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1. Introduction

*Helicobacter pylori (H pylori)* is a spiral-shaped bacterium that is attached to or just above the gastric mucosa. The organism can persist in the stomach indefinitely and may not cause clinical illness for many years after infection [1]. Indeed, a large number of infected patients never develop any symptoms [2, 3]. However, literature has also associated *H pylori* infection with gastritis and gastric malignancies (gastric adenocarcinoma and MALT-lymphoma) [4]. Chronic *H pylori* infection has also been associated with several extra intestinal diseases, such as autoimmune thrombocytopenia, sideropenic anemia and chronic urticaria but the pathogenesis is still not known [5]. *H pylori* gastric infection is one of the most prevalent infectious diseases worldwide with an estimate of 40%-50% of the world population [3]. Remarkable differences are due to geographical, socio-economic and demographic factors [6]. *H pylori* transmission is still not completely understood. In addition, among infected patients, the reasons why only some develop symptoms is still a matter of speculations. Studies suggested that *H pylori* is also transmitted from domestic animals like cat and sheep, but humans are the primary reservoir of *H pylori* infection [7, 8]. Several tests are available to detect *H pylori* in patients with ulcer or dyspepsia. The more commonly used tests are the evaluation of biopsy specimens during upper gastrointestinal tract (GI) endoscopy, the detection of serum anti *H pylori* antibodies and breath tests with 13C-labeled urea (9-11) The discovery that most upper gastrointestinal diseases are the consequence of *H pylori* infection that can be treated with antibacterials, is an important medical advance [12]. Although, in the last few decades, *H pylori* eradication has been standardized, occurrence of resistance to therapeutic regimens is a growing problem [3, 14]. The purpose of this paper is to provide an appraisal of the most
effective, current treatments available for *H pylori*, and to speculate on the potential for newer approaches in treatment and prophylaxis in future.

2. Epidemiology and prevalence

Humans are the only host for *H.pylori*, which is found in stomach, duodenum, esophagus and rectum on areas of metaplastic gastric epithelium [15]. Other *H.pylori* species have been isolated from the animals [16]. Animal models of *Helicobacter* infection have been developed due to shared characteristics of other *H.pylori* like *H.mustelae* and *H. felis* with *H.pylori*. *H.pylori* exists the world over and its prevalence in the population increases with age [17, 18]. In developed countries, prevalence increases with rate of 1% per year of age, where as it is rare in children, and reaches 70% in the seventh decade [19]. In developing countries, more than 50% children acquire the infection by the age of 10 years, and more than 80% of the population gets infected by the age of 20 years [20-22]. In asymptomatic individuals prevalence of *H.pylori* infection varies from 31%-84% [22]. *H.pylori* infection is chronic and once acquired remains life long, unless eradicated by antibiotics given for some other conditions [22]. Humoral and tissue immune response by the host is usually not sufficient to clear the infection. Though the mode of transmission is not yet well established, most probably it occurs by oral-oral or faeco-oral route and important risk factors are socioeconomic status and age [7, 8]. Overcrowding, poor socio-economic status and poor hygiene are associated with high infection rate. Re-infection rate after eradication is quite high in developing countries due to above mentioned risk factors [23].

Colonization of *H. Pylori* occurs by producing urease and gastric acid inhibitory protein. It can colonize only in gastric type epithelium and cannot stay anywhere else in the GI in the absence of gastric mucosa [24]. Metaplasia, which is present in more than 90% of patients of duodenal ulcer, occurs by replacing the columnar cells, normally covering the duodenal villi, by gastric type epithelium. Adhesion of *H.pylori* to the gastric epithelium occurs by tissue specific proteins. Colonization of the duodenal bulb by *H.pylori* leads to mucosal inflammation which makes it vulnerable to attack by acid or pepsin or bile resulting into ulceration, however, factors leading to gastric metaplasia in the duodenal bulb are not known. Stimulation of the immune system of *H.pylori* contributes to host damage and it evades the immunological clearance [25-2]).

3. Diseases associated with *H.pylori*

*H.pylori* infection is found to be associated with gastritis, non-ulcer dyspepsia (NUD), duodenal ulcer, gastric ulcer, gastric cancer, gastric lymphoma of mucosa associated lymphoid tissue (MALT) and even coronary heart disease [28, 29]. It has now been well established that *H.pylori* is the cause of almost all duodenal ulcers (DU) and chronic benign gastric ulcers (GU) which are not associated with nonsteroidal anti-inflammatory drugs (NSAID) [30]. More than 95%
of DU and 90% of GU are associated with \textit{H.pylori} infection and there is a dramatic decrease in their relapse rate after the \textit{H.pylori} eradication. Right now there is no convincing evidence that NUD symptoms are due to \textit{H.pylori} infection. Prevalence of \textit{H.pylori} infection is comparable between healthy individuals and patients with the symptoms of NUD. Recurrent abdominal pain in children suggestive of NUD subsides after the eradication of \textit{H.pylori} which indirectly associates \textit{H.pylori} infection with NUD. However, further studies are necessary to eradicate \textit{H.pylori} completely in NUD. [31]

