REVIEW

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Therapeutic and clinical regimens against *Helicobacter pylori* infections in humans: an overview

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1. Introduction

Peptic ulcer has emerged as one of the major pathological ailment affecting nearly 50% of the world population and imposes a set of challenges [1]. Peptic ulcers could be characterised by lesions and excretions of mucosa of the upper gastric region with stomach and duodenum being the most affected areas. The pathophysiology of peptic ulceration has so far been ascribed to an imbalance between corrosive factors (acid pepsin complex) and protective factors (mucosal barrier and prostaglandins). Recently, the World Health Organization (WHO) revised the pathophysiological approaches by identifying the bacterium *Helicobacter pylori* as the causative organism for peptic ulcers. *H. pylori* has been implicated in the etiology of chronic gastritis and chronic peptic ulceration, associated with an increased risk of gastric adenocarcinoma (or gastric mucosa-associated lymphoid tissue lymphoma), particularly when the organism harbors in the g.i. tract for decades [2].

Traditionally, bacterial infections have not been considered as major causes of cancer. Recently, however, a bacterial infection has been explored to be responsible for cancer involving the induction of chronic inflammation and the production of a carcinogenic bacterial metabolite [3]. The most specific example of the inflammatory mechanism of carcinogenesis is a *H. pylori* infection. *H. pylori* has been epidemiologically linked to adenocarcinoma of the distal stomach for its well known tendency to cause lifelong inflammation. The persistent inflammation in turn is thought to cause cancer probably by inducing cell proliferation and production of mutagenic free radicals and bacterial metabolites [3]. The International agency for research and cancer (IARC), recently declared *H. pylori* to be a Group I carcinogen, a definite cause of human gastric cancers [4]. The report ignited well directed interest among gastroenterologists and the pharmaceutical industries, who have prepared themselves to take up the problem seriously to evolve effective therapeutic strategies. The regimen combines efforts to raise pH in the stomach acid environment as well as to eradicate the bacterial infection.

2. Epidemiological and etiological profile of *H. pylori*

H. pylori is epidemiologically linked with a wide range of pathological disorders. The probabilities of *H. pylori* associated diseases in developed and in developing countries are presented in Fig. 1. *H. pylori* was first identified in the human mucosa with a patchy distribution overlaying the gastric epithelium [5]. It is a gram negative, spiral, microaerophilic S-shaped bacterium, which colonizes the gastric mucosa. Colonization and survival in the stomach is supported by several virulence factors. *H. pylori* produces several pathogenic elements that are critical for the establishment and maintenance of a gastric infection. Among these pathogenic determinants present in all the bacterial strains are a range of enzymes including urease [6], catalase and superoxide-dismutase [7]; the flagella [8]; and a number of adhesins (adherin proteins) that assure tissue specific colonization [9]. In addition, a subset of *H. pylori* strains produces a potent cytotoxin (VacA) associated with a surface exposed immuno-dominant antigen (CagA) [10, 11]. Isolates of *H. pylori* can be classified into two groups, characterized by whether or not they produce the vacuolating cytotoxin and contain the cytotoxin-associated gene (Type I and Type II strain respectively).

2.1. Virulence factors present in all H. pylori strains

2.1.1. Flagellins

Multiple sheathed flagellae are characteristic to all the strains of *H. pylori* allowing the bacterium to move in the viscous mucous layer of the stomach. *Helicobacter* typically moves with the help of a five- or six-polar flagella in the viscous mucous layer of the gastric epithelium. The flagellae are composed of a major (FlaA) and a minor (FlaB) flagellin (~53 kDa) proteins [12, 13]. Motile mutants of the FlaA gene express a considerable colonization of the gastric mucosa of gnotobiotic piglets. A disruption of the FlaA gene results in a non-flagellated, non-motile phenotype; whereas disruption of the FlaB gene expresses no obvious phenotype character. The function of FlaB therefore remains unclear.

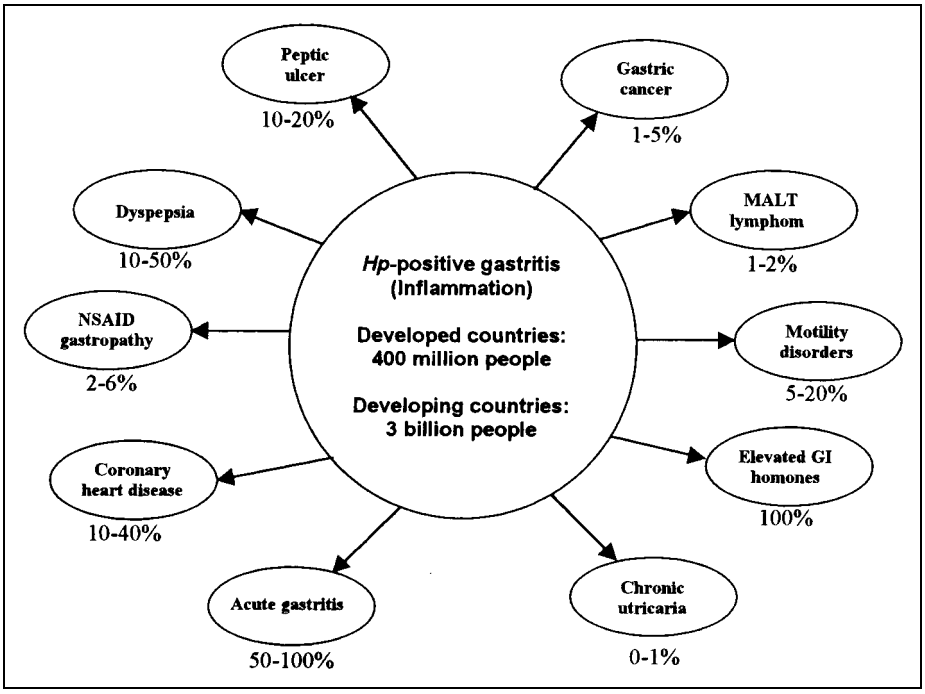

Fig. 1:
Probabilities for *Helicobacter pylori* associated diseases with their respective occurrence profile

2.1.2. Urease

H. pylori strains produce a patent urease, which is a large complex (~350 kDa) of two subunits of 26.5 (UreA) and 61 kDa (UreB) in a 1:1 stoichiometric ratio [6, 14]. Disruption of either the UreA or UreB gene results in a urease negative phenotype. In the gnotobiotic-piglet model, urease has been shown to be essential for colonization; urease negative mutants obtained by gene disruption failed to colonize. Urease may further lead to neutralization of the acidic micro-environment and elevate the pH of the gastric fluid, thus enabling the organism to survive [15]. In addition, the ammonia released by urease may also contribute to the damage of the gastric epithelium. This has led the researchers to explore the role of urease in the induction of a protective immune response.

