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Review Article

CHALLENGES AND RECENT DEVELOPMENTS ASSOCIATED WITH VACCINE ANTIGENS PRODUCTION AGAINST *HELICOBACTER PYLORI*

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ABSTRACT

Around half of the world's population faces *Helicobactor pylori (H. pylori)* infection. Enormous progress has been made to understand the bacterial pathogenesis process and pathogen interaction with eukaryotic cells but infectious diseases are still the cause of premature death of humans around the world. *H. pylori* is categorized under class I carcinogen by the WHO based on clinical study results. This review paper discusses various attempts made to establish an efficient vaccine to manage *H. pylori* infection. Some of the problems in developing an efficient vaccine against *H. pylori* are recurrent or persistent infection, insufficient knowledge about the action specifically in case of probiotics, development of antibiotic resistance, and cost of therapy are noted. This research may come up with transient *Nicotiana benthamiana* with suitable *H. pylori* genes expressed as antigenic proteins, which can be used for further studies to develop a vaccine for gastric ulcer/cancer and generate good scientific data that can be helpful for scientists and researchers in this field.

This review article for monitors' current approaches monitoring *H. pylori* infection since 1998 to 2019 using world-wide recognized journals and books, questioning its efficacies and whether these strategies help eradicate or there is a need to focus on several diversions. We provide scientific recommendations in eliminating *H. pylori* through vaccination along with addressing the preventive vaccine for this pathogen rather than using defeated treatments with plant-based nil side effects solution. The information relies on the available content in Google Scholar and PubMed using the keywords listed below.

Keywords: *Helicobacter pylori*, *Helicobacter pylori* neutrophil-activating protein (HP-NAP), Cytotoxin-associated genes pathogenicity island (*cag*PAI), VacA, UreB, Tumoural necrosis factor-alpha (TNF-α).

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INTRODUCTION

Apathogenic bacterium, *H. pylori* is a microaerophilic, gram-negative microorganism, was discovered in 1982 by Marshall and Warren in 1984. *H. pylorus has* two original morphological shapes, bacillary and coccoidal. The virulent form being bacillary form, while the protective form is the coccoid form. The bacillary form projects many unipolar flagella.

H. pylori is persistent in an infected person's stomach throughout a lifetime. It leads to chronic gastric inflammation that further causes diseases of the gastrointestinal tract like peptic ulcers, lymphoid tissue lymphoma, and gastric cancer. To avoid these ailments, this bacterial infection has to be eradicated and resistance to most of antimicrobial treatment calls for a new research in pharmaceutical science [1].

A considerable amount of evidence suggests that bacterial genotype is an important factor determining the type of induced pathology. The nature and severity of a disease depends on both-host characteristics and environmental factors. Genetically modified Plant-based treatment is an interesting substitute to existing treatment methods, which could be target-specific as well as no or least side effects to humans against *H. pylori*.

Virulence

Helicobacter produces virulence factors that lead to the development of disease symptoms. The most extensively studied factors are adhesins that control adhesion of bacteria to gastric mucosal cells, urease-which neutralizes stomach's acidic environment, CagA responsible for influencing host cell signal transduction pathways, VacA, a vacuolating toxin that regulates immune cell activity and NapA a protein that activates neutrophil.

Urease plays a role in gastric acid neutralization and metabolization of urea into ammonia and CO_2. Urease also exhibits strong immunogenic and chemotactic activity towards phagocytes, aids proinflammatory cytokines interleukin IL-6, (IL)-1β, IL-8, and tumoral necrosis factor-alpha (TNF-α) production. In the stomach, approximately 20% population of the *H. pylori* attach themselves to epithelial cells and the remaining is present in the mucosal layer [2].

CagA, being the most destructive virulence factor, shows its presence along cag Pathogenicity Island (cagPAI), is known as the first bacterial oncoprotein. Its interaction with human proteins causes many irreversible changes to host tissues such as exponential rise in cell size, elevating motility, humming bird phenotype phenomena. Along with these, CagA destroys apical junctions altering the normal epithelial morphology. At every tyrosine phosphorylation site, termed EPIYA motif, CagA links to the cytosolic proteins in a phosphorylation-dependent fashion. Also, binding of CagA with host proteins in a phosphorylation-independent method triggers to adenocarcinoma in host cells.