4. Current therapy of \textit{H.pylori} infection

The objective of \textit{H. pylori} treatment is the complete elimination of the organism from the GI of patients and once this has been achieved then the rate of reinfection is low. Development of a successful treatment for \textit{H. pylori} infection has been fraught with difficulty. The survival capabilities of the \textit{H.pylori} organism over a wide pH spectrum within the stomach make the task of eradication difficult [32]. The organism must be eradicated from each of these potential niches and this is a daunting task for any single antibiotic. Initial attempts to cure the infection showed that the presence of antibiotic susceptibility in vitro did not necessarily correlate with successful treatment. It was rapidly recognized that the therapy with a single antibiotic led to a poor cure rate and various antimicrobial mixtures were tried resulting in several effective combinations of antibiotics, bismuth and antisecretory drugs [33, 34].

It is vital that the infection be treated optimally with clinically relevant \textit{H.pylori}– eradication regimens that has an acceptably high eradication rate and without major side effects and with minimal induction of bacterial resistance. The reasonable eradication targets would be $\geq 90\%$ cure rate on per-protocol analysis, and $\geq 80\%$ cure rate on intent-to-treat analysis [35]. Such goals have not been achieved with antibiotics alone. Because of luminal acidity influences the effectiveness of some antimicrobial agents that are active against \textit{H. pylori}. In order to achieve the desirable eradication rate, the antibiotics are combined with proton-pump inhibitors or ranitidine bismuth citrate. So-called triple therapies, combinations of one anti secretory agent with two antimicrobial agents for 7 to 14 days, have been extensively evaluated, and several regimens have been approved by the Food and Drug Administration (FDA). Combination drug regimens are essential to maximize the chance of eradicating the infection and to minimize the risk of promoting antimicrobial (to metronidazole and clarithromycin) resistance [35].

The most widely used antimicrobials in these regimens are amoxicillin, clarithromycin, metronidazole, tetracycline, and bismuth. Resistance of \textit{H pylori} to the limited range of antibiotics that have efficacy in its treatment can severely affect attempts to eradicate the bacteria. Resistance to tetracycline or amoxicillin is extremely rare [36, 37]. The issue of resistance primarily concerns the nitroimidazoles (metronidazole or tinidazole) and macrolides (clarithromycin) [38, 39]. Prevalence of \textit{H pylori} resistance to metronidazole is approximately 25\% in developing countries, because of the frequent use of nitroimidazoles to treat other diseases% [40, 41]. Increasing the dosage of metronidazole administered (e.g., from 1.0
to 1.5 g/day) generally improves the results of therapy when treating metronidazole-resistant \textit{H. pylori} strains. Resistance to clarithromycin is becoming more prevalent in some European countries, where the prevalence may be as high as 17\% \cite{40, 41}. The clinical effect of clarithromycin resistance is essentially complete loss of any clarithromycin anti-\textit{H pylori} effect; outcome of therapy can generally be predicted on the basis of what could be expected if only the other antimicrobials in the regimen are used \cite{39}.

4.1. First-line therapy of \textit{H. pylori} infection

First-line \textit{H. pylori} therapy should ideally be short, easy to administer, well tolerated and relatively cheap \cite{42}. However, over and above these considerations, the prime objective of any treatment is to eradicate the infection in the maximum number of patients \cite{43}. Some of the best validated first-line treatments for \textit{H pylori} include amoxicillin or clarithromycin-based triple therapies, which consists of a proton pump inhibitor PPI (standard dose twice daily) combined with clarithromycin (500 mg twice daily) and either amoxicillin (1 g twice daily) or metronidazole (500 mg twice daily) for a minimum of 7 days. Bismuth-based quadruple therapies consisting of a histamine receptor antagonist or PPI combined with bismuth, tetracycline, and metronidazole \cite{42, 43}. Another alternative approach is to include ranitidine bismuth citrate (RBC) in place of a PPI in clarithromycin triple therapy. These regimens have the propensity to eradicate 70 to 85\% of the infection. However, the regimen, which employs a PPI with clarithromycin and amoxicillin is the most widely endorsed first-line regimen for \textit{H pylori} \cite{42, 43}. Any of the currently available PPIs may be used with equivalent treatment efficacy \cite{44, 45} with the exception of esomeprazole as it is prescribed once daily because it is imperative that the standard dose of a PPI be prescribed twice daily in order to maximize treatment efficacy \cite{46}. RBC is sometimes used in place of a PPI in countries outside the United States with at least equal and perhaps greater efficacy \cite{47}. Metronidazole can be used as an alternative to amoxicillin, particularly in the setting of penicillin allergy or intolerance \cite{42, 43}.