2.1.3. Adherin factors

The bacterium *H. pylori* adheres to the cells of the gastric epithelium using some special adhesin (adherin protein) receptors like 20 kDa *N*-acetyl-neuraminillactose binding fibrillar haemagglutinin (HPhA), a protein that binds to the membrane lipid associated phosphatidyl-ethanolamine and gangliotetraosyl ceramide, and Lewis^b (Le^b) blood group antigens [9, 16]. Soluble glyco-proteins belonging to the Le^b blood-group antigens or antibodies against the Le^b could inhibit bacterial binding to the human gastric mucosa. This finding in part may reveal the possibility of occurrence of gastric infections in individuals with blood group 'O'. A plethora of putative attachment factors has been investigated in *H. pylori* to establish the bacterium in the forbidding environment of the gastric domains [9]. In addition, α-2,6-linked terminal sialic acid recognizing lectin present on the surface of *H. pylori*, polyglycosyl ceramides and acidic glyco-sphingolipids/sulfatides (I_3SO_3Gal cer$^-$ and GM_3^- ganglioside) may act as receptor molecules for the organism [17].

2.2. Virulence factors expressed specifically by the Type I strain

Most of the strains of *H. pylori* elaborate and release a potent cytotoxin VacA (vacuolating antigen A ~94 kDa), which produces vacuoles in cultured gastric cells. These vacuoles resemble histologically the lesions in patient biopsy material. This vacuolating action of VacA has been attributed to a destruction of the gastric epithelium, although in the absence of an appropriate animal model, the role of this protein could not be realized with experimental supports. The strains of *H. pylori* also express and present a protein CagA (cytotoxic associated gene A ~130 kDa) and a surface exposed genome expressing VacA. Strains of *H. pylori* could be classified into two broad groups [18] based on the cytotoxic activity of the bacterial cytotoxins. Type I strains are the strains that express both VacA and CagA proteins; Type II strains do not express cytotoxin VacA and the genome expressing VacA, i.e., CagA. Data from animal models and serological studies with the CagA protein (evaluated as second-generation diagnostic for *H. pylori*) suggest that Type I strains predominate in patients with ulcers [19] and gastric adenocarcinoma [20]. Fig. 2 explains the role of bacterial virulence factors in infection, which could serve in future as a basis for vaccine development [21].

Table 1: Various types of gastritis with their etiology and pathogenesis

Gastritis	Pathogenesis	Etiology	Diagnosed as
Type A	Auto-immune disease	Immune responses	Asymptomatic gastritis
Type B	Bacterial infections	*Helicobacter pylori* colonization	Glandular atrophy with some risk of adenocarcinoma
Type C	Chemical injury	NSAID's, alcohol, enterogenic reflexes	Epithelium degeneration and gastric hyperplasia

* Adapted with modification from [22]

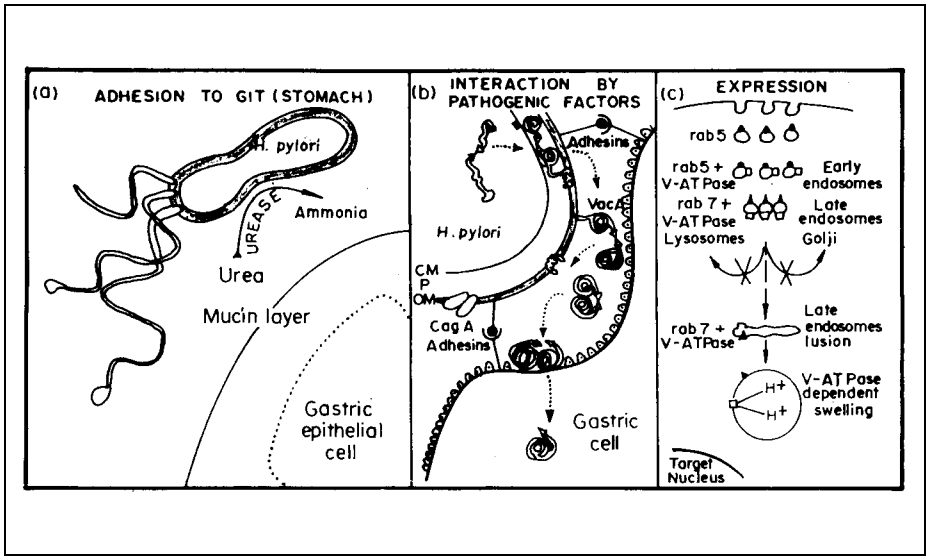

Fig. 2:
Schematic representation of different stages of infection by Type I *H. pylori* bacteria. (a) Bacterium swims in the gastric mucous layer (through flagella) with the production of ammonia (cytotoxic?) which neutralizes the low pH of gastric lumen. (b) Interaction of the bacteria with the target cells using some adhering proteins and cytotoxic proteins. The cytotoxin precursor inserts in the outer membrane and mediates export of the 94 kDa cytotoxin monomer, which subsequently gets processed to interact with the target cells. (c) A model of the effect of intracellular and sub-cellular interaction of the cytotoxins with the target cells. Following the fluid phase pinocytosis pathway, the late endosomes, which accumulate rab7, fuse with each other to form the large vacuole mediated through the activity of V-ATPase proton pump and approaches the target cells. Abbreviations: CM, cytoplasmic membrane; P, periplasm; and OM, outer membrane