The *H. pylori* protein (HP-NAP) triggers neutrophil activation aids in the essential process of bacterial growth by helping it in capturing. HP-NAP is significant in pathogenesisas it induces mononuclear and polymorphonuclear phagocyte adhesion and chemotaxis [3]. NADPH oxidase enzyme activates reactive oxygen species production (ROS) in the presence of HP-NAP [4]. HP-NAP additionally triggers the release of cytokines IL-23 and IL-12 by neutrophils and monocytes that have inflammatory effects [5]. Properties of HP-NAP facilitate increased inflammation of gastric mucosa and ROS persistently harming gastric cells.

VacA shows significant factors promoting the virulence of the *H. pylori* strains with pathogenic properties. It forms pores in host cell membranes, promoting chlorine, pyruvate, bicarbonate ions, and urea to exit, which helped in its characterization [6]. VacA is generally a water-soluble protein residing on membranes to initiate the formation of a hexameric anion-selective pore. Reports of interference with the of *in vitro* antigen presentation process [7], apoptosis induction in epithelial cells [8] and inhibiting the process of *in vitro* T and B cell

activation and proliferation. One of the functions of VacA comprises of its initial activity of nutrient supplying for infection establishment. VacA facilitates infection persistence and chronicity by contributing to immune cell inhibition and cellular turnover balance alteration; it also leads to an increase in proliferation of cells and supports the establishment of many mutated cells, further contributing to the process of carcinogenesis.

The cagA gene is on the cytotoxin-associated genes pathogenicity island (*cag*PAI), which induces severe inflammatory responses. The interaction of the immune system of the host with *H. pylori* leads to years of persistent infection and the chronic inflammation of gastric mucosa.

Infection in childhood, *H. pylori* infection remains throughout life, despite of the host showing a spontaneously immune response by coating the invading pathogen with antibodies [9]. This bacterium develops processes to evade effective host immunity leading to longevity.

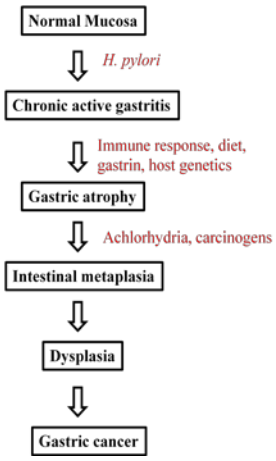

Fig. 1: Model representing the role of *H. pylori* and other factors in gastric carcinogenesis

Current status of *H. pylori* vaccine R and D activities

Vaccine development for the eradication of *H. Pylori* infection has been extremely difficult [10]. Delivery systems and adjuvants can achieve a decent reduction in levels of colonization by *H. pylori.* Strategies for the production of suitable sterilizing immunity are yet to be worked upon. Mice which is immune after therapeutic vaccination is one of the first demonstrations for the efficacy of a therapeutic vaccine against bacterial pathogens. With a single exception, all attempts to convert this success from animal models towards clinical trials have turned out to be unsuccessful. Urease along with varied adjuvants, mechanism, delivery systems, served as the focus for most of the early clinical trials for *H. pylori* vaccine, generally showing no efficacy in humans. Novartis tried a unique approach by combining three *H. pylori* virulence factors (vacA, cagA, and NAP), which were then intramuscularly introduced with alum. This vaccine displayed immunogenic properties and was taken to the phase 1/2 trial but was not pursued further [11].

All vaccines for *H. pylori* currently are in very early stages of development (Phase 1/2 or preclinical). Purified and recombinant constituents of *H. pylori* antigens together with an adjuvant, constitute these vaccines.

Preclinical

EpiVax *H. pylori* vaccine is an epitope-based vaccine that has displayed therapeutic protection in mice [12]. It is an initiation vaccination along the DNA and peptide-liposome. Helicovaxor has two approaches, one with engineering a *Vibrio cholera* strain of vaccine having non-pathogenic properties to express *H. pylori* antigens (HpaA, UreB and FlaA), and a strain of *H. pylori* which has been inactivated. Limited protection in BALB/c mice has been shown in two initial tests of epitope-based *H. pylori* antigen vaccines (Lp220) [11]. Probiotics are been identified as a delivery system for vaccines in recent years. When mice were administered oral dosage of *Lactococcus lactis* showing recombinant expression of cholera toxin B subunit and *H. pylori* urease epitopes, they showed some specific protection but this did not show improvement in the protection generally observed with standard recombinant antigen strategies [13]. Murdoch Children's Research Institute (MCRI) is working to develop a vaccine that targets and inhibits inflammatory effects caused by this disease instead of targeting the eradication of *H. pylori*.