Bismuth-based quadruple therapy is another option in penicillin allergic patients which yields similar eradication rates to clarithromycin triple therapies \cite{48, 49}. A recent meta-analysis including five randomized trials reported intention-to-treat (ITT) and per protocol (PP) eradication rates of 79\% and 85\% for clarithromycin triple therapy and 80\% and 87\% for bismuth quadruple therapy, respectively \cite{50}. Recently, simplified twice-daily dosing regimens for bismuth quadruple therapy have been successfully used in clinical trials \cite{51}. It is worth noting that the dosing of metronidazole used in the various bismuth quadruple therapies has not been consistent across studies. As higher doses of metronidazole (500 mg) may provide better cure rates than lower doses (250 mg), caution must be exercised when interpreting the data from comparative studies and pooled analyses involving quadruple therapies. Although there is no universal standard, there has been a desire to decrease the duration of therapy, particularly in countries outside the United States where treatment durations of at least 7 days have been recommended \cite{42}. Until very recently, the recommended treatment duration in the United States has been 10–14 days due to lower eradication rates with 7-day regimens \cite{52}. However, a large randomized US trial, rabeprazole-based triple therapy for 7 days was found to be as effective as 10 days of therapy (ITT eradication rates of
77% [71%–83%] vs. 78% [72%–84%], respectively) [53]. Based upon these results, 7-day rabeprazole-based triple therapy has been approved by the Food and Drug Administration for use in the United States. However there is some minor disagreement on the duration of treatment; the US guidelines recommend a 14-day course, whereas in Europe, a 7-day course is considered to be sufficient and Canadian and Asia–Pacific guidelines correspond largely to the Maastricht 2–2000 guidelines [54].

4.2. Second-line therapy of *H. pylori* infection

The choice of second-line treatment depends on which treatment approach was used initially, because retreatment with the same regimen is not recommended. If a clarithromycin-based regimen was used initially, a metronidazole-based regimen should be used as follow-up (in combination with a PPI, tetracycline, and bismuth), and vice versa. Prolonging the treatment period to 14 days is probably necessary. Because bacterial resistance to metronidazole or clarithromycin results primarily from previous treatment failures, first-choice treatment should never combine clarithromycin and metronidazole in the same regimen [55]. Second-line therapy has been extensively reviewed by several authors [55-57]. The assessment of *H. pylori* sensitivity to antibiotics may be useful only after failure of the second-line therapy [58].

The most widely recommended second-line treatment for persistent *H. pylori* infection is quadruple therapy with a PPI (standard dose twice daily), bismuth salt (subsalicylate or subcitrate 120 mg 4 times daily), metronidazole (500 mg thrice daily) and tetracycline (500 mg 4 times daily) for a minimum of 7 days. Further failures should be managed by specialists. The Table 1 summarizes the suggested therapeutic regimens for eradication of *H. pylori* infection. No other second-line treatment strategies have been widely endorsed; however, rifabutin-based triple therapy and furazolidone-based triple therapy [59, 60] have been investigated and suggested as alternatives to bismuth quadruple therapy (Table 1). As second-line therapy, the Maastricht 2-2000 Consensus Report suggests a quadruple therapy based on bismuth (120 mg, q.i.d.), tetracycline (500 mg, q.i.d.), metronidazole (500 mg, t.i.d.) and antisecretory agent (PPI, b.i.d.) for a minimum of 7 days [34]. Further trials have shown that replacing the proton pump inhibitor and the bismuth compound of the quadruple therapy by RBC also achieves good results, with an eradication rate ranging between 57%-95% [61, 62]. The failure of second line quadruple therapy is associated with its discontinuation because of high incidence of side effects (6%-68%) [63]. A triple therapy with the combination of levofloxacin, rabeprazole and tinidazole or amoxicillin has been proposed as an alternative to Maastricht. This protocol shows an eradication rate higher than 90% compared to quadruple therapies given for 7 days (63%) with a lower incidence of side effects [64]. Rifabutin has been shown to have a good eradication rate (87%), if administered at a high dose (300 mg) in combination with amoxicillin and PPI, as compared to quadruple therapy. Wong et al [65] showed that a combination of levofloxacin, rifabutin and rabeprazole has a high efficacy with an eradication rate >90%. Furazolidone is also used to replace metronidazole in quadruple therapy [66]. Different *in vivo* studies have confirmed the efficacy of regimens containing a high-dose furazolidone [200 mg, b.i.d.] as the second-line therapy in patients with metronidazole-resistance [66]. Many other combinations have been used with various rates of success.
Bacterial eradication may fail in up to 40% of cases after the suggested second-line regimens. As a consequence, to treat patients who have already undergone the first- and second-line therapies is a common challenge [67]. A recent rifabutin based triple therapy shown eradication rates of 100 and 87.5 % (Per Protocol and Intention-to-Treat analysis) in primary resistance to clarithromycin and tinidazole and 82.2 and 78.5% in secondary resistance [68].

4.3. Third-line rescue therapy for persistent H.pylori infection

Patients who fail both first- and second-line therapy are a clinical challenge. New modified eradication regimes involve the substitution of antibiotics used with other drugs, such as rifabutin, levofloxacin, and furazolidone [58-65]. These antibiotics should be considered for third-line treatment. Currently, no widely endorsed treatment regimens are available, for persons with persistent H pylori infection despite two or more previous courses of antibiotics (third-line therapy). A number of third-line regimens such as bismuth quadruple therapy, rifabutin-based triple therapy, levofloxacin-based triple therapy furazolidone-based triple therapy and Doxycycline-based therapy have been investigated and shown in Table 2.