3. Pathophysiology of gastric infections caused by *H. pylori*

Infection of the stomach due to *H. pylori* can occur as early as at the age of infancy, presumably through fecal-oral or salivary transmissions, and may persist for lifetime. These ulcerations can be fatal in nature. They are causative of acute gastritis associated with an increased risk of gastric adenocarcinoma, especially in the developing countries where socio-economic and hygienic conditions are not appropriate as devised. All *Helicobacter* species often cause some degree of persistent inflammation during their proliferation in the mammalian stomach. Gastritis is found in virtually all infected human hosts. Various types of gastritis with their pathogenesis and etiology [22] are presented in Table 1. Persistent inflammation can eventually lead to a destruction of the normal epithelium, loss of mucous layer, and increased cell turnover, a condition referred to as atrophic gastritis with a serious risk factor for gastric cancer [3]. In fact, gastric adenocarcinoma is 10–12 times more likely to develop in individuals infected with *H. pylori*, whereas the latter has been linked to the development of low-grade, B-cell lymphomas of gastric mucosa-associated lymphoid tissues (MALT) [23]. An existence of a positive correlation between chronic persistence of *H. pylori* infection and patho-physiology could be highlighted [3] as indicated in Fig. 3.

4. Pathogenesis of peptic ulcers

Recent evidences relate *H. pylori* to the pathogenesis of chronic duodenal ulceration in more than 95% of the cases [24]. The organism grows only over the gastric (mainly antral) epithelium with areas of intestinal metaplasia spread in the antrum. The initial event in the pathogenesis may be a gastric metaplasia due to hyperacidity subsequently followed by a *H. pylori* induced gastritis [25]. The infection is paradoxically facilitated by a defense response against hyperacidity. If, however, the infection is once established, it surpasses and effectively deters the mucosal defense (mucosal injury). This in turn may obscure the presence of any infected gastric metaplasia, depending on the survival and harbouring of the invading organism with regard to the period of time (opportunistic pathogenesis). The pathogenesis of *H. pylori* is explained [25] schematically in Fig. 4.

Progress in the understanding of the pathogenesis of *H. pylori* infections has been obscured due to the non-availability of suitable animal models to study the relatively rare ulceration associated carcinogenesis due to *Helicobacter* infections. At present, several *Helicobacter* species are known and almost all except *H. pylori* are of animal origin [26]. *H. felis* can be administered into experimental mice to cause intense colonization and inflam-

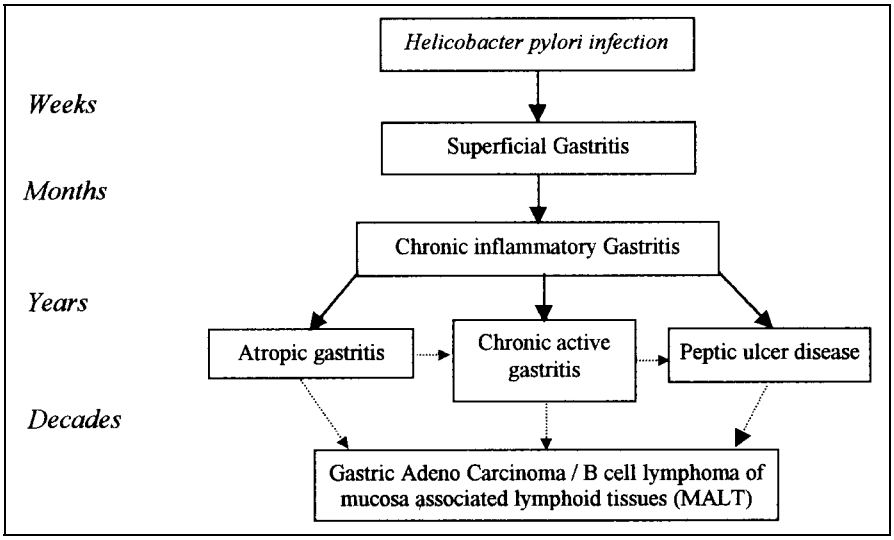

Fig. 3:
Schematic diagram showing the progression of *H. pylori* infection. Opportunistic pathogenesis of the bacteria may lead to the gastric infections ranging from the superficial gastritis to peptic ulcer and/or ultimately to the gastric lymphoma, depending upon the survival period of *H. pylori* in the mammalian host

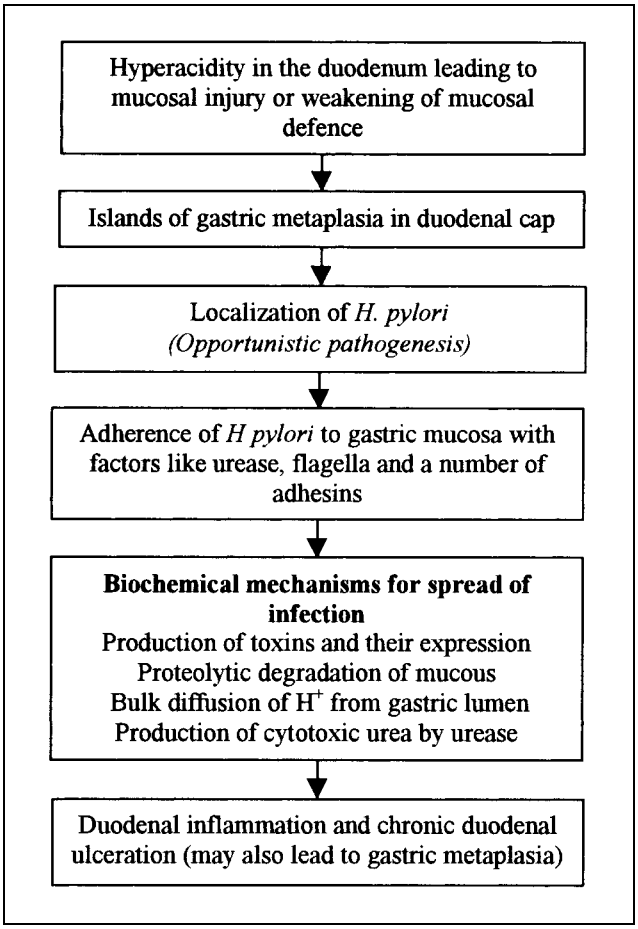

Fig. 4: Pathogenesis based on *Helicobacter pylori* infection. Various gastric adherence factors and biochemical mechanisms involved in the pathogenesis are presented

