Helicobacter Pylori infection; the challenging task of improving eradication rates in light of rising antibiotic resistance, a literature review

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Abstract

Helicobacter pylori (H. pylori) infection continues to be a major public health issue worldwide. A global systematic review shows that in 2015, approximately 4.4 billion individuals worldwide were estimated to be positive for H. pylori. Overwhelming evidence has demonstrated that H. pylori infection is associated with significant medical conditions including peptic ulcer disease, stomach cancer and MALToma. The consensus report from the Kyoto H. pylori conference in 2015 recommended that all H. pylori infections should be eradicated whenever they are found unless there are compelling reasons not to do so. However, increasing H. pylori antibiotic resistance has been reported globally over the past two decades coinciding with a continuous decrease in eradication success rates. To tackle this global problem, we need new antimicrobial drugs and treatment strategies but also a better understanding of the emergence and spread of resistant bacteria as well as improved diagnostic tools to guide clinicians in further optimizing currently available regimens.

Key words: Helicobacter pylori, eradication, antibiotic resistance,

Introduction and Epidemiology

Helicobacter pylori (*H. pylori*) infection continues to be a major public health issue worldwide. A global systematic review shows that in 2015, approximately 4.4 billion individuals worldwide were estimated to be positive for *H. pylori* (1). Infection is more frequent and acquired at an earlier age in resource-limited countries compared with industrialized nations(2). Other studies have also found that the risk of acquiring *H. pylori* infection is related to socioeconomic status and living conditions early in life. Factors such as density of housing, overcrowding, number of siblings, sharing a bed, and lack of running water have all been linked to a higher acquisition rate of *H. pylori* infection(3–6).

The problem of recurrence of H. pylori infection after successful treatment also appears closely associated with socioeconomic and sanitary conditions. In a systematic review and meta-analysis of 132 studies that included 53,934 patient-years, globally; the recurrence rate was inversely related to the human development index and directly related to *H. pylori* prevalence (7).

Microbiology

H. pylori is a spiral shaped, micro-aerophilic, multi-flagellate gram-negative bacterium measuring approximately 3.5 microns in length and 0.5 microns in width(8). It can be biochemically characterized as catalase, oxidase, and urease positive. Urease, produced in abundance making up more than 5 percent of the organism's total protein weight, appears to be vital for its survival and colonization. It is also clinically important because it forms the basis for several invasive and noninvasive tests to diagnose infection. The organism's urease activity, motility, and ability to adhere to gastric epithelium are factors that allow it to survive and

proliferate in the gastric milieu (9). Disruption of urease activity, bacterial mobility, or attachment prevents H. pylori colonization (10). Bacterial urease hydrolyzes gastric luminal urea to form ammonia that helps neutralize gastric acid and form a protective cloud around the organism, enabling it to penetrate the gastric mucus layer (11).

Disease Association

Although overwhelming evidence has demonstrated that *H. pylori* infection is bad for humans, in the past some have questioned the wisdom of eradicating the infection in all those infected. Their arguments are largely based on hypothesis and circumstantial evidence:
1) Less than 20% of all *H. pylori* infected persons will develop significant clinical consequences in their lifetime.
2) *H. pylori* strains are highly diverse at a genetic level and are of different virulence.
3) The antiquity of *H. pylori* infection in humans and their co-evolution suggests that *H. pylori* may be a commensal to humans. Eradication of *H. pylori* may remove some beneficial bacterial strains and may provoke oesophageal disease or gastric cancer at the cardia. However, careful review of the literature confirms that *H. pylori* infection is a serious pathogen albeit in a minority of those infected. It remains for carefully designed prospective studies, rather than hypothesis, to make changes in the current consensus position(3).

Below is an overview of some of the evidence linking H. pylori to clinically significant medical conditions.

Peptic Ulcer disease
Various aspects of *H. pylori* infection suggest strong association with Peptic Ulcer disease; firstly, *H. pylorus* is present in most patients who have a duodenal ulcer that is not related to NSAID use. Early studies noted a high incidence of *H. pylori* infection in patients with duodenal ulcers(12). Subsequent reviews confirmed that *H. pylori* is detectable in 80 to 95 percent of these patients(13,14). These data were supported by reports which found that the prevalence of *H. pylori* is negligible in populations in which ulcer disease is rare (15).

Secondly, *H. pylori* infection is detectable before the occurrence of duodenal ulcers and appears to be a risk factor for the disorder. Several trials have found that pre-existing *H. pylori* infection is a risk factor for the development of duodenal ulcers(16–18).

Thirdly, treatment of *H. pylori i*nfection in patients with duodenal ulcers decreases the incidence of ulcer recurrence. One meta-analysis examined the recurrence rate for duodenal ulcer after at least six months of follow-up. The recurrence rate was 6 percent if *H. pylori* was eradicated and 67 percent if it was not(19). A second meta-analysis found recurrence rates of 20 and 56 percent, respectively(20).

