

Antibiotic Treatment Strategies for *Helicobacter pylori* Infection

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Abstract: In the third decade of the *Helicobacter pylori* era several informations are available on its pathogenetic mechanisms, as well as on therapeutic approaches. A 7-14 day triple or quadruple regimens (proton pump inhibitor together with 2 antibiotics) are currently suggested as first-line treatment, but the success rate following these therapy is constantly decreasing worldwide. Therefore, new drugs are needed to treat such a widespread infection. Several patents of new antibiotics have been claimed in the last 5 years, and some of them showed a very powerful antibacterial activity *in vitro*, even against clarithromycin and metronidazole resistant strains. Among the new compounds, thienylthiazole derivatives, benzamide derivatives and pyloricidins should be regarded as very promising molecules.

Keywords: Antibiotics, *Helicobacter pylori*, therapy.

1. INTRODUCTION

Helicobacter pylori (*H. pylori*) is a Gram negative, spiral, microaerophile, multi-flagellate bacterium found on the human gastric mucosa. It causes a long-lasting, transmissible, worldwide spread infection which is involved in the pathogenesis of different gastroduodenal diseases, such as chronic active gastritis, duodenal and gastric ulcers, and gastric neoplasia [1]. Indeed, several studies have recognised that *H. pylori* may cause gastric low-grade mucosal-associated lymphoid tissue (MALT) lymphoma in susceptible patients, and a whole regression has been reported following bacterial eradication when such a neoplasia is in the early stage [2]. Furthermore, *H. pylori* is a proved environmental risk factor for gastric carcinoma, and it has been recognised as a definite type I carcinogen [3]. Although, the role of this infection in non-ulcer dyspepsia is still debatable, two comprehensive reviews showed a small, but significant, benefit of eradicating *H. pylori* in such patients [4,5]. Moreover, a recent, prospective, very large study found a long-term advantage in curing such an infection in these patients [6]. In the Fig. 1, all recognized *H. pylori*-associated disease are provided.

Obviously, as for several other bacterial diseases, the clinical manifestations of *H. pylori* infection mainly depend on the interaction between the host and the bacterium. Indeed, a genetic predisposition, an early age of infection, and the presence of concomitant risk factors (i.e. bile reflux in stomach) on one hand, and virulent factors (i.e. CagA cytotoxin), bacterial density or distribution in the gastric mucosa (i.e. confined in the antrum or involving the whole stomach), on the other, are important factors involved in the disease development [7-9].

The present review aimed to provide a perspective on the different therapeutic alternatives to cure *H. pylori* infection, and to explain why various agents should or should not be

used. It also includes information on novel molecules, some of which will be probably available in next future.

2. CONSIDERATIONS ON TREATMENT

H. pylori has evolved several efficacious mechanisms to enable the colonization of human gastric epithelium. Due to its production of urease and the presence of 3-7 flagella, it is able to survive over a wide pH spectrum and to efficiently penetrate the gastric mucous layer reaching the underlying gastric epithelium, where it can be found strongly attached to cells and even within cells [10]. Gastric juice has also been shown to contain viable *H. pylori* organisms, even when pH is below 3.0. These gastric luminal bacteria are transiently attacked with oral antibiotics, but are probably best treated by drugs that are secreted in gastric juice or saliva. The pH near the lumen of the stomach is maintained at 2.0, whereas the cell-mucus interface is more alkaline, with a pH value of approximately 5.5. Only very few antibiotics are active in both these pH extremes. Indeed, several antibiotics, which own a strong bactericidal efficacy *in vitro*, are readily inactivated by the low pH values encountered in the stomach. Moreover, oral agents reach very high concentrations in gastric mucus, but levels quickly fall as the stomach empties after a meal. These observations clearly demonstrate how it is mandatory to administer drugs able to significantly increase gastric pH – as a proton pump inhibitor (PPI) – together with antibiotics in order to enhance their efficacy [11].