mation of the stomach [27]. Such models have been used to study vaccine adjuvants, delivery systems and therapeutic vaccination [28]. Marchetti et al. [29] described a mouse model of persistent *Helicobacter* infection, which provides a novel opportunity to examine the interaction between *H. pylori* and a conveniently available mammalian host. Recently, standardized CagA and VacA positive mouse models have been introduced, named as Sydney strain of *H. pylori* (strain SS1), with high colonizing ability. These models serve for vaccine development, compound screening and pathogenesis explorations [30].

5. Current drug regimes

The realization that peptic ulcer is an infectious disease caused by *H. pylori* has revolutionized the approaches of diagnosis and therapy of peptic ulcers. Treatment of peptic ulcers with H_2-receptor antagonists, proton pump inhibitors and mucosal protectants has been replaced partially or totally by antibiotics or antimicrobials or a combination of these. As a result of empirical clinical investigations it has been realized that various antimicrobial drugs possess activity against *H. pylori*. Monotherapy with antimicrobials like tetracycline, amoxycillin, clarithromycin, ciprofloxacin, metronidazole, tinidazole and bismuth compounds (tricitrato-bismuthate, TDB or bismuth-subcitrate, BSC); or with antisecretory agents like omeprazole, lansoprazole, ranitidine, cimetidine and famotidine; provide promising results *in vitro*. However, an encouraging *in vivo/in vitro* correlation could not be achieved [31, 32]. Limitations of mono-therapy are attributed to the ecological niche of *H. pylori*, living not strictly within the gastric mucosa, but in the mucosal layer below the epithelium, where adequate penetration and localization of antimicrobials is difficult [33]. Another factor could be the rapid development of drug resistance by the organisms, owing to sub-therapeutic concentrations due to an impermeability of the bacterial envelope to the antimicrobial, or due to inactivation of potent drugs from hydrolysis by β-lactamases or exogenous enzymes [34]. Improved success rates and a suppression of resistance have been recorded when drugs were administered in combination [35, 36]. Precise comparisons between different regimens cannot be made with confidence due to a lack of prospective randomized studies. Triple therapy with a combination of antibiotics, antimicrobials and/or antisecretory agents is the most widely accepted regimen worldwide [37–40]. The addition of an antisecretory agent like omeprazole or a H_2-receptor antagonist to the basic "triple antimicrobial therapy" improves efficacy with a cure rate rising over 95% following a 7-day treatment regimen [37, 40, 41]. This quadruple therapy regimen is generally reserved particularly for refractory or large *H. pylori* based gastric infections [42]. All therapeutic regimens reported in the literature are summarized in Table 2 with their present status and representative multiple combinations.

5.1. Triple therapy regimen

Triple therapy is a well-established therapeutic regimen against *H. pylori* induced gastric infections. The choice of appropriate drugs depends on the characteristics of the *H. pylori* infection, the localization depth in the gastric mucosa, the physico-chemical properties of the gastric medium especially acidity (which deactivates antimicrobials), slow bacterial growth and organism sensitivity to antibiotics. The antiinfectious treatment is mainly based on a triple drug regimen combining an antisecretory drug (proton pump inhibitors or H_2 receptor antagonist) and two well-optimized antimicrobials: tetracycline or clarithromycin and/or associated amoxycillin or an imidazole derivative (metronidazole or trinidazole). Triple therapy based on tetracycline/clarithromycin (500 mg), in combination with metronidazole (250 mg) and tri-potassium dicitrato-bismuthate (TDB, 240 mg) each four times daily for two weeks has been found more effective than monotherapy [37–39, 43]. Using this regimen, infection has been successfully eliminated in 85–95% of the cases [40]. Schutze and Hentschel [44] evaluated the efficacy and safety of a one week triple therapy without bismuth and metronidazole. The pilot study with lansoprazole, amoxycillin and clarithromycin has been found to be highly effective in terms of duodenal ulcer healing and symptomatic relief. On the other hand, Berstad et al. [45] advocated the pharmacodynamically significant role of spiramycin (Rovamycin) as an alternative to tetracycline in the therapy of *H. pylori* associated peptic ulcer. Triple therapy with spiramycin (1.5 MIU) in combination with BSC (150 mg q.d.s.) and metronidazole (400 mg t.d.s.) for 10 days was found to be effective in an open pilot study with a 12 month followup. Adamek et al. [46] reported that a short term triple therapy regimen consisting of pantoprazole (40 mg twice daily), clarithromycin (500 mg daily) and metronidazole (500 mg twice daily) is highly efficacious in curing *H. pylori* based gastric infections. An interesting randomized controlled pilot study with an one year follow up was conducted by Saberi-Fironzi et al.