The new fluoroquinolone, levofloxacin, has shown an excellent activity against a variety of Gram-positive and Gram-negative organisms which are resistant to the established agents Matsuzaki et al [69] studied several strains and reported that levofloxacin had an excellent and wide spectrum antibacterial activity compared with other fluoroquinolones and antibiotics tested [69]. A previous study has shown efficacy and safety of levofloxacin based triple therapy in H pylori infection first-line therapy [70]. Recently, Gatta et.al [71] have proposed a third-line treatment after two eradication failed courses without fluoroquinolones, with standard dose of PPIs (b.i.d.), levofloxacin (250 mg, b.i.d.) and amoxicillin (1 g, b.i.d.) for 10 days. The eradication rates of 76.2% and 84.6% according to ITT and PP analysis, respectively, have been achieved. The levofloxacin-based treatment could eradicate most of the strains (92.3%) which are resistant in vitro to both clarithromycin and metronidazole, but susceptible to levofloxacin. Furthermore, this drug combination, successfully employed as rescue therapy, is well tolerated and has no major side-effects [72].

Rifabutin, a spiroperiperidyl derivative of rifamycin, has been shown to exhibit high in vitro activity against H pylori [73, 74]. Furthermore, clinical trials have suggested that rifabutin may be a possible candidate for second or third line eradication combination therapy [75]. Furthermore, rifabutin is chemically stable at a wide pH range [74]. Recently, rifabutin-based rescue therapies (twice-daily standard-dose PPI plus amoxicillin 1 g twice daily or levofloxacin 500 mg once daily plus rifabutin 300 mg daily, for 7 days) have been shown to represent an encouraging strategy for eradication failures because they are effective against H pylori strains resistant to clarithromycin or metronidazole [75, 76]. However, rifabutin is very costly, and concerns still remain about the widespread use of this drug because of the possibility for accelerating development of drug resistance. Results of a recent study [59] suggest that a 10-day rescue therapy regimen based on the use of rabeprazole (20 mg twice daily) plus amoxicillin (1 g twice daily) plus levofloxacin (500 mg once daily) is more effective than standard quadruple regimen as a second-line option for H pylori eradication. Additionally, a 7-day quadruple-therapy regimen containing amoxicillin and tetracycline has recently been proven
more effective than standard quadruple therapy with metronidazole and tetracycline to rescue failed triple therapy, by overcoming the antimicrobial resistance of *H. pylori* [77-81].

Doxycycline is a widely used tetracycline antibiotic for eradicating several infections. With respect to tetracycline, Doxycycline requires the administration of only two tablets per day, leading to a better compliance in patients undergoing eradication therapies. Furthermore, Heep et al. [82] have found no secondary resistance to doxycycline in *H.pylori* patients who failed one or more eradication therapies. Quadruple regimens represent the most widely used rescue therapy. Induction of metronidazole resistance has suggested a new protocol, namely replacing tetracycline with doxycycline (because it requires the administration of only two tablets per day) and metronidazole with amoxicillin (because its resistance is less 1%), one week quadruple therapy with doxycycline (100 mg, b.i.d.), amoxicillin (1 g, b.i.d.), omeprazole (20 mg, b.i.d.) and bismuth salts (120 mg, two tablets b.i.d). This treatment has proved to be a highly effective third-line ‘rescue’ therapy, achieving 91% eradication rate in patients harbouring metronidazole and clarithromycin resistant *H. pylori* strains (by ITT analysis) [83]. This regimen shows excellent compliance (99%) with mild side-effects.

1. Bismuth subsalicylate (120 mg q.i.d)+ metronidazole (500 mg t.i.d)+ tetracycline (.500 mg q.i.d)+ proton pump inhibitor ( standard dose b.i.d) for a minimum of 7 days
2. Levofloxacin (250 mg b.i.d)+ amoxicillin (1g mg b.i.d)+ proton pump inhibitor ( standard dose b.i.d) for 10 days

Table 1. Second line therapy regimens of *H. pylori* infection.

<table>
<thead>
<tr>
<th>Regimen</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Rifabutin (300 mg o.d)+ amoxicillin (1g b.i.d)+ proton pump inhibitor ( standard dose b.i.d) for 10 days</td>
</tr>
<tr>
<td>2. Furazolidone 200 mg bid + amoxicillin (1g b.i.d)+ proton pump inhibitor ( standard dose b.i.d) for 14 days</td>
</tr>
<tr>
<td>3. Furazolidone 200 mg bid + tetracycline ( 1g b.i.d)+ proton pump inhibitor ( standard dose b.i.d)+ bismuth (140 mg b.i.d) for 7 days</td>
</tr>
<tr>
<td>4. Doxycycline (100 mg, b.i.d.)+ amoxicillin (1g b.i.d)+ omeprazole (20 mg, b.i.d)+ bismuth salts (120 mg, b.i.d) for 7 days</td>
</tr>
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</table>

Table 2. Third line rescue therapy regimens of *H. pylori* infection.