In clinical trial

Imevax, a vaccine that consists of *H. pylori* antigen gamma-glutamyl transpeptidase (GGT), a protein from the outer membrane and a mucosal adjuvant. IMX101 has completed phase I of the clinical trial. Previously developed vaccines have failed to provide complete protection because of the strategies of immune evasion shown by *H. pylori* [14]. GGT is one of the most important vaccine approaches that display significant potent immunosuppressive activity [11]. Therefore targeting and neutralizing of the defense mechanism of and allowing a more efficacious immune action to be generated rose against other antigenic vaccine components. This trial is attempting to develop an effective vaccine-mediated response by overcoming one mechanism of *H. pylori* evading host immunity. It is still to be established whether targeting one such mechanism is enough as these bacteria possess many such defensive strategies but it seems like a valid strategy to investigate. The trial has been completed, but results are yet to be available.

Table 1: Adhesins and virulence-associated proteins of *H. pylori*

Protein/gene clusters	Predicted role	Associated with *H. pylori*-related diseases	References
BabA	Binds to fucosylated-Le^b blood group antigen on cells	babA2 allele has implicated in peptic ulcer disease and gastric cancer	[15, 16]
SabA	Binds to sialyl-Le^{x}and sialyl-Le^{a}antigens and is involoved in activation of neutrophils	None	[17]
SabB	Binding specificity is unknown	Absence of SabB expression via phase variation is associated with duodenal ulcers	[18]
OipA	OipA has been reported to assist in IL-8 induction, but this association is not universal	Expression of OipA is linked with cag status and development of duodenal ulcers ad gastric cancer	[19]
AlpA and AlpB	Inactivation of these genes reduced adherence to gastric epithelial cells	Unknown	[20]
Hp-NAP	Activates neutrophils and promotes adhesion to mucin, potential to protect *H. pylori* DNA or iron storage	Unknown	[21]
Plasticity region	Unknown	Presence of plasticity region is accociated with development region of gastric cancer, MALT lymphoma, duodenal ulcers	[22]
IceA	This encodes CATG-recognizing restriction endonuclease	IceA1 associated with peptic ulcers, but is not universal	[23]
DupA	This encodes VirB4 ATPase homolog	It is associated with duodenal ulcers but also reduces risk of gastric atrophy and cancer	[24]

Classical whole-cell inactivated vaccines

Vaccines constitute the most economical tool for prophylaxis of infectious diseases. In case of individual patients, *H. pylori* vaccination is more effective than antibiotic therapy decreasing the infection at population level leading to the eradication of the pathogen. Three main categories form active vaccines: killed vaccines, attenuated vaccines and subunit vaccines. Infected and non-infected volunteers were subjected to oral administration of *H. pylori* cells killed by formalin along with a mucosal adjuvant-a mutated form of heat liable *E. coli* toxin which is known to stimulate Th1 and Th2 immune responses [25]. Specific antigen secreting cell production was induced by the vaccine but it was not able to eradicate pre-existing infection.

Subunit helicobacter vaccines-antigen selection

Traditional methods used for identifying vaccine candidates are selected based on analyzing known pathogen virulence factors with concerning their immunogenicity. Methods of genetic engineering were developed, allowing genomic libraries to be constructed and screening them with specific antibodies obtained from patients and immunized animals. Recent progress in bacterial genome sequencing has reinforced the current approaches in identifying new, potential subunit vaccines. Genome sequencing of two *H. pylori* strains-26695 and J 99-have been done [26]. *In silico* analysis and genome comparison of various *H. pylori* isolates by microarray shows that their genomes contain 1111-1281 common genes [27]. Few out of these are considered for developing a vaccine. Assessing bacterial genome diversity, analyzing gene sets expressed *in vivo*, pathogen antigen screening and further assessing their uses in vaccine development.