Although the immune system produces antibodies against the bacterium virtually in all infected patients, immunoglobulin activity is largely inhibited by the gastric acid. Moreover, *H. pylori* is able to minimise the exposure of antigens by masking flagella with lipopolysaccharides, which mimic host carbohydrates. These abilities can explain, at least in part, the complexity in generating an efficient vaccine [12]. Furthermore, due to its motility, the bacterium may be inaccessible to direct contact with immune cells, such as neutrophils, which can migrate only slowly across the gastric mucosa. In addition, its catalase activity is able to destroy lysosomal hydrogen peroxide before it can generate the oxygen radicals necessary for a bactericidal effect [13]. Recent studies have also shown that CagA-negative

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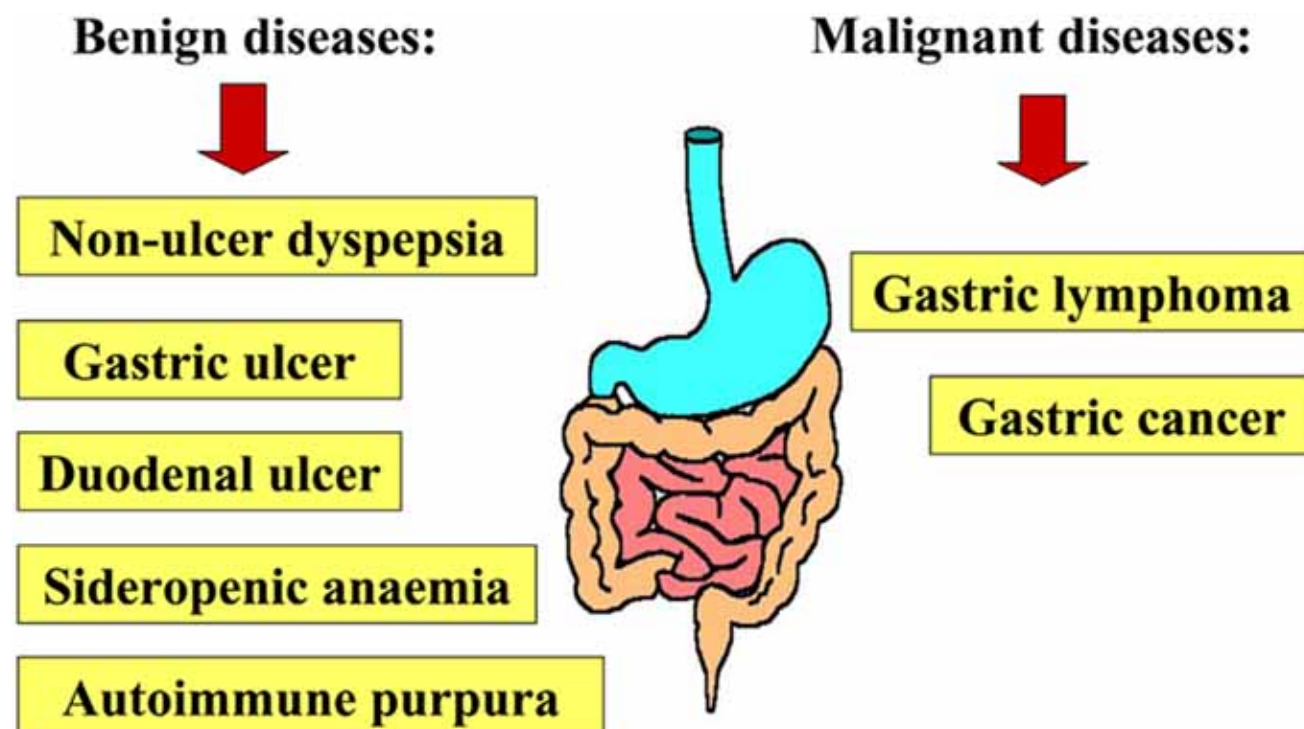


Fig. (1).