4.4. Failure of therapy

The reasons behind failure of these antibacterial treatments are not very clear, but are likely multifactorial [85-87]. Failure of therapy is most frequently associated with drug resistance and non-compliance, due to complexity of regimens and associated side effects, e.g., nausea, diarrhea, taste disturbances, mucositis, and pseudo membranous colitis. The continuing
emergence of resistance to the conventional antibacterials used to treat \textit{H. pylori} infections is of major concern [85]. Resistance to nitroimidazoles (e.g. metronidazole) is extremely high in the developing world and rates of resistance (>50%) have been described in Western countries [39-41]. Strains of \textit{H. pylori} which are resistant to clarithromycin are also now widespread e.g., 10–17% in most part of Europe [39].

5. Future therapeutic strategies

Resistance in \textit{H. pylori} is anticipated to increase because the currently effective drugs are used to treat many other infections where also develops the drug resistance in body. So there is an urgent need for improvement over current regimens due high resistance over no of antibiotics using in current therapy and moreover current triple and quadruple therapies are may not be desirable for widespread eradication of \textit{H. pylori} infection in future. The next generation of \textit{H.pylori} therapeutic regimens should be simpler, novel and specific. There are some novel approaches available to achieve this goal, such as development of therapeutic vaccine, geonome bsed drug discovery, pathogen–host tissue adhesion inhibitor, and novel site specific drug delivery at specific site of \textit{H.pylori} infection.

5.1. Vaccine development

The success of eradication of \textit{H.pylori} by antibiotic therapy is being hampered by the increasing occurrence of antibiotic resistance \textit{H.pylori} strains and patience compliance. Consequently investigative attention has been focused on development of an effective therapeutic vaccine. From mathematical [88] and animal models [89] of \textit{H. pylori} colonization, the host response has been suggested to play an important role in bacterial growth regulation. Immunization against \textit{H. pylori} may thus be considered as a strategy to deviate the immune response programmed by infection, i.e., the re-direction of a host response from natural infection leading to persistence of infection and gastric disease, to another state in the host response capable of attenuating or eliminating \textit{H. pylori} and its associated gastric inflammatory sequelae. Evidence from both animal models and humans implicate \textit{H. pylori} in the activation of B cell and T cell functions leading to heterogeneous systemic and local antibody responses, lymphoid follicle hyperplasia, and significant recruitment of CD4+ and CD8+ T cells [90, 91].

Vaccination against \textit{H. pylori} has been performed in several animal models. Although several studies showed the benefit of prophylactic as well as therapeutic vaccination in animals, bacterial eradication was not observed in humans [92]. Knockout studies in mice revealed that the Th2 response can be absent but immunization is still possible when using urease as an antigen [93, 94].

A number of novel approaches to delivery of \textit{H.pylori} vaccine have been reported recently. Smythies et.al [95] reported on \textit{H.pylori} vaccination based on a modified polio virus vector where the capsid genes are replaced with \textit{H.pylori} urease B. Poliovirus UreB replicons were co-administered with a recombinant vaccinia virus engineered to express polio virus capsid proteins, resulting in a vaccine which can only undergo one round of infection. Mice which
are transgenic for the human poliovirus receptor (C57BL/6/DAB) are susceptible to infection with poliovirus via the systemic route. Replicon vaccination resulted in clearance of an established \textit{H. pylori} infection in 73\% of mice compared to 31\% of vector-immunized controls. Furthermore, immunization prevented an infection from becoming established in 80\% of immunized mice. Bacterial ghosts (Gram-negative bacterial cell envelopes, devoid of cytoplasmic envelopes) have also been shown to have good adjuvant properties [96]. \textit{H. pylori} ghosts induced protection in a mouse model without the use of an additional adjuvant, although batch-to-batch variations were observed and improvements are therefore required before this approach could have practical applications [97]. Sodium alginate microbeads have also been tested for controlled release of a model \textit{H. pylori} vaccine [98]. Alginate beads are widely used for encapsulation of drugs, and the mild formulation conditions, and their reported muco-adhesive properties should make them ideal carriers for vaccine antigens. Recombinant urease encapsulated in alginate beads was administered to mice via the subcutaneous, nasal and oral routes. Unexpectedly, only subcutaneous delivery induced a significant antibody response and led to reductions in \textit{H. pylori} colonization (as determined by urease test) indicating that this approach also needs further improvement [98].