It was found that only a small part of only 10 out of 400 examined *H. pylori*, antigens revealed protection in preclinical experiments [28]. The most promising candidates to construct an efficient vaccine might be abundant, surface-located, conserved, and seroreactive antigens. Conventional methodologies were used to select a few *H. pylori* antigens (CagA, VacA, HspA, NapA, catalase and urease sub-unit A and B) to evaluate their protective potential. None of these antigens fully protected humans despite showing immunogenic properties and protective properties in animal models (mice or gerbils) [29]. CagA and VacA, present in several prototypes of vaccines, have high polymorphic properties and are not conserved. Certain clinical isolates lack fully or partially the part of pathogenicity island formed by CagA gene. All genomes contain vacA gene expressed at different levels. CagA and VacA expressed by type I *H. pylori* strains, cause the most dangerous pathogenic effects. The majority of candidates for subunit vaccines that are analyzed consist of one or two antigens, but recent studies show that the efficacy of a vaccine can be increased by including more antigens. Clinical isolates of *H. pylori* show vast genetic diversity in recent studies. Thus, it is important to study the protein level comparison of different strains.

Immunoproteomics is a novel strategy combining standard proteomics with immunological screening and is currently the method of choice for identifying new antigens of diagnostic and protective values. It is proposed that highly specific antigens could be used as biomarkers of different pathologies induced by *H. pylori* infections, whereas novel, highly immunogenic, conserved, abundant and surface-located proteins could facilitate construction of an efficient anti-Helicobacter vaccine. Although proteomics is considered useful in evaluating the total protein content in bacterial cells and in studying protein-protein interactions, they display some significant limitations. One of the major problems is the fact that low-abundant and hydrophobic membrane proteins are undetectable by standard methods [30].

Bacterial cells (attenuated pathogenic bacteria and lactic acid bacteria) and viruses as carriers for *H. pylori* antigens

Most pathogenic microorganisms are either restricted to mucosal membranes or need to cross them to achieve their proper infectious niche [31]. For years it was considered that due to the apparent compartmentalization of the mucosal and systemic immune systems, vaccines administrated parenterally are less effective in protection against mucosal pathogens than mucosal immunization. However, more recent data indicate that the protective mechanism can be also stimulated parenterally. The effect seems to be dependent on the antigen delivery system and type of adjuvant used for immunization. Moreover, it should be noticed that although *H. pylori* colonizes gastric mucosa, vaccine prototypes administrated parenterally were also evaluated [10, 32]. Delivery of vaccine antigens via the mucosal route can be carried out using different strategies. The best-examined strategy used both attenuated and commensal microorganisms as bacterial carriers [33]. Delivery of vaccine antigens by live bacterial cells has resulted in the elucidation of both mucosal and systematic immune responses. Several attenuated *Salmonella* strains have been exploited as delivery systems for *H. pylori* antigens, mainly for urease sub-units A and B [34]. The antigens were expressed either as cytoplasmic-or surface-located proteins. Rhiozo *et al.* showed that exposure of UreA on the surface of *Salmonella* cells by employing part of the *E. coli* adhesion AIDA-1 greatly reduced the level of *H. pylori* colonization compared to cytoplasm-located UreA [35]. Recently Smythies documented that the genetically engineered poliovirus can be employed as a carrier of *H. pylori* antigens (UreB) [36]. Vaccination of mice with a replicon construct resulted in the clearance of established *H. pylori* infection in 73% of animals compared to 31% of mice immunized with the vector alone. A few attempts have been also undertaken to evaluate lactic acid bacteria potential as carriers for *H. pylori* antigens [37, 38].

DNA Vs. antigen vaccination

One of the two main deterrants to develop an effective vaccine for *H. pylori* is its unique characteristics and the other is the persistence of its infection. Antibiotic therapy and vaccination are the two major approaches to defeat the microorganism. Several attempts have been undertaken to estimate the strategy potential of developing a vaccine against *H. pylori*. Plasmid DNA containing urease, catalase or heat-shock protein-encoding genes as well as genes encoding immunogenic protein derived from *H. pylori* genomic library was administered to mice by different routes (intramuscularly, subcutaneously or intranasally) [34, 39].