H. pylori strains seem to be less susceptible towards therapy as compared to CagA-positive bacteria [14].

On the other hand, both primary and acquired bacterial resistance against different antibiotics has been clearly demonstrated, and it is increasing in the last decade in several countries [15]. In addition, a co-infection with different *H. pylori* strains is not infrequent, and a mixture of colonies differing in their susceptibility to antibiotics have been also observed in some cultures [16]. Furthermore, it has been observed that there is a frequent discrepancy between antibiotic susceptibility *in vitro* and bacterial eradication *in vivo*, so that the role of bacterial culture with antibiotic susceptibility assessment in clinical practice has been questioned [17].

3. ACTIVITY OF DIFFERENT ANTIBIOTICS

Since its discovery, the bactericidal activity of several compounds has been tested against *H. pylori* either *in vitro* or *in vivo* [18]. The initial attempts to cure the infection by using a single drug were largely disappointing. Therefore, it became evident that a combination of drugs was required to treat this infection [19]. There are some agents (penicillins, tetracyclines, nitrofurans) that lead to secondary antibiotic resistance less frequently than others (macrolides, nitroimidazoles, quinolones) and, therefore, they may be re-used in the same patients with other drug combination.

Penicillins

Undoubtedly, amoxicillin is the most used penicillin for *H. pylori* therapy. The high susceptibility of *H. pylori* to amoxicillin *in vitro* contrasts with the low efficacy of this antibiotic *in vivo*, with an eradication rate of less than 20 % when administered as monotherapy. However, when amoxi-

cillin is administered together with a PPI for 7-14 days, an eradication rate higher than 50-60 % may be achieved [20]. Indeed, high concentrations of amoxicillin are obtained in gastric juice and mucosa during oral or intravenous therapy with 2 g daily, the molecule being actively secreted into the stomach, especially through the gastric body mucosa [21]. Moreover, besides its bactericidal effect, amoxicillin offers other advantages for *H. pylori* therapy. It acts destroying the bacterial membrane, and this effect is of paramount importance, even if the bactericidal effect is not directly achieved. In fact, an inducible synthesis of ATP-ase-dependent pumps on the bacterial membrane has been shown to cause a rapid efflux of the antibiotics in resistant strains contrasting their bactericidal action [22], and it has been regarded as an alternative mechanism of clarithromycin resistance [23]. Intriguingly, the ability of amoxicillin in destroying the bacterial wall seems to impair such a transmembrane efflux system, resulting into an intracellular entrapment of the macrolide. The highest intracellular macrolide concentration - due to both a higher access and a lower efflux through an already riddled membrane - could overcome the rescue attempt engineered by the bacterium to prevent the fatal clarithromycin-ribosomal binding. In fact, there is evidence that pre-treatment with amoxicillin reduce the onset of secondary clarithromycin resistance [24].

Other penicillins, such as ampicillin, are largely inactivated in the stomach and, therefore, are largely useless for *H. pylori* therapy. Similarly, the amoxicillin/clavulanic acid combination does not offer any advantage over amoxicillin alone, since *H. pylori* is not a beta-lactamase producer. By excluding a specific Italian area (Sardinia) and Korea where an amazing 18.5-26 % rate was observed [25,26], both primary and secondary amoxicillin resistance is

rare worldwide, being estimated to be as low as 0-1.3 % in several countries [27].

Tetracyclines

Tetracycline is acid stable and active at acid pH, achieving high concentrations in the gastric mucosa and exceeding minimal inhibitory concentration (MIC) of *H. pylori* for several hours. It is ineffective in eradicating this infection, but it is helpful for prolonged bacterial suppression. Some evidences suggest that its efficacy is improved by co-administration of bismuth salts. Such a phenomenon seems to be due to a higher concentration of tetracycline achieved on gastric mucosa during bismuth salts therapy, probably due to a "trapping" effect [28]. Indeed, tetracycline is generally administered together with bismuth salts and metronidazole. Although, both primary and secondary tetracycline resistances are infrequent [27], high doses (1.5-2 g) should be employed for *H. pylori* treatment, so that a large number of tablets is needed. This is a major limitation for this drug, only 250 mg/tablets being available in different European countries. Unfortunately, bismuth salts are no more available in some countries, further restricting the use of tetracycline for *H. pylori* therapy.