Table 2: Clinical drug regimens with their current status reported in the literature

Therapy/mode	Status/comments	Drug(s) regimen	Ref.
Mono therapy	Rapid development of resistance, high relapse rates, GIT disturbances, Hypergastrinemia on long term therapy, Increased risk of intestinal transmitted infections	Metronidazole	[81]
		Clarithromycin	[82]
		Amoxycillin	[83]
		Tetracycline	[32]
		Ranitidine/cimetidine	[84, 85]
		Omeprazole	[33]
		Bismuth compounds	[86]
Dual therapy	Development of resistance, relapse may delay for several months but recurrence is due after termination of therapy, better regimen than monotherapy	Bismuth (TDB) + amoxycillin	[39]
		Bismuth (TDB) + metronidazole	[36]
		Bismuth (BSC) + erythromycin	[52]
		Clarithromycin + omeprazole	[55]
		Clarithromycin + amoxycillin	[35]
		Omeprazole + amoxycillin	[87]
		Amoxycillin + tinidazole	[37]
Triple therapy	Best among the all therapeutic regimens, Better tolerability, Effectively balancing clinical efficacy, Patient compliance, Cost effective regimen	BSC + metronidazole + tetracycline	[40]
		BSC + metronidazole + amoxycillin	[38]
		BSC + metronidazole + clarithromycin	[38]
		BSC + furazolidone + amoxycillin	[39]
		Omeprazole + amoxycillin + tinidazole	[49]
Quadruple therapy	Reserved for refractory or large *H. pylori* positive ulcers, optimal *H. pylori* therapy, Costly regimen	Omeprazole plus triple antimicrobial therapy	[88]
		Omeprazole plus triple antimicrobial therapy	[89]

[47]. Different regimens studied and compared by these workers are: (i) dual therapy regimen based on omeprazole (2×40 mg) and amoxycillin (4×500 mg) for two weeks, (ii) a triple therapy regimen with omeprazole (20 mg) together with amoxycillin (4×500 mg) and tinidazole (2×500 mg) for two weeks and (iii) an extended triple therapy regimen conducted with bismuth nitrate (4×375 mg), metronidazole (4×250 mg) and tetracycline (4×500 mg) daily for two weeks and ranitidine (150 mg) for one week and bismuth nitrate (4×375 mg) for another two weeks. The two week triple therapy following an additional two week treatment with a bismuth derivative appeared to be an effective and economic treatment not only for the eradication of *H. pylori* but also for the healing of acute duodenal ulcers. deBoer et al. [48] critically evaluated the efficacy and side effect profile of two currently advocated treatment regimen, i.e., dual and quadruple therapy for eradicating *H. pylori* in ulcer patients. The results of the randomized prospective single centre study clearly revealed that a two week dual therapy (omeprazole plus amoxycillin) was relatively less effective in providing the cure when compared against a week of quadruple therapy (omeprazole, colloidal bismuth, tetracycline and metronidazole). The latter was well tolerated and highly effective against metronidazole sensitive as well as metronidazole resistant strains of *H. pylori*.

Recently a triple therapy regimen [49] has stirred the Indian drug marked. It is based on amoxycillin (500 mg), tinidazole (650 mg) and omeprazole (10 mg) each twice a day for one week and found to be superior among the existing modes of therapies for the *H. pylori* based gastric pathogenicity. Two antibiotics (clarithromycin and amoxycillin) as well as three antisecretory agents (lansoprazole, omeprazole and ranitidine) have been approved in France for triple therapy regimens of 1 or 2 weeks leading to approx. 90% eradication [50]. Multiple antimicrobial regimens may impose patient compliance problems, a development of resistance of *H. pylori* towards potent antimicrobials and are expensive.

5.2. Antimicrobial versus antisecretory agents

The results of many independent studies reveal that both duodenal and gastric ulcers unrelated to antiinflammatory drugs are healed as effectively by antimicrobal therapy as by antisecretory agents. The distinctive advantage of *H. pylori* eradication with antimicrobials enables a short-term treatment regimen to greatly reduce ulcer relapse rates. Antisecretory agents including omeprazole and H_2 receptor antagonists, on the other hand, reduce relapse rates as long they are given as maintenance therapy. They do not eradicate *H. pylori* and they may even aggravate the gastritis associated with the antral infection [51]. Perhaps for this reason, pretreatment with omeprazole has been shown to decrease the efficacy of antimicrobial therapy in some [52] but not in all [53] cases. This disadvantage, however, is not evident when antisecretory agents are given simultaneously with antimicrobials. Indeed, the addition of omeprazole to triple therapy improved efficacy in several studies. A cure rate over 95% following a 7-day regimen was found even in the presence of metronidazole resistant strains [54]. The somewhat marginal advantage could be gained by adding an antisecretory agent to the basic "triple therapy". The inclusion of an antisecretory agent however increases the daily drug costs by some ten folds and as a result the regimen is mainly reserved for refractory or large *H. pylori*-positive ulcers. Recently a dosage regimen consisting of clarithromycin and omeprazole has been advocated for the eradication of *H. pylori* [55]. The regimen offers no advantages in regard to relative effectiveness over other less expensive combinations. Nevertheless, it may be better tolerated than multiple antibiotic regimens with better patient compliance.

5.3. H. pylori infections in children

H. pylori is a major cause of antral gastritis is children, which is not necessarily presented symptomatically. The exception to this occurs in duodenal ulcer disease where *H. pylori* is symptomatically linked to the disease in chil-

dren. Duodenal ulcers are not commonly recurred in growing children following eradication of *H. pylori* infection. The importance of asymptomatic carriage of *H. pylori* in children, particularly in regard to the duration of this infection and the subsequent development of gastric cancer, remains to be established. *H. pylori* is associated with both hypochlorhydria and persistent diarrhea in children in developing countries, but the pathological significance of this association is still unclear.

Chong et al. [56] diagnosed children with *H. pylori* infections showing non-classical symptoms of recurrent abdominal pain (RAP syndrome) with positive serological tests using the cell-associated protein specific enzyme immunoassay kit. Although there is no consensus on the optimal regimen for treating *H. pylori* infections in children, dual therapy with amoxycillin and bismuth subcitrate for 2 weeks followed by monotherapy with bismuth-subcitrate for a further six weeks eradicates *H. pylori* infections in the majority of children. Those who relapse may be treated with a repeat course plus metronidazole for 4 weeks [57]. Compliance with such regimens is a problem and as a result shorther treatment courses that are equally effective in children need to be redefined.

6. Protective mucosal immunization

An intense medical campaign is being waged to cure *H. pylori* infection in patients with documented ulcer diseases and in patients at high risk for cancer. Antimicrobials and antibiotics within a versatile range of delivery systems, are not a long-term answer, rather, efforts are being made to provide protective (prophylactic) mucosal immunization. After continual and considerable efforts, researchers succeeded in adapting molecular biology and biochemistry to study this fastidious organism. This has triggered intensive research and led to a rapid increase of knowledge concerning the basics of *H. pylori* virulence (as well as that of experimental pathogens *H. felis* and *H. mustelae*) and the development of molecular methods for diagnosis and epidemiological research.

