by Antibiotic Class	n	Risk of Quadruple Therapy Treatment Failure (Incidence Proportion)	
		%	95% CI
Nitroimidazoles			
1-4 Prescriptions	18	17	4, 41
0 Prescriptions	36	8	2, 22
Macrolides			
1-5 Prescriptions	20	25	9, 49
0 Prescriptions	34	3	0.07, 15
β-lactams			
1-8 Prescriptions	34	9	2, 24
0 Prescriptions	20	15	3, 38

Tetracyclines			
1-3 Prescriptions	7	14	0.4, 58
0 Prescriptions	47	11	4, 23
Fluoroquinolones			
1-6 Prescriptions	7	14	0.4, 58
0 Prescriptions	47	11	4, 23
Nitrofurans			
1-4 Prescriptions	6	50	12, 88
0 Prescriptions	48	6	1, 17
Rifamycins			
0 Prescriptions	54	11	4, 23

Table A5.6. Incidence of quadruple therapy failure by exposure to specific antibiotic drugs among 54 *H. pylori*-positive individuals who were treated with bismuth-based quadruple therapy and who completed a post-treatment UBT.

Exposure by Specific Antibiotic Drug	**n**	**Risk of Quadruple Therapy Treatment Failure (Incidence Proportion)**	
		%	**95% CI**
Metronidazole			
1-4 Prescriptions	18	17	4, 41
0 Prescriptions	36	8	2, 22
Clarithromycin			
1 Prescriptions	3	33	0.8, 91
0 Prescriptions	51	10	3, 21
Amoxicillin			
1-7 Prescriptions	18	17	4, 41
0 Prescriptions	36	8	2, 22
Tetracycline			
0 Prescriptions	54	11	4, 23
Ciprofloxacin			
1-6 Prescriptions	7	14	0.4, 58
0 Prescriptions	47	11	4, 23
Levofloxacin			
0 Prescriptions	54	11	4, 23
Nitrofurantoin			
1-4 Prescriptions	6	50	12, 88
0 Prescriptions	48	6	0.1, 17
Rifampicin			
0 Prescriptions	54	11	4, 23

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Table A5.7. Incidence of treatment failure by exposure to antibiotic class for among 179 *H. pylori*-positive individuals who were treated with sequential therapy, standard triple therapy, or bismuth-based quadruple therapy, who completed a post-treatment UBT and who had complete data for all selected confounding variables.

Exposure by Antibiotic Class	n	Risk of Treatment Failure (Incidence Proportion)	
		%	95% CI
Nitroimidazoles			
1-7 Prescriptions	42	21	10, 37
0 Prescriptions	137	28	20, 36
Macrolides			
1-5 Prescriptions	61	33	21, 46
0 Prescriptions	118	23	16, 32
β-lactams			
1-9 Prescriptions	116	28	20, 37
0 Prescriptions	63	24	14, 36
Tetracyclines			
1-3 Prescriptions	12	25	0.5, 57
0 Prescriptions	167	26	20, 34
Fluoroquinolones			
1-6 Prescriptions	14	29	8, 58
0 Prescriptions	165	26	20, 33
Nitrofurans			
1-6 Prescriptions	13	46	19, 75
0 Prescriptions	166	25	18, 32
Rifamycins			
0 Prescriptions	179	26	20, 33

Table A5.8. Incidence of treatment failure by exposure to specific antibiotic drugs among 179 *H. pylori*-positive individuals who were treated with sequential therapy, standard triple therapy, or quadruple therapy, who completed a post-treatment UBT and who had complete data for all selected confounding variables.