Kanamycin and minocycline were found to own powerful bactericidal effect against *H. pylori* strains *in vitro* [29]. Recently, the efficacy of a minocycline-based triple therapy has been also evaluated in both first- and second-line regimens [30]. Unluckily, the minocycline-amoxicillin combination achieved an eradication rate as low as 38.5 % when administered in patients never previously treated for *H. pylori*, even if primary minocycline resistance was absent. On the contrary, the minocycline-metronidazole triple therapy achieved an acceptably high (82.5 %) cure rate as second-line treatment [30].

Nitrofurans

Nitrofurans may be used as gastric luminal antibacterial agents. As far as *H. pylori* infection therapy is concerned, furazolidone and nifuratel have been used in combination with other drugs, generally in quadruple therapy as a "rescue" treatment. However, an acceptable high (> 85 %) eradication rate has been reported in first-line therapy by using a quadruple combination with proton pump inhibitor (PPI), bismuth salts, amoxicillin and either furazolidone or nifuratel [31]. This could be due, at least in part, to low *H. pylori* primary resistance towards these compounds. Indeed, some studies found a furazolidone resistance rate as low as 0-8.7 % [32,33]. Nifuratel seems to be better tolerated than furazolidone, a prevalence of side-effects of 3 % and 21 %, respectively, being reported in a comparative study in children [31]. Nitrofurans offer the advantage of a low cost but, unfortunately, their use is not allowed in European countries.

Bismuth Salts

Bismuth salts (subcitrate and subsalicylate) were the first molecules successfully used as a monotherapy for *H. pylori* eradication in peptic ulcer patients [34]. They act detaching the organism from the mucosa and causing their lysis. These compounds have been generally used as a first-line triple therapy with tetracycline and metronidazole for 2 weeks

(American therapy) or as a second-line quadruple therapy with the same antibiotics and PPI for 7 days (European therapy) [35]. Because of its short-half life in gastric mucus, bismuth should be administered frequently, at least 3-4 times daily. Unfortunately, bismuth compounds - including ranitidine bismuth subcitrate - are no more available in several European countries due to their potential neurotoxic effect. Indeed, a study found that 9 % of patients receiving the quadruple regimen had very high blood bismuth concentrations within the Hillemand alarm level [36].

Macrolides

Currently, clarithromycin is the most powerful drug against *H. pylori*, with MIC values as low as 0.01 mg/L [27]. Like to amoxicillin, clarithromycin is actively secreted in the gastric juice [21]. A dual therapy with IPP or ranitidine bismuth subcitrate and clarithromycin 1 g daily was proposed for 2 weeks in the nineties, with a cure rate of 60-80 %. However, it was observed that secondary clarithromycin rapidly developed in all eradication failure patients. Moreover, primary clarithromycin resistance is remarkably increased in the last five years worldwide, being quoted as high as 10 % (range: 2-25 %) [27], and constantly higher than 15 % in more recent evaluations [15,16], even in children [37]. Therefore, clarithromycin (500 mg twice daily) is currently used only in triple or quadruple combination for 7-4 days.

Azithromycin (500 mg u.d for 3 days) has been used instead of clarithromycin as triple therapy in combination with PPI and either amoxicillin [38], tinidazole [39], or levofloxacin [40] administered for 7 days. Moreover, it has been administered as quadruple therapy with PPI, bismuth and amoxicillin. However, all these studies found eradication rate < 75 % and, therefore, the use of this macrolide does not offer any advantage as compared to clarithromycin, further considering that a 32.3 % primary resistance rate has been recently reported [26].

Erythromycin is available as base, stearate, ethylsuccinate, and erythromycin estolate. Although very low MIC values have been observed *in vitro*, *H. pylori* eradication rates achieved *in vivo* were low, mainly due to the high instability of erythromycin at low pH values. It has been used as enteric-coated erythromycin base delivered as small granules within a capsule and erythromycin-base-film-coated tablets, but 8 tablets daily are required to achieve a 80 % eradication rate in quadruple therapy [41], heavily limiting the compliance of patients.