6.1. Identification of coadate antigens

Modern approaches towards vaccine design involve the identification of genetically detoxified protective antigens of the non-toxic and highly purified forms, hence eliminating the risk of reversion to virulence of the organism and/or more or less severe side effects. In the design of a vaccine against the bacterial pathogens, protective immunogens are usually sought among the virulence factors involved in induction of the disease.

Strategies for vaccine development are based on the molecular and biochemical etiology of the bacterium. Evidence for a direct role of the cytotoxin in the pathogenesis comes from studies in mice. Orally immunized mice with *H. pylori* antigens, including VacA and urease, were protected from subsequent challenges with viable Type I *H. pylori* strains. These results suggest that not only the vaccination might prevent *Helicobacter* infection. They also provide a cheaper and simpler animal model for the investigation of related factors, including the CagA associated gene products, that may be useful as vaccine antigens [58]. Thus, urease [59], flagella proteins [14], and *H. pylori* adherins, as well as cytotoxins, CagA and VacA [58], can be examined as potential vaccine candidates in experimental models. Intragastric administration of the extracts of type I, CagA positive, cytotoxic strains of *H. pylori* caused severe epithelial erosion, ulceration and inflammatory cell infiltration into the lamina proporia. Administration of type II, nontoxic strains caused only mild gastritis. Administration of highly purified, active VacA caused epithelial erosion similar to that caused by the extracts but did not result in inflammatory cell infiltration [59]. Hence, VacA is likely to play a major role in the epithelial damage caused by *H. pylori* infection of humans, and as such, after suitable detoxification it is considered to be a major candidate for inclusion in a vaccine regimen against *H. pylori* associated gastric infection. The etiological profile of the bacterium clearly reveals two possible and workable approaches for vaccination: genetically detoxified and antigenically active CagA protein, and, antibodies developed against the cytotoxic gene or genome responsible for the expression. A resent application of molecular biology in this area is the use of recombinant proteins for the development of an *H. pylori* vaccine [60].

On the other hand, other factors appear to be involved in the massive inflammation associated with an infection by type I strains. Recent evidences indicate that the gene products expressed only by these strains contribute to the production of factors, which induce interleukin-8 (IL-8) expression in gastric epithelial cells [61]. This cytokine is a potent neutrophil chemo-attractant and may be involved in inflammatory cell filtration. Furthermore, the CagA protein itself appears to be a dominant antigen for CD + 4 T cells isolated from gastric biopsies of peptic ulcer patients. The majority of the CagA specific clones isolated were of the T-helper cell-I (Th-I) type which produce tumor necrosis factor-α and interferon-γ, both potent inflammatory cytokines [62]. These immuno-dominant antigens could further be identified as potential candidates for immunization.

6.2. Assessing vaccine candidates

The potential for vaccination against *Helicobacter* infection was investigated using mice infected with the related species. *H. felis* was among the first organism revealing that oral immunization with lysates in combination with cholera toxin (CT) could induce protection against an infection with *Helicobacter* species. Protein antigens are generally poorly immunogenic when administered orally due to the intrinsic tolerance of the gastrointestinal system to ingested materials. Cholera toxin, and the very closely related heat labile toxin (HLT) from enteropathogenic *Escherichia coli* however, are not only highly immunogenic when administered orally but they also confer immunogenicity to coadministered antigens. Subsequently, it has been demonstrated that purified *H. pylori* urease could induce protection against *H. felis* when administered along with cholera toxin [68]. Until recently, several purified antigens have been shown to induce protective immunity in either *H. felis* or recently introduced *H. pylori* infected mice models. In addition to urease, purified VacA, recombinant VacA, CagA and 60 kDa heat shock protein (Hsp 60) and several other adherins induce protection on administration with mucosal adjuvant [63]. Combinations of purified antigens have also been tested. With a combination of recombinant urease (B subunit) and heat shock protein A (HspA), a complete protection against *H. felis* infection has been achieved in mice [64]. Moreover, an association of VacA and native purified urease was observed to confer full protection against *H. pylori* infection in mice [65].

6.3. Vaccines developed or proposed against H. pylori

An exhaustive account of the potential antigens and/or mucosal adjuvants is presented here to explain future vaccine strategies against *Helicobacter* species based gastric infections.

6.3.1. Safe mucosal adjuvants

Protection of germ free mice from gastric infection by *H. felis* after oral or passive IgA immunization could be initiated. In an *H. felis*, germ free mouse model, oral immunization with bacterial antigens and co-adjuvants like cholera toxin (CT) or its nontoxic subunit B (CTB) resulted in elevated serum, gastric and intestinal anti-*H. felis* antibody titres with subsequent protection of mice from acute infection [2, 65]. The observation that immunity could be induced with a mucosal adjuvant like cholera toxin B-subunit whole vaccine, opened the way for human studies with orally administered *H. pylori* vaccines.

To date, cholera toxin and *E. coli* heat labile toxins are the only known mucosal adjuvants capable of conferring antigenicity to the orally co-administered antigens. The toxicity of these molecules, however, effectively limits their use in humans. Recently the B-sub-unit of cholera toxin lacking the enzymatically active sub-unit was found to function as an adjuvant, but subsequent experiments showed that the adjuvancity of the commercially available preparations of sub-unit B was due to a contamination by small quantities of active toxins [65, 66]. More recently, genetically detoxified mutants of *E. coli* toxins have been isolated and were found to be completely free of toxic activity but exhibited the capacity to confer antigenicity to candidate antigens. One of these mutants (LTK 63) which contains a single amino acid constitution in the active site of the enzyme, has been proved successful in both prophylactic and therapeutic immunization against *H. pylori* when evaluated in animal models (Table 3) [67]. The nontoxic mutant functioned equivocally to the wild type toxin, as an adjuvant for the urease, CagA and VacA antigens.