DNA vaccines are a potentially attractive approach to vaccination, and a genomic library approach has shown encouraging preliminary results in mice [99]. Two recent studies have investigated the adjuvant properties of CpG motifs in the context of DNA immunization. Interestingly, a prototype immunization construct encoding the UreB subunit which included CpG motifs [100] induced significant increases in the expression of IL-10 and beta-defensins in the gastric mucosa. In an approach that aimed to induce and modulate the immune response by triggering a specific Toll-like receptor (TLR), Sommer et.al [101] immunized C57BL/6 mice with \textit{H. pylori} lystate mixed with a synthetic CpG oligonucleotide targeted at TLR-9 (CpG oligonucleotide 1688). Immunization induced a Th1-biased immune response as expected, and immunized mice had 10-fold reduced levels of \textit{H. pylori} in the gastric mucosa after challenge. Synthetic CpGs have recently been approved for human use as a therapy for genital warts [102], and so given the encouraging results from mice, this approach might also be applicable for a human \textit{H. pylori} vaccine. However, DNA vaccination studies in human volunteers have reported only suboptimal immune responses [103] and it appears that the barriers to DNA uptake may be more difficult to overcome in humans [104]. To return to our original question regarding what we have learned from the mouse model, the data from animal models of \textit{H. pylori} infection support the feasibility of both therapeutic and prophylactic vaccination, for neonates and adults. Furthermore, a variety of routes of application and adjuvants are effective. It is, however, clear that only a better understanding of the underlying immune mechanisms will make it possible to improve efficacy and to address the issue of post immunization gastritis.

5.2. Genome-based drug discovery

The recent availability of the genome sequences of two different isolates of \textit{H. pylori} [105, 106] has provided much stimulus to research aimed at discovering and developing novel therapeutics to eradicate \textit{H. pylori}. \textit{H. pylori} is a relatively simple organism with a small genome of
circa $1.7 \times 10^6$ nucleotides, varying slightly according to the strain of the organism, and encoding around 1600 genes [107]. Of these, depending on the stringency of the comparison, roughly 55% have homologs of putative known identity in other organisms, while another 10% have database homologs of unknown identity. The remaining 35% are unique to H. pylori at this time. This complement of 1600-1700 gene contain a relatively small number which encode proteins whose functions are essential to the viability of the bacterium. A genome-based strategy facilitates the expeditious advanced selection of novel lethal targets not used by current antimicrobials, thus providing the opportunity to identify novel classes of antibacterials. In the case of vaccine discovery and development, comparison of two genomes has allowed the identification of a “common set” of H. pylori genes, among which genes encode antigens [108].

The principle underlying genome based drug development is to identify those essential proteins which are specific to H. pylori, and then to isolate, identify and synthesize a small molecule chemical which inhibits the essential activity of such proteins [105]. This approach is designed both to identify a drug that will work as a monotherapy, which would be specific for H. pylori and would not interfere with other organisms. In case of H. pylori drug discovery, the task is to identify those essential proteins which are specific for and conserved in H. pylori. Identification of essentiality involves a process whereby attempts are made to mutate a gene performed using an allelic recombination method via homologous recombination and so “knock-out” its function [106]. If a particular mutant can be generated, the disrupted gene does not code for a biological process that is required for viability in vitro. “Knock-out” mutagenesis experiments with selected members of the “common set” of H. pylori genes have allowed the identification of targets for drug discovery in a variety of H. pylori physiological functions [107, 108]. These targets include proteins involved in cell envelope synthesis/integrity, cell division, protein synthesis, nucleic acid biosynthesis, gene expression and regulation, cell metabolism and energetics, as well as proteins encoded by H. pylori genes of unknown function [109]. Because many essential proteins are enzymes, it is possible to establish in vitro assays to detect the ability of compounds to interfere with their enzymatic activity. The selected essential protein target is overproduced in E. coli, purified, and employed to identify inhibitory compounds by High Throughput Screening [110, 111]. This involves the automated micro assay screening of the ability of large numbers of compounds (>200,000) to inhibit the enzymatic activity of the target protein. Conformed “hits” are then tested for their ability to selectively kill H. pylori in vitro, and to eradicate H. pylori infection in an animal model. Lead compounds are subjected to chemical structure-activity relationship studies to improve antibacterial potency and selectivity, as well as solubility, oral bioavailability, duration of action and pharmacokinetic properties [101]. Understanding the mechanism of action of a particular target enzyme or molecular modeling based on three-dimensional crystal structure of the protein, with or without bound inhibitor(s), can be used to facilitate medicinal chemistry studies [112, 113]. As lead compounds are identified, the classical medicinal chemistry and combinatorial chemistry approaches are applied to improve the potency and the desired microbiological properties, with the ultimate goal of producing compounds suitable for human trials.
5.3. Novel drug delivery approaches

One of the reasons for incomplete eradication of *H. pylori* is probably due to the short residence time of antimicrobial agents in stomach so that effective antimicrobial concentration cannot be achieved in the gastric mucus or epithelial cell surfaces where *H. pylori* resides [114-116]. Conventional tablets and capsules are, in general used for eradication therapy but these do not remain in stomach for prolonged time therefore it is difficult to reach minimum inhibitory concentration in gastric mucus where *H. pylori* colonize. To overcome the problem, a new concept is proposed based on gastroretentive concept (Floating drug delivery systems and Mucoadhesive drug delivery systems) with site specific drug delivery [117]. It is expected that the topical delivery of narrow spectrum antibiotic through floating and bioadhesive drug delivery system may result in complete removal of organism from stomach.