Nitroimidazoles

Nitroimidazoles are active drugs for anaerobic infections and differ from each other in dosage and half-life. They act by altering membrane redox potential. Contrarily to other antibiotics, nitroimidazoles are quite stable in the gastric juice, and this characteristic is of paramount importance for *H. pylori* therapy. Among imidazole-derivatives, both metronidazole and tinidazole are largely used for *H. pylori* treatment as triple or quadruple therapy [11,35]. Some data suggest that the clarithromycin-imidazole is more effective than amoxicillin-imidazole combination. However, primary metronidazole resistance is very high, with values ranging from 20 % to 40 % both in Europe and USA, and with values

as high as 70-80 % in developing countries, especially in female [27]. This depends on the use of nitroimidazoles for parasites or vaginal infection.

Quinolones

Several quinolones exert *in vitro* a bactericidal action against *H. pylori*. However, some of these, such as ciprofloxacin or norfloxacin, are largely inactivated in gastric juice. Undeniably, the most experienced fluoroquinolone is levofloxacin. It has been used both for first- and second-line therapy, in a triple combination with either amoxicillin, tinidazole or clarithromycin, generally achieving acceptably high eradication rates [42, 43]. Unfortunately, levofloxacin is quite expensive, and *H. pylori* primary resistance seems to be increasing worldwide, with a prevalence ranging from 9.7 % to 21 % [44,45]. Therefore, its use should be reserved as a "rescue" triple therapy [46], being significantly more effective than standard quadruple therapy [47,48].

Moxifloxacin-based triple therapies either with clarithromycin [49], tinidazole or amoxicillin [50] have been recently proposed as first-line *H. pylori* treatment. All these regimens achieved an eradication rate > 90 %, but data of only two hundred patients, all enrolled in a single centre, are available. Moreover, it should be considered that moxifloxacin is similarly expensive to levofloxacin. Akin to levofloxacin, a moxifloxacin-based triple therapy has been recently tested as second-line regimen with an eradication rate significantly higher as compared to a quadruple therapy [51].

Interestingly, garenoxacin administered alone for 14 days at oral doses of 400 mg demonstrated activity against *H. pylori*, and it was safe and well tolerated [52], whilst not impressive results have been observed by using a gatifloxacin-amoxicillin combination [53,54].

Rifamycines

Rifabutin is a rifamycin-derivative with a powerful activity against *H. pylori* strains *in vitro*. A therapeutic approach based on a rifabutin-amoxicillin combination has been proposed for patients who failed two or more courses of standard proton pump inhibitor-based triple regimens, with an eradication of 71-86.6 % after a 7-day regimen [55,56] and 90 % following a 12-day therapy [57]. However, this is an expensive antimycobacterial drug particularly useful for tuberculosis treatment (e.g. in AIDS patients) and, therefore, resistance development for this drug should be avoided as much as possible. Moreover, the onset of myelotoxicity has been recently observed following rifabutin-based regimen for *H. pylori* eradication, suggesting more caution in this approach [58]. Therefore, such a drug should be employed after failure of other therapeutic approaches, exclusively in patients with multi-resistant strains in whom *H. pylori* cure is strongly indicated (MALT-lymphoma, complicated peptic ulcer, etc.).