The studies in the meta-analyses used endoscopic findings to define ulcer recurrence. It should be noted that the rate of symptomatic recurrence is lower.

Gastric cancer
The International Agency for Research on Cancer estimates that 36 and 47 percent of all gastric cancers in developed and developing countries, respectively, are solely attributable to *H. pylori* infection. This accounts for almost 350,000 gastric cancers annually worldwide. One report indicated that of the 12.7 million new cancers occurring in 2008, the population attributable fraction due to infections was over 16 percent for *H. pylori*(21).

Two meta-analyses of cohort and case control studies examining the relationship between *H. pylori* sero-positivity and gastric cancer found that *H. pylori* infection was associated with a twofold increased risk for developing gastric adenocarcinoma(22,23).

One of the largest prospective studies addressing *H. pylori* and cancer risk included 1,526 Japanese patients of whom 1,246 had *H. pylori* infection(24). Patients underwent endoscopy with biopsy at enrollment and then again between one and three years after enrollment. During a mean follow-up of 7.8 years, 36 patients developed gastric cancer (2.9 percent), all of whom were *H. pylori* infected. No uninfected patient developed cancer(24).

MALToma
Multiple studies have demonstrated an association between *H. pylori* infection and MALToma, (mucosa (gut)-associated lymphoid tissue tumor) and have begun to elucidate the mechanisms underlying this association(25–31).

The most dramatic evidence supporting a pathogenetic role for *H. pylori* in MALToma is remission of the tumor following eradication of *H. pylori* with antibiotic therapy(32–39).

Rationale for H. Pylori eradication

In the absence of an effective vaccine, treatment of chronic *H. pylori* infection has emerged as the main strategy for reducing the spread of bacteria in the population, for resolving gastric lesions in infected patients, and for preventing subsequent gastric cancer development(40). Furthermore, the consensus report from the Kyoto H. pylori conference in 2015 signaled a fundamental shift in thinking and recommended that all *H. pylori* infections should be eradicated whenever they are found unless there are compelling reasons not to, such as co-morbidities, high re-infection rates in the country and competing health priorities of society(41,42).

Treatment strategies

The American College of Gastroenterology guidelines for *H. Pylori* infection recommend that the choice of initial antibiotic regimen to treat *H. pylori* should be guided by the presence of risk factors for macrolide resistance and the presence of a penicillin allergy(43). A resistance threshold of ≥15 percent in the community is commonly used for choosing alternative empiric antibiotic regimen for *H. pylori*(44,45).

Clarithromycin triple therapy consisting of a PPI, Clarithromycin, and Amoxicillin or Metronidazole for 14 days remains a recommended treatment option in regions where *H. pylori* Clarithromycin resistance is known to be <15% and in patients with no previous history of macrolide exposure for any reason(43). In regions where the rate of Clarithromycin resistance is known to be high or if a patient has previously been treated with macrolides for any reason, Bismuth quadruple therapy should be strongly considered as the initial treatment choice(43).

Unlike Clarithromycin triple therapy, the efficacy of Bismuth quadruple therapy is not affected by Clarithromycin resistance. Also, although Metronidazole resistance does have an impact on the efficacy of Bismuth quadruple therapy, it is not nearly as profound as that of Clarithromycin resistance on Clarithromycin triple therapy(46).

Other regimes have been trialled and suggested like hybrid, sequential and concomitant(47–49).

However, the complexity of the treatment regimen has limited its use as a first-line regimen in the treatment of *H. pylori.*

Role of probiotics

There is growing interest in the United States of probiotics as adjuvant therapy in the treatment of *H. pylori* infection. Emerging evidence suggests an inhibitory effect of Lactobacillus and Bifidobacterium species on *H. pylori*(43). The addition of probiotics to *H. pylori* therapies has been acknowledged as improving eradication rates whilst reducing the adverse effects of regimens and counteracting the harmful effects of antimicrobials on the gut microbiota. In different meta-analyses, compared to events noted with control therapies, probiotics globally improved *H. pylori* eradication rates by >10% while preventing >50% of total drug-related adverse effects and antibiotic-associated diarrhoea(50–52).

A recent meta-analysis of 10 clinical trials of adjuvant probiotics in patients with *H. pylori* infection demonstrated increased cure rates with probiotic supplementation (pooled OR, 2.07; 95% CI, 1.40–3.06) (53). Probiotics also reduced the incidence of total side effects (pooled OR, 0.31; 95% CI, 0.12–0.79).

The issue of antibiotic resistance

Increasing *H. pylori* antibiotic resistance has been reported globally over the past two decades coinciding with a continuous decrease in eradication success rates(54–57).