Exposure to Specific Antibiotic Drug	n	Risk of Treatment Failure (Incidence Proportion)	
		%	95% CI
Metronidazole			
1-7 Prescriptions	42	21	10, 37
0 Prescriptions	137	28	20, 36
Clarithromycin			
1 Prescriptions	5	20	0.5, 72
0 Prescriptions	174	26	20, 34
Amoxicillin			
1-8 Prescriptions	47	32	19, 47
0 Prescriptions	132	24	17, 32

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Tetracycline			
0 Prescriptions	179	26	20, 33
Ciprofloxacin			
1-6 Prescriptions	14	29	8, 58
0 Prescriptions	165	26	20, 33
Levofloxacin			
0 Prescriptions	179	26	20, 33
Nitrofurantoin			
1-6 Prescriptions	13	46	19, 75
0 Prescriptions	166	25	18, 32
Rifampicin			
0 Prescriptions	179	26	20, 33

Appendix A6

Incidence Proportion of Anti*-H. pylori *Treatment Failure by Antibiotic Susceptibility to Specific Antibiotic Drugs

Table A6.1. Incidence of anti-*H. pylori* treatment failure by antibiotic susceptibility to specific antibiotic drugs among 114 *H. pylori*-positive individuals treated with standard triple therapy, sequential therapy, or bismuth-based quadruple therapy who had samples of *H. pylori* tested for antibiotic susceptibility.

Susceptibility by Specific Antibiotic Drug	n	Risk of Treatment Failure (Incidence Proportion)	
		%	95% CI
Metronidazole			
Susceptible	75	31	21, 42
Resistant to ≥1 antibiotic	39	28	15, 45
Clarithromycin			
Susceptible	95	27	19, 37
Resistant to ≥1 antibiotic	19	42	20, 67
Amoxicillin			
Susceptible	114	30	22, 39
Tetracycline			
Susceptible	113	29	21, 39
Resistant to ≥1 antibiotic	1	100	3, 100*
Ciprofloxacin			
Susceptible	112	29	21, 39
Resistant to ≥1 antibiotic	2	50	2, 99
Nitrofurantoin			
Susceptible	113	30	22, 39
Resistant to ≥1 antibiotic	1	0	-
Rifampicin			
Susceptible	113	30	22, 39
Resistant to ≥1 antibiotic	1	0	-

One-sided, 97.5% CI

Table A6.2. Incidence of anti-*H. pylori* treatment failure by antibiotic susceptibility to specific antibiotic drugs among 26 *H. pylori*-positive individuals treated with standard triple therapy who had samples of *H. pylori* tested for antibiotic susceptibility.

Susceptibility by Specific Antibiotic Drug	n	Risk of Triple Therapy Treatment Failure (Incidence Proportion)	
Metronidazole		%	95% CI
Susceptible	18	50	26, 74
Resistant	8	50	16, 84
Clarithromycin			
Susceptible	25	48	28, 69
Resistant	1	100	3, 100*
Amoxicillin			

Susceptible	26	50	30, 70
Tetracycline			
Susceptible	26	50	30, 70
Ciprofloxacin			
Susceptible	26	50	30, 70
Nitrofurantoin			
Susceptible	26	50	30, 70
Rifampicin			
Susceptible	26	50	30, 70

*One-sided, 97.5% CI

Table A6.3. Incidence of anti-*H. pylori* treatment failure by antibiotic susceptibility to specific antibiotic drugs among 48 *H. pylori*-positive individuals treated with sequential therapy who had samples of *H. pylori* tested for antibiotic susceptibility.

Susceptibility by Specific Antibiotic Drug	**n**	**Risk of Sequential Therapy Treatment Failure (Incidence Proportion)**	
		%	**95% CI**
Metronidazole			
Susceptible	29	38	21, 58
Resistant	19	26	9, 51
Clarithromycin			
Susceptible	39	28	15, 45
Resistant	9	56	21, 86
Amoxicillin			
Susceptible	48	33	20, 48
Tetracycline			
Susceptible	47	32	19, 47
Resistant	1	100	3, 100*
Ciprofloxacin			
Susceptible	46	33	20, 48
Resistant	2	50	2, 99
Nitrofurantoin			
Susceptible	47	34	21, 49
Resistant	1	0	-
Rifampicin			
Susceptible	47	34	21, 49
Resistant	1	0	-

*One-sided, 97.5% CI

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Table A6.4. Incidence of anti-*H. pylori* treatment failure by antibiotic susceptibility to specific antibiotic drugs among 36 *H. pylori*-positive individuals treated with quadruple therapy who had samples of *H. pylori* tested for antibiotic susceptibility.