5.4. Floating drug delivery systems (FDDS)

Floating drug delivery systems have a bulk density less than gastric fluids and so remain buoyant in the stomach without affecting the gastric emptying rate for a prolonged period of time. The FDDS can be divided into effervescent and non-effervescent systems. Effervescent floating dosage forms are matrix type systems prepared with the help of swellable polymers such as methylcellulose and chitosan and various effervescent compounds, such as sodium bicarbonate, tartaric acid, and citric acid. They are formulated in such a way that when comes in contact with the acidic gastric contents, CO2 is liberated and gets entrapped in swollen hydrocolloids, which provides buoyancy to the dosage forms. Non-effervescent floating dosage forms use a gel forming or swellable cellulose type of hydrocolloids, polysaccharides, and matrix-forming polymers like polycarbonate, polyacrylate, polymethacrylate, and polystyrene [118]. The formulation method includes a simple approach of thoroughly mixing the drug and the gel-forming hydrocolloid. After oral administration this dosage form swells in contact with gastric fluids and attains a bulk density of < 1. The air entrapped within the swollen matrix imparts buoyancy to the dosage form. The so formed swollen gel-like structure acts as a reservoir and allows sustained release of drug through the gelatinous mass. While the system floats on the gastric contents, the drug is released slowly at the desired rate from the system. After release of drug, the residual system is emptied from the stomach. The FDDS greatly improve the pharmacotherapy of the stomach through local drug release, leading to high drug concentrations at the gastric mucosa for eradicating *H. pylori* from the submucosal tissue of the stomach, making it possible to treat stomach and duodenal ulcers, and gastritis [119-121].

In our previous study [114] we have developed the floating in situ gelling system of amoxicillin and evaluated in vivo efficiency in Mongolian gerbil model, the result indicated that the floating in situ gel has promising potential for eradicating *H. pylori* infection. In this study the gelled solution floated in stomach for >20 h, and the in vivo *H. pylori* clearance efficiency was 10 time more than that of amoxicillin solution in gerbils model.

Yang et al [119] developed a swellable asymmetric triple-layer tablet with floating ability to prolong the gastric residence time of triple drug regimen (tetracycline, metronidazole, and clarithromycin) in *H. pylori*-associated peptic ulcers using hydroxy propyl methyl cellulose.
(HPMC) and poly ethylene oxide (PEO) as the rate-controlling polymeric matrix. The design of the delivery system was based on the swellable asymmetric triple-layer tablet approach. Tetracycline and metronidazole were incorporated into the core layer of the triple-layer matrix for controlled delivery, while bismuth salt was included in one of the outer layers for instant release. According to the authors, the aim of such a device was to obtain a simple regimen for a standard triple therapy. Indeed, they obtained in vitro a duration of buoyancy and sustained release of metronidazole and tetracycline over prolong time of period. The rapid effect of the device would be due to the rapidly dissolving layer containing the bismuth salt, which disintegrated within 10–15 min in vitro. However, no in vivo data are available concerning the floating characteristics of the drug delivery system or its effect against H.pylori. Indeed, they obtained in vitro a duration of buoyancy and sustained release of metronidazole and tetracycline over prolong time of period.

Umamaheshwari et al [120] developed several drug delivery systems especially designed to improve efficiency against H.pylori. In all of them, they used an antiurease drug, acetohydroxamic acid (AHA), as an active agent against the bacterium. H. pylori urease hydrolyses urea present in the gastric juice and extracellular fluid to generate ammonia and bicarbonate, which effectively neutralize an acidic pH in its environment [121, 122]. Thus, urease inhibitors hinder the bacterium to protect itself against low pH and avoid thus the problem of treatment of antibiotic-resistant strains [123].

The same authors developed polycarbonate microballoons by an emulsion (o/w) solvent evaporation technique. In vitro (i.e. in simulated gastric fluid) 74% to 85% of microballoons stayed buoyant up to 12 h and exhibited a sustained drug release profile. In vitro and in vivo growth inhibition studies were performed using cultures of H. pylori and H. pylori-infected Mongolian gerbils, respectively. Microballoons showed 10 times higher anti-H. pylori activity compared with AHA solution [124].

Umamaheshwari et al. [125] developed cellulose acetate butyrate coated floating microspheres of cholestyamine for targeting to H.pylori infection. In vitro (drug release, buoyancy) and in vivo studies (gastric mucoadhesion in the rat stomach) led the authors to conclude that this drug delivery system possessed both floating and bioadhesive properties, and may be successful in the treatment of H.pylori. Katayama et al [126] developed a sustained release liquid preparation of ampicillin using sodium alginate for the treatment of H.pylori infection. The gastroretentive property of the device was provided by the ability of sodium alginate to form a firm gel when an acid or di-or trivalent metal ions (Ca$^{2+}$, Ba$^{2+}$, Sr$^{2+}$) were added. The authors expected the solution to be able to spread out, adhere to the gastric mucosa, and release the antibiotic continuously (ampicillin). The alginate formulation gelled in stomach and delivered ampicillin locally in stomach and showed significant anti H. pylori effect in rat model.