4. CURRENT THERAPIES

Triple therapy has the advantage of luminal and systemic activity. A 14-day combination of bismuth, tetracycline, and metronidazole (American triple therapy) was effective in up to 90 % of patients, but patient compliance resulted to be low due to both the very high number of tablets administered in a

four daily schedule and the high incidence of side-effects [35]. A 7-day triple therapy, comprising a proton pump inhibitor (or ranitidine bismuth citrate), clarithromycin and amoxicillin (French therapy) or tinidazole (Italian therapy) is advised as a first-line therapy in the current European guidelines [35]. However, two very large meta-analysis showed that standard triple therapies fail to eradicate *H. pylori* in up to 20 % of patients [59,60]. In the forthcoming update European guidelines, the use of a 14-day triple therapy or a 7-day quadruple therapy (PPI, bismuth salts, tetracycline and metronidazole) has been suggested. However, only a minor therapeutic gain (+ 6-8 %) has been achieved by prologing the standard triple to 14 days [61], whilst the cost of therapy is doubled. Moreover, a meta-analysis found that even the 7-day quadruple therapy does not offer any significant advantage as compared to 7-day triple therapy, bacterial infection being cured in 80 % of 559 and in 79 % of 569 patients, respectively [62].

In order to improve the eradication rate of standard triple therapies, a different combination of the available antibiotics has been recently conceived, consisting in a novel 10-day sequential regimen [63]. This schedule is a simple dual therapy (PPI plus amoxicillin) given for the first 5 days followed by a triple therapy (PPI, clarithromycin, and tinidazole) for the remaining 5 days. Interestingly, such a therapy regimen was proved to be highly effective in a large Italian multicenter study, irrespectively of gastroduodenal disease, smoking habit, CagA status, and primary clarithromycin resistance [64,65].

5. NEW MOLECULES

In the last 5 years, several compounds owing antibacterial activity *in vitro* against *H. pylori* have been claimed, and few of these novel antibiotics have been also experienced *in vivo* either in animal models or humans.

In this field, quinolones certainly remain the most investigated drugs. It has been observed that both sparfloxacin and tosulfloxacin exhibit a similar bactericidal activity *in vitro* against *H. pylori*, including clarithromycin and metronidazole resistant strains, with the same MIC₉₀ of levofloxacin [44]. The efficacy of gemifloxacin against some *H. pylori* strains has been investigated *in vitro* [66]. Interestingly, such a drug showed MIC₉₀ values of 0.13 mg/L, exhibiting a more powerful action as compared with other fluoroquinolone derivatives. Moreover, both sitafloxacin and clinafloxacin were tested for activity, showing MIC₉₀ values as low as 0.008 and 0.12 mg/L, respectively [67]. Finally, a new fluoroquinolone (Y-904) was found to own an *in vitro* antibacterial activity similar to that of clarithromycin, showing an equal strong activity at pH values 5.5 and 7.0 [68]. Interestingly, this drug cleared *H. pylori* infection in *Mongolian gerbils* with a potency 30-fold higher than that of clarithromycin and levofloxacin.

The *in vitro* activities of mupirocin, quinupristin/dalfopristin, linezolid, eperzolid, have been tested [67]. Among these drugs, only mupirocin was very active at both pH values 7.4 and 5.4 with MIC₉₀ of 0.25 and 0.12 mg/L, respectively. Among new molecules, the efficacy of novel polycyclic compounds against some *H. pylori* strains has been investigated *in vitro* [69]. Of note, such agents showed