6.3.2. Recombinant urease (R-Urease)

Oral immunization with recombinant *H. pylori* urease induces secretory IgA antibodies and protects mice from challenges from *H. felis* [68–70]. Intestinal IgA and serum IgA and IgG antiurease antibody responses were highest in the case of recombinant urease (R-urease) when orally immunized in the mice at the termination of the experiment. Mice immunized with R-urease were protected against infection on subsequent challenge with *H. felis* 2 or 6 weeks after oral immunization. Mice in the control group, on the contrary, developed multifocal mucosal lymphoid follicles. It was suggested that the gastric corpus may function as an effector organ of the mucosal immune system reflecting antigenic stimulation and mononuclear inflammatory response. Recently, Weltzin et al. [71] introduced novel intranasal immunization techniques for *H. pylori* urease with mucosal adjuvants to generate circulating and secretory antibodies. Repeated daily intranasal (i.n.) administration of antigen without an adjuvant elicited high levels of specific IgG in serum, and IgA in serum, saliva and feces. Once weekly i.n. immunization with co-administration of CT or *E. coli* heat labile toxin as an adjuvant elicited somewhat lower antibody levels to urease. When challenged with *H. felis*, only mice immunized with urease in the presence of adjuvants were protected against gastric infections.

6.3.3. Intracellular proteins

Proteins, normally intracellular, such as catalase as present on the surface of *H. pylori*, are potential epitopes for im-

Table 3: Prophylactic vaccination against *H. pylori*: Current status of mouse studies

H. pylori antigen	Adjuvant	Infection	Protection	Ref.
Prophylactic or protective immunization				
Sonicate	CT, LT, LTK 63	*H. pylori*	80–100	[29, 90]
Sonicate	CT, LT	*H. felis*	≅70	[91]
Urease (Ure)[a]	CT, LT, LTK 63	*H. pylori*	≅80	[29, 90]
Urease (Ure)[a]	CT, LT	*H. felis*	≅70	[65, 91]
UreB subunit	LT, CT	*H. felis*	25–70	[91, 93]
R Urease	LT	*H. felis*	60–100	[69]
R Urease + sonicate	CT	*H. felis*	≅100	[68]
HspA	LT, CT	*H. felis*	≅50	[64]
HspB	LT, LTK 63	*H. pylori*	≅50	[64]
HspB	LT, CT	*H. felis*	≅50	[64]
UreB + HspA	LT	*H. felis*	≅50	[64]
VacA	LT, CT, LTK 63	*H. pylori*	≅80	[65, 91]
Catalase	CT	*H. pylori*	≅80	[72]
R Catalase	CT	*H. pylori*	≅90	[72]
R GroES like Hsp	–	*H. felis*	≅80	[75]
R GroES + UreB subunit	–	*H. felis*	≅100	[75]
Sonicate	CTB	*H. felis*	≅26	[65]
CagA	LTK 63	*H. pylori*	≅70	[64]
VacA + Urease[a]	LT, LTK 63	*H. pylori*	≅100	[65]
Therapeutic immunization				
Sonicate	CT	*H. felis*	70–90	[94]
Urease B subunit	CT	*H. felis*	≅50	[95]
Sonicate	LTK 63	*H. pylori*	70	[90]
Recombinant CagA	LTK 63	*H. pylori*	–	[90, 96]
Recombinant VacA	LTK 63	*H. pylori*	90	[96]

munization. The efficacy of an orogastric vaccine comprised of purified *H. pylori* catalase and a mucosal adjuvant, i.e., cholera toxin (CT) was examined against *H. pylori* and *H. felis* mouse models [72]. Groups of mice were challenged with the Sydney strain of *H. pylori* and were subsequently immunized using native/recombinant *H. pylori* catalase plus CT (vaccine antigens). Protective immunization in the animal models was found to be significant for R-catalase as compared against the native catalase. The study explores that intracellular proteins/enzymes may be presented as candidates for future vaccines.

6.3.4. Adherin proteins

Adhesins or adherin proteins expressed by *H. pylori* are promising handles to negotiate protective immunization. The *H. pylori* 19.6 kDa adhesin protein could be associated both with human and rabbit erythrocytes as well as with human-buccal epithelial cells. Polystyrene microspheres coated with the adherin-protein were found to agglutinate human, horse and rabbit erythrocytes, further exploring the role of adhesin to mediate adhesion between *H. pylori* and eukaryotic cells [73]. The ability to act as an adhesin may make this protein a vulnerable virulence factor for *H. pylori* and hence offer a potential target for the development of vaccine therapy. Major flagellin protein FlaA, an adherin determinant, was also investigated as an important vaccine candidate [14].

6.3.5. Heat shock protein

Exported proteins have a significant role in the pathogenesis of the bacterium and are ideal candidates for vaccine development. Identification of the *H. pylori* genes encoded proteins has been highlighted where the plasmid-vector pJEM11 based gene fusion has been adapted as a strategy [74]. Ferrero et al. [75] assessed the heat shock protein 60 expressed (HSP 60) on the cell surface of *H. pylori* as potential protective antigen in a murine model of gastric *Helicobacter* infection. Orogastric immunization of mice with recombinant *H. pylori* GroES- and GroEL-like heat shock proteins protected the *H. felis* infected mice. The same group proposed GroEs-like heat shock protein and urease B-sub-unit proteins as potential components of a *H. pylori* sub-unit vaccine.