5.5. Mucoadhesive drug delivery systems (MDDS)

Bioadhesive drug delivery systems are used to localize a delivery device within the lumen to enhance the drug absorption in a site-specific manner. Mucoadhesive drug carrier may prolong the residence time of drug in stomach because they adhere to mucus surface, resulting in an effective localizing drug concentration. Mucoadhesion is a very complex process and several theories have been put forward to explain the mechanism, including electrical, adsorption, and wetting and diffusion theories [127]. Because the mucosal surface is negatively
charged, a positive charge on polymer might facilitate the mucoadhesion process. Some of the most promising charged excipients used commonly in these systems include polycarbophil, carbopol, lecithins, chitosan, carboxy methyl cellulose and gliadin, etc. Some investigators have tried a synergistic approach between floating and bioadhesion systems [120, 124, 128]. Among the mucoadhesive formulation, mucioadhesive microspheres have gained considerable attention due to their ability to adhere to mucus layer as well as release drugs locally at the infected cell line.

Nagahara et al [128] formulated mucoadhesive microspheres containing amoxicillin. They dispersed the drug and bioadhesive polymers (carboxyvinyl polymer and curdlan [polysaccharide]) in melted hydrogenated castor oil. They compared these microspheres with an amoxicillin suspension in infected Mongolian gerbils under feeding conditions. The microspheres with an amoxicillin dose of 1.0 mg/kg provided the same clearance rate (20%) as the amoxicillin suspension with a dose of 10 mg/kg. This means that the amoxicillin-microspheres provided 10 times greater anti-\textit{H. pylori} activity than the amoxicillin suspension. Moreover, adhesion of microspheres on the stomach wall was observed (47% and 20% remained in the stomach after 2 and 4 h, respectively). The authors concluded that these mucoadhesive microspheres containing an appropriate antimicrobial agent should be useful for the eradication of \textit{H. pylori}.

Wang et al. [129] studied positively charged gelatin microspheres as gastric mucoadhesive drug delivery system for eradication of \textit{H. pylori}. Umamaheshwari et al. [130] developed nanoparticles bearing amoxicillin using gliadin polymer and evaluated in-vivo the anti \textit{H. pylori} efficiency of nanoparticles in gerbil model, results were found to be promising molecule to eradicate of \textit{H. pylori} infection. Radiet et al. [131] developed tetracycline-loaded crosslinked chitosan microspheres to increase the local concentration of the antibiotic in the stomach and, thus eradicate \textit{H. pylori} infection. The microspheres were examined for gastric residence time and local tetracycline concentrations in fasted gerbils. The microspheres were found to reside in the stomach even after 10 h of administration.

Recently, Zhepeng et al. [132] also published a study on mucoadhesive microspheres containing amoxicillin by an emulsion/evaporation method, using ethylcellulose as matrix and carbopol 934P as a mucoadhesive polymer. This work showed that free amoxicillin was rapidly degraded in acidic medium; however, amoxicillin entrapped in the microspheres microspheres kept stable. Furthermore, studies on the in vivo clearance of \textit{H. pylori} revealed that, in a single-dosage administration, the mucoadhesive microspheres had a better effectiveness than that of amoxicillin powder. Finally this study showed a complete eradication of \textit{H. pylori} with microspheres in five of six rat stomachs, whereas amoxicillin powder showed four times less effectiveness. The authors found a tendency for an effective anti-\textit{H. pylori} activity induced by mucoadhesive microspheres, but concluded that larger groups of animal are required to confirm these results.

Among all the drug delivery approaches (Dosage forms) described herein, some provide interesting solutions, although many of them present drawbacks. In the particular case of \textit{H. pylori} eradication, the ideal dosage form should, to be really effective, not only stay in the stomach, but also target the bacterium. However, knowledge about this pathogen discovered twenty years ago is still poor. More data are necessary, for example, to identify an “ubiquitous”
receptor (i.e. for all *H. pylori* strains) at the surface of the bacterium, which could provide a strong interaction with a ligand [133-135]. Thus, the development of an efficient gastroretentive dosage form against *H. pylori* is closely linked to a better understanding of its pathogenicity mechanisms.

6. Conclusion

Considerable advances have been made in understanding the evolution of the organism and pathogenesis of disease. Although combination therapies have high rates of eradication, the preferred therapy would be one which use a low dose of a single drug with a short duration treatment and without any adverse effect. The sequencing of two strains of *H. pylori* has provided a wealth of data that will be useful in understanding the pathogenesis of disease, microbial evolution and highlight potential therapeutic targets and potential vaccine candidates. In future, it is likely to advance understanding of disease progression and aid in the development of novel therapies. First aspect of research is that genomic studies are arousing considerable interest, and will certainly be followed up in future research for development of new therapies. A second area of research that is becoming increasingly important is the development of novel targeted drug delivery systems for *H. pylori* infection. The floating and mucoadhesive formulation of antibacterial drugs have shown good *H. pylori* clarence effect in both *in-vitro* and in-vivo animal model studies. However, extensive clinical studies are required in human to establish a correlation.

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References