MIC values of 0.20-0.39 mg/L, exhibiting a similar efficacy of tetracycline. Patents of pleuromutilin derivatives have been also claimed [70]. The activities of three compounds were determined against *H. pylori* isolates, and some of these molecules exhibited a powerful bactericidal activity. In detail, I-valnemulin showed MIC values of < 0.0125 to 0.5 compared with MIC values of 2-128 mg/L and 0.2-0.4 mg/L for metronidazole and tetracycline, respectively, tested on the same *H. pylori* strains [70]. A prototype carbamate 12a of benzimidazol-derivatives has been found to display a very potent and selective antibacterial activity *in vitro* against *H. pylori*, showing *in vivo* a pharmacokinetic comparable with those of other antimicrobials currently used in triple therapy [71]. Noteworthy, these compounds were also active against those *H. pylori* bacterial strains with both metronidazole and clarithromycin resistance. Moreover, the antibacterial activity of a new benzamide derivative (BAS-118) against *H. pylori* has been investigated [72]. Of note, this molecule showed *in vitro* MIC₉₀ values as low as 0.013 mg/L, without a significant difference between strains with either clarithromycin or metronidazole resistance and those susceptibles. It has been demonstrated that a new family of natural antibiotics (pyloricidin A, B, and C) exhibits a potent and highly selective bactericidal activity against *H. pylori* with a MIC₉₀ value of 0.013 mg/L [73]. Recently, several arylthiazole analogues have been tested showing a potent bactericidal effect against *H. pylori*. Among these compounds, thienylthiazole derivative-44 exhibited the strongest activity, with MIC₉₀ values as low as 0.0065 mg/L [74]. In addition, such compounds cleared the infection in 60 % of Mongolian gerbils upon 7-days, twice daily, oral administration as monotherapy. A novel cephem derivative (FR 193879 8a) has been found to exert a potent therapeutic efficacy against *H. pylori*, showing an excellent safety in dogs [75]. Patents of enteroperoxide containing compounds, able to inhibit the growth of ferrous-dependent bacteria, have been also claimed [76]. In detail, artemisinin and artesunate have been tested *in vivo* in an animal model. Of note, two weeks following infection the mice were orally administered with 50 mg/kg t.i.d of these molecules for 8 days. The number of colony forming units derived from homogenized stomach on days 4 and 8 was significantly reduced as compared to placebo. Bismuth salt of antibiotics of the moenomycin group (the so called phophoglycolipid antibiotics) have been tested *in vitro*, but the MIC₉₀ value (0.5 mg/L) against *H. pylori* has been not impressive [77]. Finally a novel oral dosage composition comprising an antibiotic that belongs to the nitrofurans class and a second antibiotic that belongs to macrolide or tetracycline class have been claimed [78]. When these compounds were administered for 7-14 days together with a proton pump inhibitor to 10 patients, *H. pylori* infection was cured in all the cases. However, it should be noted that the use of nitrofurans derivatives is not permitted in Europe.

The urease production is crucial for viability of *H. pylori* in the stomach. Some plasmids able to inactivate the UreA gene have been prepared [79]. The inactivation of urease production by these plasmids has been found to strongly reduce the viability of *H. pylori* strains. New anti-urease and anti-flagella antibodies obtained from eggs of immunised hens have been claimed [80]. These antibodies have been

found to be effective for eradicating *H. pylori* adhered to gastric mucosa and suppressing the occurrence of gastritis in animal models.

6. CURRENT & FUTURE DEVELOPMENTS

H. pylori infection is a worldwide spread disease which causes several, and potentially lifethreatening, gastro-duodenal disease. Although it remains asymptomatic in a large percentage of subjects, several patients undoubtedly need to be currently treated for such an infection. The treatment is mainly based on a combination of proton pump inhibitor together with 2 or 3 different antibiotics. However, the therapy regimens suggested have given disappointing eradication rates in several countries. To find out new drugs in order to improve *H. pylori* eradication rate is of a paramount importance in primary medical care. Indeed, the management cost of this infection deeply depends on the efficacy of first-line therapy, the "rescue" treatments being generally more expensive and less effective.

Several patents of new antibiotics have been claimed in the last years, some of which showed a very potent antibacterial activity *in vitro*. Different molecules among quinolones have been tested, with preliminary interesting results. Among new patents, both benzamide derivatives and pyloricidins should be regarded as very promising molecules, due to their high efficacy even towards clarithromycin and/or metronidazole resistant strains. Moreover, a thienylthiazole derivative showed a very impressive anti-bacterial activity against *H. pylori*, showing the strongest activity *in vitro*. Other molecules have been found to exhibit a similar efficacy at both neutral and pH 5, and this characteristic is a clear advantage for *H. pylori* therapy, since antibiotics must act in the gastric juice. Finally, some compounds seem to be safe and effective also *in vivo*, although data are still preliminary.

In conclusion, several interesting patents have been claimed in the last 5 years and, therefore, it is foreseeable that the arsenal against *H. pylori* will be increased in the next future.

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