In some experiments, a decrease in the bacterial load in the stomach and induction of the humoral immune response was noticed. Hatzifoti, *et al.*, also suggested that vaccination also results in an up-regulation of the IL-10 level, whereas the detection of $-defensin in the stomach indicated that immunization modulates both innate and adaptive immune responses [30]. Attempts to employ attenuated *Salmonella* strains for anti-Helicobacter DNA vaccination have also been undertaken. *Salmonella* is an enteroinvasive pathogen which can target plasmid DNA, carrying heterologous genes cloned under the control of eukaryotic promoters, to antigen-presenting cells (APC), specifically to dendritic cells (DC) the major target cells processing the antigen. Expression of foreign antigens results in antigen presentation by class I MHC and stimulation of both Th1 and Th2, T lymphocytes [41, 42]. *Salmonella* expressing *H. pylori* hpaA and napA genes cloned into *eukaryotic* expression vectors showed high immunogenicity when evaluated in the murine model. Hatzifoti *et al.*, [40] using the urease B DNA vaccine pointed out the role of an innate immune response in reducing *H. pylori* colonization of the murine gastric mucosa.

Attempts with probiotics

Several probiotic therapies in *H. pylori* treatment leads to an improved tolerance by prevention of outdated treatment and their side effects. Researchers reveal the acceptance of different probiotics usage against *H. pylori* along with the detailed mechanism of probiotic action against *H. pylori*, such as mucosal barrier efficacy, competition for adhesion, and immunomodulatory mechanisms [43, 44].

Probiotics administration in *H. pylori*-related diseases has shown consistent improvement of symptoms in patients. However, its efficacy is related considerably on factors such as *H. pylori* strain, the colonization size, density and the extent of inflammation. Studies reveal that extent of infection of this bacterium determines the risk of development of peptic ulcer disease and gastric cancer. Thus, medication of probiotics at the very beginning could bypass

permanent damage by *H. Pylori* related diseases [45]. The main issues with considering probiotics to manage *H. pylori* are insufficient knowledge of their action mechanism and inconsistent application findings [46].

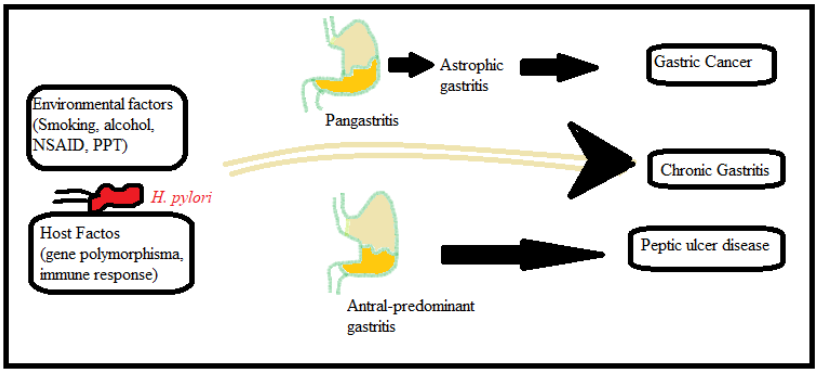

Fig. 2: Schematic representation of the factors contributing in *H. pylori* infections [38]

Recombinant protein expressions in plants

With notable damage and development of resistance towards existing treatment, antibiotics along with triple therapy with clarithromycin is not the best option for *H. pylori* infection, especially in remote areas where the local resistance to this antibiotic is 20% or higher. Therefore, alternate treatments have been proposed for the eradication of *H. pylori*.

Recombinant protein production on an industrial scale is in high demand due to their applications as diagnostic reagents, vaccines, and therapeutic agents. Heterologous proteins can be expressed in different systems through recombinant DNA technology. Earlier prokaryotic hosts were chosen due to their low overall cost and short production time scale. Protein classes that can be produced in prokaryotic systems are limited and post-translational modifications are not performed by them [47]. Therefore eukaryotic hosts: yeast, insects, and mammalian cell cultures and transgenic animals are focused upon. Some downsides of mammalian cell cultures and yeast, which are the main expression systems used, are cost, scalability potential, pathogenicity risk, and authenticity. Plants are a simple and inexpensive alternative to produce recombinant proteins at a scalable level allowing the development and production of safe and effective new vaccines and pharmaceutical compounds for populations that are in dire need of them. There are many advantages of recombinant proteins obtained from plants that are engineered genetically. Recombinant protein accumulation inside a plant cell in specific plant organs (E. G. in seeds) or in specific plant organelles (E. G. chloroplasts) occurs throughout the entire plant and is dependent upon the promoters used. Target for an accumulation of recombinant protein is seeds because of the high content of protein in seeds [48].