6.3.6. Bacterial ghost

Bacterial ghosts are used as non-living candidate vaccines and represent an alternative to heat or chemically inactivated bacteria [76]. Oral, aerogenic or parenteral applications of recombinant ghosts (R-ghosts) in experimental animals could induce specific humoral and cellular immune responses against bacterial components, thus could be exploited to provide protective mucosal immunization. Expression of cloned PhiX174 gene E in Gram-negative bacteria results in the lysis of the bacteria by formation of an E-specific trans-membrane tunnel structure built through the cell-envelope complex. In recombinant ghosts, foreign proteins can be inserted into the inner membrane prior to E-mediated lysis via specific N-, or C-, or N- and C-terminal anchor sequences. The export of proteins into the periplasmic space or the expression of recombinant S-layer proteins vastly extends the capacity of ghosts or R-ghosts as carrier for foreign epitopes or proteins. Bacterial ghosts have been produced from a variety of bacteria including *E. coli* and *H. pylori*. The most relevant advantage of ghosts and R-ghosts as immunogens is that no activation procedure that might denature relevant immunogenic determinants could be employed in the production of ghosts which are used as vaccines or as carriers of relevant antigens [76]. Thus, the novel bacterial ghosts for *H. pylori* could be proposed as multifunctional vaccine particles.

7. Therapeutic immunization

Prophylactic vaccination regimens, clearly demonstrate that, using appropriate immunization techniques, an immune response capable of preventing colonization is possible, whereas natural infection failed to elicit the appropriate response. Bacterial lysate and purified antigens such as recombinant VacA have also been shown to eradicate *H. pylori* from chronically infected mice and to induce protective memory against a subsequent reinfection. Table 3 presens the current status of mouse studies for protective and therapeutic immunization.

Therapeutic vaccination is a new concept which is not explored until recently. Vaccination has traditionally been considered as a prophylactic measure. Vaccination of an already infected individual has been considered potentially dangerous and non-ethical for example vaccination gainst *Mycobacterium* infections. The success obtained in the animal model of *H. pylori* infection indicates that if the immune system can be stimulated in a correct way against the antigens, a therapeutic response can be induced. The efficacy of most commercially available vaccines can be predicted by their capacity to induce a specific serum immunoglobulin response (induced immunity could potentially eliminate the bacterial infection). This does not seem to be the case for *H. pylori* since natural infection induces strong serum immune responses in the absence of protection. On the other hand, secretory immunoglobulin A (sIgA) may play an important role in protection from infections of the mucosal surface. In fact, a correlation has been established between specific secretory IgA levels against urease antigen and protection [68]. Whether sIgA response is the major mechanism of protection or whether other aspects of the immune system such as IgG from mucosa infiltrated B cells or cell mediated responses play a role remains to be explored.

Recently, Ghiara et al. reported therapeutic vaccination in a mouse model with persistent infection exploiting mouse-adapted *H. pylori* strains [96]. The group reported that an otherwise chronic *H. pylori* infection in mice can successfully be eradicated by intragastric vaccination with *H. pylori* antigens like recombinant VacA and CagA, which were administered together with a genetically detoxified mutant of the heat labile enterotoxin of *E. coli* (LTK 63). The results represent a strong evidence of the feasibility of the therapeutic use of VacA and CagA-based vaccine formulations against *H. pylori* infections in an animal model and provide substantial preclinical support to the adaptation of the approach for clinical trials in human volunteers.

8. Future perspectives

8.1. Delivery systems directed towards the pathogenic site(s)

It is necessary to design drug delivery systems, which not only curtail and alleviate the shortcomings of conventional

delivery vehicles, but also place the antimicrobial to the infected cell lines. Very few drug delivery systems have been designed which are specifically targeted to the GI cell linings. Most of them are intended simply to achieve controlled gastrointestinal absorption; since the stomach is rarely used as a site of absorption strategies for effective and selective stomach targeting have been neglected. Orally delivered finely divided ion exchange resins may form a useful delivery system for the topical treatment of infected gastric mucosal cell lines [77]. This mucoadhesive system with uniform intragastric distribution was further proposed to target *H. pylori* infected sites more effectively and could serve to optimize antibiotic monotherapy of *H. pylori* based gastric infections. Sialic acid binding specificity and affinity of the *H. pylori* isolates could be used as recognition portals for their binding to sialic acids containing glyco-sphingolipids. Lelwala et al. [78] detected and characterized sialic acid-specific haemagglutinins/lectins (SALs) in *H. pylori* isolates (recognize alpha-2,3-terminal sialic acid) which bind sialo-glycoconjugates [17]. Secondly, a sialic acid based specificity mediated through polyglycosyl-ceramide has been explored on the surface of *H. pylori*. These sialic acid specific lectins can be presented as carbohydrate handles or epitopes to be specifically targeted to the carbohydrate recognition domains exposed on the bacterial biofilm [17].

8.2. Genomic research

The marginal potential of developing an antibiotic or vaccine regimen provoked efforts to explore the genetic make-up and genomic trait of the bacteria *H. pylori*. The genomics of *H. pylori* have been sequenced from several clinical isolates by the Genome Therapeutic Corporation (licensed to ASTRA, Cambridge, MA) and by the Institute for Genomic Research (TIGR, Rockville, MD) [79]. Both these groups revealed the existence of repetitive sequences within a family of expressed genes, which were predicted to encode outer membrane proteins (OMPs). OMPs are either major antigens or adhesins that determine serotype specificity; or porins (pore forming antigens, Hop) that are involved in mediating the passage of the antimicrobial into the cell and determine its susceptibility. Three pairs of genes that encode the OMPs have been analysed [80]: Omp 16 and Omp 17 were 85% identical; Omp 12 and Omp 22 were 99% identical; and Omp 5 and Omp 29 were 100% identical. The composition of the outer membrane of *H. pylori* having a large array of moderately expressed proteins coupled with genomics of OMPs may present a sequence signature for regulating and processing the genetic manipulations. Thus the potential for capitalizing on these genomics may lead to the discovery of novel vaccines and therapeutic strategies.

In conclusion, both prophylactic and therapeutic oral immunization have been shown to be effective against *H. pylori* in transgenic mouse models. In addition, several *H. pylori* proteins have been identified as potential vaccines. A phase I clinical trial has been completed [70] demonstrating the safety and tolerability of urease as an antigen. Such antigens in combination with a safe mucosal adjuvant could be used in the form of an oral vaccine administered during childhood before exposure to *H. pylori* in order to prevent infection. In addition, therapeutic immunization alone or as an adjunct to antimicrobial therapy may be promising attempts towards achieving a radical cure.

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