Resistance by Specific Antibiotic Drug	**n**	**Risk of Quadruple Therapy Treatment Failure (Incidence Proportion)**	
		%	**95% CI**
Metronidazole			
Susceptible	24	8	1, 27
Resistant	12	17	2, 48
Clarithromycin			
Susceptible	27	7	0.9, 24
Resistant	9	22	3, 60
Amoxicillin			
Susceptible	36	11	3, 26
Tetracycline			
Susceptible	36	11	3, 26
Ciprofloxacin			
Susceptible	36	11	3, 26
Nitrofurantoin			
Susceptible	36	11	3, 26
Rifampicin			
Susceptible	36	11	3, 26

Appendix A7

Distribution of Selected Variables Among Individuals Included in Analysis 1 and/or Analysis 2, and Among All* H. pylori*-positive Individuals Enrolled in CAN*Help Community Projects

Table A7.1. Distribution of select variables across study participants included in Analysis 1 and Analysis 2 compared to all *H. pylori*-positive individuals enrolled across CAN*Help* community projects.

Variables	**Analysis 1 n (%) Total = 169**	**Analysis 2 n (%) Total = 179**	**All *H. pylori*(+) n (%) *Total = 559**
Age Range in Years [Mean]	11-80 [42]	12-78 [44]	1-92 [40]
<35 Years	66 (39)	56 (31)	232 (42)
35-54 Years	61 (36)	68 (38)	181 (32)
>=55 Years	42 (25)	55 (31)	146 (26)
Sex			
Female	89 (53)	94 (53)	295 (53)
Male	80 (47)	85 (47)	264 (47)
Education			
Less than Grade 12	95 (56)	92 (51)	262 (47)
Grade 12 or Equivalent	33 (20)	33 (18)	84 (15)
Certificate/Diploma/Degree	38 (22)	54 (30)	136 (24)
Missing Data	3 (2)	0 (0)	77 (14)
Ethnicity			
Gwich'in	86 (51)	91 (51)	254 (45)
Inuvialuit	67 (40)	68 (38)	177 (32)
Other Aboriginal	9 (5)	14 (9)	34 (6)
Non-Aboriginal	4 (2)	6 (3)	16 (3)
Missing Data	3 (2)	0 (0)	78 (14)
Household Income			
High	63 (37)	65 (36)	156 (28)
Medium	32 (19)	33 (18)	96 (17)
Low & Very Low	39 (23)	46 (26)	118 (21)
Missing Data	35 (21)	35 (20)	189 (34)
Community			
Aklavik	106 (63)	90 (50)	232 (42)
Old Crow	39 (23)	36 (20)	135 (24)
Fort McPherson	24 (14)	42 (23)	133 (24)
Tuktoyaktuk	0 (0)	11 (6)	59 (11)
Previously Treated for *H. pylori*			
None	132 (78)	141 (79)	392 (70)
One or More	37 (22)	38 (21)	62 (11)
Missing Data	0 (0)	0 (0)	105 (19)

*CAN*Help* project participants across all community projects who are *H. pylori*-positive by all methods (using the HP classification algorithm).

Appendix B

Qualitative Questionnaire for Antibiotic Exposure Project

This questionnaire aims to ascertain participants' perspectives on the value and potential harms of using antibiotics to treat infections and on factors that influence adherence to prescribed antibiotic regimens.

Interview Transcript:

We are interviewing people because we want to understand their views on the value, possible harms and problems that people have with taking antibiotics. Antibiotic treatment of bacterial infections, such as *Helicobacter pylori* (*H. pylori*), may require many pills to be taken at specific times throughout the day for many days. Many people are not able to always take their pills as prescribed because:

- Some people get busy and forget to take their pills;
- Some people find it hard to take their pills according to all instructions, such as "with meals," or "on an empty stomach," or "with plenty of fluids";
- Some people skip pills to avoid side effects;
- And some people do not like to take any medicines prescribed by doctors.