H. pylori genes showing pathogenic properties have been greatly studied to initiate a disirable treatment for its complete removal from the human system. Many researchers are working on virulent genes of *H. pylori*, vector systems to introduce in suitable plant cells to obtain genetically altered traits to aid the treatment of *H. pylori*. A total of 30 regenerated rice plants with hygromycin resistance were obtained when UreB (urease subunit of *H. pylori*) was introduced in rice plants via Agrobacterium-mediated transformation. This way, the expression of urease was curtailed consuming using transgenic rice, which could not neutralize the acidic medium in the stomach leading to bacterium death [49].

Another approach was laid by Zhang, *et al.*, who initiated gene transformation of UreB in carrot through Agrobacterium-mediated transformation, got successful after the expressional traits of UreB proteins (25mug/g) in roots) could activate an immune response in mice. This result suggests that the UreB transgenic carrot could be a potential vaccine for *H. pylori* infection [50].

Using the tree *Nicotiana benthamiana*

Several plants, such as *Polygonum minus* [51] and transient *Nicotiana benthamiana* have proved to be an aid for *H. pylori* treatment. *Nicotiana benthamiana* with *cagA, vacA and NapA* genes expressed as antigenic proteins can be used for further studies to develop a vaccine for gastric ulcer/cancer and other ailments caused by *H. pylori.* This tree has already added scientific appreciation towards its role in plant biology, therapeutic and tremendous biotechnology applications [52]. Recently, the rise of research on this tree is noted for its purpose as a useful tool in the study of plant microbiology and protein-protein interactions, pathogen-host interactions. Since it can easily exhibit foreign traits, it is considered as one of the best choices for plasmid-encoded virus interactions, screen for gene functions with virus-induced gene silencing (VIGS), and transiently express genes by leaf agroin filtration.

Table 2: Genetically modified organisms to treat *H. pylori*

Transgenic plant	Virulent gene from *H. pylori*	References
Callus of Carrot	UreB gene	[52]
Nicotiana benthamiana	UreB gene	[52]
Nicotiana tobacum	Heat shock protein A (HspA)	[53]
Arachis hypogaea L. (peanut)	UreB	[54]
Arabidopsis Thaliana	HP1341 (iron-dependent siderophore transporter protein)	[55]
Acacia nilotica and *Calotropis procera,* oregano and cranberry, *Calophyllum brasiliense,*	Urease	[56, 57]
Paeonia lactiflora roots,	UreB	[58]

One distinctive feature added with three notable technologies has strongly recommended the rise and popularity of *N. benthamiana*. These are its easy susceptibility to plant viruses, development viral vectors to study foreign genes expression, the development of virus vectors in silencing genes signals placed on the borders of a T-DNA plasmid, and are delivered into the leaf via Agrobacterium in filtration, and to develop visual reporter genes. 72 base-pair of *N. benthamiana* showcases an insertion in its RNA-dependent RNA polymerase gene

(Rdr1), which easily dysfunctions after it terminates the gene's open reading frame. This makes it easily susceptible to a viral infection making it an excellent host for virus-delivered gene expression and gene silencing [59, 60]. So, significant interest has developed to utilize this plant to exhibit desired genes traits to obtain a suitable cure to nullify *H. pylori* virulence in humans.

CONCLUSION

H. pylori have proved to be a significant public health risk pathogen and several attempts to completely eradicate this bacterium have not been achieved so far. Yet, no target-specific, complete removal of the infection is found. Plant-based novel approaches are been researched to aim for complete cure from pylori caused diseases.

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AUTHORS CONTRIBUTIONS

Contributions; is in order of Authors name.

CONFLICT OF INTERESTS

Declared none

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