In a systematic review and meta-analysis that included 178 studies, comprising 66,142 isolates from 65 countries, primary and secondary resistance to Clarithromycin, Metronidazole, and Levofloxacin were high (≥15 percent) in the majority of WHO (World Health Organization) regions. Resistance to Clarithromycin was significantly associated with failure of *H. pylori* eradication with a Clarithromycin-containing regimen (odds ratio, 6.97; 95% CI, 5.2-9.3) (57). Local surveillance data are needed to guide the choice of eradication regimens.

Many studies have shown this rising resistance to have direct impact on the efficacy of the most commonly used eradication regimes. In a meta-analysis, data from 93 studies with 10,178 participants was analysed. For triple therapies, Clarithromycin resistance had a greater effect on treatment efficacy than Nitroimidazole resistance. Drug resistance was a strong predictor of efficacy across triple therapies for the eradication of *H. pylori* in adults(58).

Multiple factors have been reported to play a role in this rapid development of primary antibiotic resistance in *H. pylori*. These include limited choice of effective therapeutics; the extensive use of certain antibiotics in the general population (such as Clarithromycin for respiratory infections); the exceptional adaptation ability of the species(55,59). Thus, since 2017, *H. pylori* has been listed by the WHO among the 20 pathogens that pose the most serious threat to human health because of their drug resistance(60).

The worrying issue of Multi-drug resistance

The alarming presence of *H. pylori* strains with a MDR (Multi-drug resistant) profile, such as simultaneous resistance to three or more drug families, is increasingly noted within the global emergence of antibiotic resistance in the species(61).

Clinical implications of drug resistance

The main clinical implication of bacterial resistance in vitro is a substantial decrease in *H. pylori* treatment efficacy with, theoretically, a subsequent increase in clinical complications such as gastric cancer or peptic ulcers following increased persistence of infections(57). Pretreatment antibiotic resistance has been identified as the most important factor in non-response to *H. pylori* treatment(62,63).

To tackle this global problem, we need new antimicrobial drugs and treatment strategies but also a better understanding of the emergence and spread of resistant bacteria as well as improved diagnostic tools to guide clinicians in further optimizing currently available regimens(64).

Molecular mechanisms of drug resistance

Understanding the mechanistic and biological attributes that drive antibiotic resistance in *H. pylori* species has increased greatly over the past two decades, mainly for resistance against specific families of antimicrobials that are commonly used in *H. pylori* eradication therapies (that is, β-lactams, fluoroquinolones, macrolides, nitroimidazoles, tetracyclines and rifamycins) (64). For example in the case of Amoxicillin, *H. pylori* species, despite encoding several putative PBPs (Penicillin-binding proteins) and β-lactamase-like proteins, develop Amoxicillin resistance mainly by reducing the binding affinity to a specific PBP named PBP1A without generating substantial β-lactamase activity(65–67). Among macrolides, Clarithromycin has been used in front-line regimens for H. pylori eradication

given its two pharmacokinetic advantages in the stomach: acid stability and improved absorption in the gastric mucus layer(68,69). In *H. pylori,* the main mechanism underlying Clarithromycin resistance is point mutations in domain V of the 23S rRNA gene, namely A2142G/C and A2143G mutations(70–72).

Detection of antibiotic resistance

With the growing prevalence of global antibiotic resistance in *H. pylori* infection, antibiotic susceptibility testing (AST) is becoming increasingly needed for guiding decisions about appropriate therapies in individuals and treatment policies in populations(41,54,73). Several testing techniques have been developed in *H. pylori* for dealing with various challenges in terms of timing, costs and different required analytical specimens. These techniques can be categorized into culture-based and molecular-based AST techniques(64).

Culture-based AST techniques

Culture-based techniques are the standard AST for *H. pylori* using either the agar dilution method or the Epsilometer test (E-test) method(54). Culture-based techniques provide in vitro phenotypic susceptibly information. These techniques are useful quantitative methods for determining the minimum concentration of an antimicrobial agent that kills (bactericidal activity) or inhibits the growth (bacteriostatic activity) of *H. pylori* after around 72 hours of incubation. The agar dilution method can be adapted for testing several strains at once on the same medium plate, enabling a high-throughput AST. The E-test method is less technically challenging and less time-consuming(64).

However, both methods have important limitations in H. pylori. First, they raise challenges owing to the pathogen's fastidious growth requirements, restricting the phenotypic AST assay to only well-equipped laboratories with well-trained technicians. Second, interpretation of phenotypic outcomes is strongly dependent on the experimental conditions and hence can be subjective whilst not being always reproducible(74). For instance, Metronidazole susceptibility testing can potentially be affected by redox variations in the test medium(62). Finally, assessing the phenotypic resistance in *H. pylori* is time consuming and delivers results, after strain isolation, in about one week in the best case(75,76). It is therefore particularly challenging to implement these procedures in clinical practice, especially in regions with high *H. pylori* prevalence, as all these factors prevent regions with limited resources from performing routine phenotypic AST of *H. pylori*, and more rapid and cost-effective molecular methods that enable reliable prediction of phenotypic drug resistance are needed(65,77).