Please provide the best answers you can to the following questions, as accurately as you can. There are no right or wrong answers. No one will judge your answers. We just want to know your views on antibiotic treatments and obstacles to taking them. If you do not want to answer a question, please tell me you would like to skip the question, and I will go on to the next question.

1) In your experience, what do people use antibiotics for?

   PROBE: For what type of illnesses would people take antibiotics?

   ☐ Views expressed ☐ No experience / no idea ☐ Skip requested

2) Can you tell me the names of any antibiotics you know about?

   ☐ Yes ☐ No ☐ Skip requested

   PROBE: Can you tell me about some of the antibiotics you or a family member took before? Do you remember the names of those antibiotics?

   ☐ Antibiotics mentioned ☐ Doesn't know/remember ☐ Skip requested

3) Have you ever been prescribed antibiotics in the past?

☐ Yes ☐ No ☐ Skip requested

(If YES), What do you remember doctors/nurses telling you about taking them?

☐ Instructions mentioned ☐ Does not remember ☐ Skip requested

Skip Question 4 if the answer to Q3 is NO.

4) Was there ever a time you didn't finish taking all the antibiotics prescribed to you?

☐ Yes ☐ No ☐ Skip requested

(If YES), Why didn't you finish all of the antibiotics prescribed?

☐ Reasons mentioned ☐ Does not remember ☐ Skip requested

Skip Question 5 if the answer to Q3 is NO.

5) Have you ever experienced side effects during antibiotic treatment?

☐ Yes ☐ No ☐ Skip requested

(If YES), What side effects did you experience?

☐ Side effects mentioned ☐ Does not remember ☐ Skip requested

6) As far as you know, [*if relevant state:* beyond your own experience], what kinds of side effects can people get from taking antibiotics?

PROBE: Has anyone you've known had a bad reaction to antibiotics? If so, what side effects did they experience?

☐ Side effects mentioned ☐ Does not know ☐ Skip requested

7) If a doctor prescribed antibiotics for an illness you had, would you be worried about side effects?

☐ Yes ☐ No ☐ Skip requested

(If YES), What side effects would you be worried about?

☐ Side effects mentioned ☐ No specifics ☐ Skip requested

8) If you were taking antibiotics for an illness, what kinds of side effects would make you stop taking the treatment?

☐ Would not stop ☐ Side effects mentioned ☐ No specifics ☐ Skip requested

9) Do you ever share left over antibiotic pills with other family members or friends when they are ill?

☐ Yes ☐ No ☐ Skip requested

(If YES,) Please tell me about that time.

☐ Experiences mentioned ☐ No specifics ☐ Skip requested

10) Have family members or friends ever shared left over antibiotic pills with you when you weren't feeling well?

☐ Yes ☐ No ☐ Skip requested

(If YES,) Please tell me about that time.

☐ Experiences mentioned ☐ No specifics ☐ Skip requested

11) Have you ever heard about antibiotic resistance?

☐ Yes ☐ No ☐ Skip requested

(If YES), What have you heard about antibiotic resistance?

(If NO), Have you ever heard about antibiotics not curing an infection because the infection is resistant to the drugs?

☐ Experiences mentioned ☐ No specifics ☐ Skip requested

Appendix B

Now I'd like to ask about antibiotics we use to treat *H. pylori* infection.

12) Have you ever been treated for *H. pylori*?

☐ Yes ☐ No ☐ Skip requested

(If YES), What treatment did you get?

(If YES), How did you feel about taking this treatment?

☐ Experience mentioned ☐ No specifics ☐ Skip requested

Skip Questions 13-16 if the answer to Q12 in NO.

13) Was the treatment successful in curing the infection?

☐ Yes ☐ No ☐ Skip requested

14) Did you experience any side-effects or health problems from this treatment?

☐ Yes ☐ No ☐ Skip requested

15) Did you experience any difficulties in taking this treatment?

☐ Yes ☐ No ☐ Skip requested

16) Were there any pills left over after you finished this treatment?

☐ Yes ☐ No ☐ Skip requested

(If YES), Why were there pills left over?

PROBE: (If YES), Were there specific reasons that you were not able to take specific pills?

☐ Experience mentioned ☐ No specifics ☐ Skip requested