Molecular-based AST techniques

Advances in the understanding of basic molecular aspects of drug resistance in *H. pylori* have enabled the development of several methods for rapid detection of resistance during clinical infections. These methods rely mainly on the detection of specific *H. pylori* mutations encoding resistance, but differ in their respective turn-around times, types of analytical specimen and performance(64).

Importantly, these assays can either be culture-based when performed on cultured isolates or culture-free when directly applied on various types of biological specimens such as fresh, frozen or paraffin-embedded gastric biopsy samples, stool samples and gastric juice(78–88). These assays can be highly reliable but their accuracy might be affected by the condition of the samples, and by possible DNA contamination or degradation(86).

For instance, stool samples in which there might be a low amount of *H. pylori* DNA require powerful DNA extraction techniques to achieve good results. Paraffin embedding of gastric biopsy samples can lead to false-negative results because the fixative can break DNA into small pieces(87–89).

Meanwhile, the clinical relevance of molecular assays can be affected by their high sensitivity that can detect dead or non-cultivable microorganisms(86).

Moreover, DNA sequencing by the Sanger method, the gold standard for identifying mutations following PCR, is not cost-effective in a routine setting(87).

Numerous PCR-based methods are available for detecting only a few specific mutations and offer cost-efficient advantages, namely for resistance to Clarithromycin, Tetracycline and Levofloxacin. In particular, several molecular-based kits that detect both the presence of *H. pylori* and the mutations associated with Clarithromycin resistance are commercially available, and can provide a result in a few hours and can be performed by any microbiology laboratory(87).

By contrast, establishing assays for detecting resistance to Metronidazole and Amoxicillin has remained difficult probably due to the wide diversity of underlying molecular mechanisms.

NGS (Next-generation sequencing) Technology:

NGS technologies have emerged as a powerful and fast tool for antibiotic resistance prediction and surveillance(65, 90–95). To predict antibiotic resistance in *H. pylori*, NGS approaches would be applied in combination with bacterial culture for bacterial whole-genome sequencing (WGS) or with other molecular techniques (such as PCR) for deep-amplicon sequencing. NGS-based methods present several advantages over traditional molecular methods. Antibiotic resistance in clinical *H. pylori* isolates often arises from scattered sequence positions (such as mutations in genes e.g. *pbp1A* and *rdxA*), requiring the molecular-based approach to cover a sufficient sequence length to reach its highest performance. Classic PCR-based methods (PCR with Sanger sequencing) face limitations as they can accurately target only a limited number of nucleotides and cannot theoretically cover all possible complex structural variants of resistance-related

genotypes encountered in *H. pylori* (for example, large deletions or insertions in *rdxA* gene). These methods are hence prone to false-negative results.

By contrast, NGS-based approaches provide a more comprehensive view of bacterial genotypes and are particularly relevant for tracking complex or nested genetic factors driving antibiotic resistance with interesting potential for the discovery of novel or rare resistance mechanisms in clinical isolates(65,91,92,94–96).

In addition, NGS can be performed within a clinically relevant timeframe (24–72 hours) (65,75,90).

Unlike deep amplicon sequencing, whole-genome sequencing (WGS) methods require an initial cultivation of *H. pylori*, but this limitation is ready to be overcome with new advances in metagenome-assembled genomics(65,75,97,98).

Currently, several NGS technologies can probably be afforded even by laboratories located in low-income regions. Exploiting bacterial genomics via WGS is therefore a highly attractive option for tracking *H. pylori* antibiotic resistance in diagnostic microbiology laboratories compared with phenotypic AST and antibiotic resistance genotyping by classic PCR-based molecular methods(75).

However, in general, given the analytical challenges raised by high-throughput NGS data, further work is currently needed for standardization and implementation of easy-to-use computational tools for detecting resistance-related genetic determinants(65,91,92,94,95).

Conclusion

Based on the lack of a readily available vaccine in the very near future, other directions for counteracting H. pylori-related drug resistance have to be considered. The first option is to optimize the effectiveness of available empirical regimens for *H. pylori* eradication(64).

While we wait for an effective vaccine and further development of the novel susceptibility testing techniques for specific drugs, we should use the most likely effective first line combination therapy. This may vary according to local resistance data and antibiotic use and local guidelines should be developed for this. This will minimise delay in treatment while at the same time avoiding recurrent failure to rescue therapies, ultimately leading to the overuse of antibiotics, worsening resistance. If this fails to achieve eradication, then local strategies should be available for the patient to undergo endoscopy and sensitivities before using further empirical therapies.

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