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Inside *Helicobacter pylori*: A Serious Threat to Humans

Abdul Rehman[1,*]

[1]Department of Microbiology and Molecular Genetics, University of the Punjab, Quaid-e-Azam Campus, Lahore, Pakistan

[*]*Corresponding author*: Abdul Rehman, Department of Microbiology and Molecular Genetics, University of the Punjab, Quaid-e-Azam Campus, Lahore, Pakistan. E-mail: a.rehman137@outlook.com

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Abstract

Helicobacter pylori is a close relative of Campylobacter species, with the ability to colonize the human gastrointestinal tract. This gastric pathogen is a flagellated, Gram-negative bacterium. Chronic gastritis, mucosa-associated lymphoid tissue (MALT) lymphoma, duodenal ulcer, and gastric ulcer are the outcomes of persistent infection with this pathogen. Recent studies have shown a direct relationship between *H. pylori* and development of gastric adenocarcinoma. A 7- to 14-day course of broad-spectrum antibiotics is required for the elimination of *H. pylori*. Treatment failure due to chromosomally encoded antibiotic resistance is increasing rapidly, which underlines the importance of new regimens against this pathogen. The vast diversity of natural compounds in living microorganisms such as algae, as well as various dietary components in herbs and foods, provides a new opportunity for the establishment of therapeutic compounds. The majority of intra- and extracellular metabolites in algae have potent inhibitory effects on *H. pylori*, leading to the development of novel therapeutic agents for gastric ulcer. Application of bioinformatics-based tools has encouraged the scientific community to find novel targets and have led to the development of *in silico* drugs against the pathogenic elements of *H. pylori*. Further research on metabolite-based therapeutic agents with the aid of modern tools can be a milestone in the management of the emerging risk of gastric ulcer.

Keywords: Gastric Ulcer, Algae, Bioinformatics, Antibiotic Resistance, *Helicobacter pylori*

1. Introduction

Gastric ulcer is a clinical dilemma with increasing prevalence, which has become a constant threat to public health. This infection/disorder appears to be a global hidden epidemic, which is silently emerging as a health concern, indicated by the development of resistance against the current therapeutic agents. In this review, we evaluated major factors associated with *Helicobacter pylori*, involved in the development of gastric ulcer. Essentially, some novel approaches for the treatment of this extremely dangerous pathogen were also introduced.

1.1. Helicobacter pylori

Helicobacter pylori is an important cause of gastric ulcer in humans. Biochemically, it is a Gram-negative rod, which has the ability to evade and colonize in the harsh environment of the stomach (1). Biochemical characterization indicates this pathogen as catalase, urease, and oxidase positive. Also, microscopic analysis has revealed the spiral shape of this pathogen, with 3 to 5 polar flagella for motility (2). The work of B. Marshall and R. Warren on the discovery of *H. pylori* and its role in stomach disorders was acknowledged in 2005, and both scientists were awarded the Nobel Prize (3).

1.2. Helicobacter Genome

Around 1.65 million bases comprise the genome of *H. pylori*, encoding 1500 proteins. The process of horizontal gene transfer has enabled bacteria to change genomes in order to cope with extreme environments. *H. pylori* genome has the capacity to develop variations by acquiring chunks of foreign DNA from other members of *H. pylori* in the development of chronic inflammation (4). The genome of *H. pylori* has an average G + C content of 35.2 mol%. Around 40% of *H. pylori* strains contain plasmids with a size range of 1.5 - 23.3 kb. Moreover, 23S and 16S r-RNA genes are present in the duplicate form.

Genomic rearrangements have led to diverse variations in the genome of *H. pylori*, detected by genomic mapping. Some key genes, which encode proteins involved in colonization and survival of *H. pylori* (such as urease, flagella, CagA and vacuolating toxin), have revealed extensive variations. The potential of *H. pylori* to uptake exogenous DNA is the main explanation for the sequence diversity (5) (Figure 1).

1.3. Epidemiology of H. pylori

Today, *H. pylori* infection is known as a worldwide problem and a major cause of gastric ulcer in different coun-

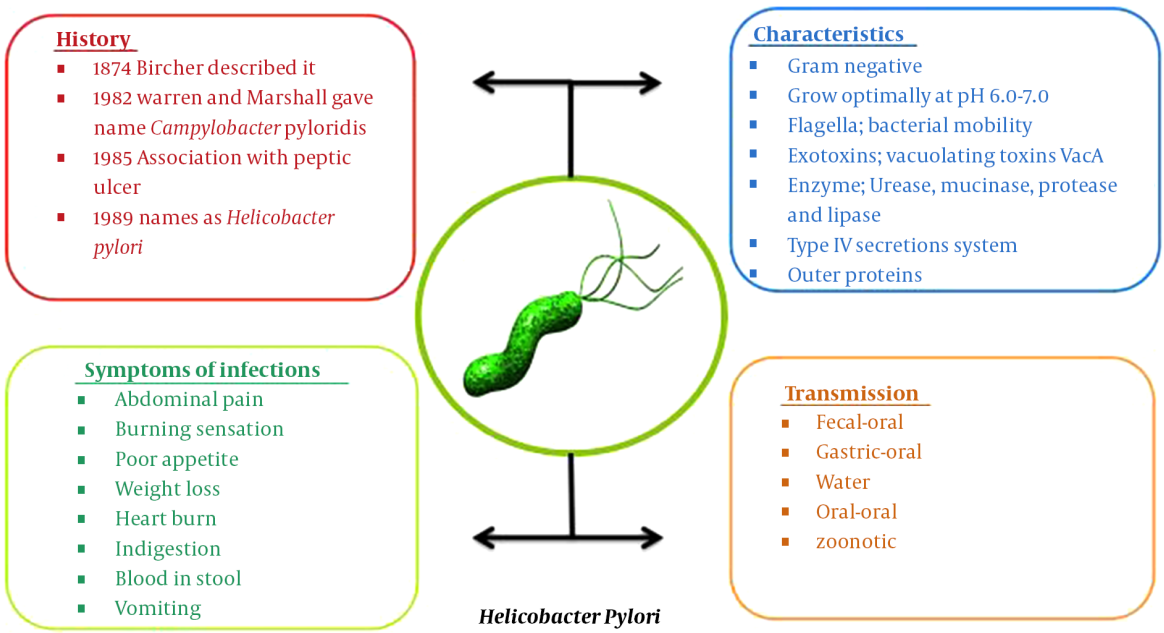

Figure 1. Different Aspects of *Helicobacter pylori*

tries around the world (6). A wide majority of the world's population carries this peptic pathogen. Nonetheless, the geographical distribution of *H. pylori* varies between developed and underdeveloped countries. The infection rate is continuously increasing in developing countries and is reaching up to 90% (1). The prevalence of *H. pylori* infections remains high in devolving countries, compared to developed countries due to proper sanitation conditions and high living standards (7). In North Europe and North America, one-third of the total adult population is infected with this pathogen, while in East and South Europe, Asia, and South America, the prevalence of *H. pylori* infection is more than 50% in the populations. Poor sanitation and low socioeconomic conditions are the confirmed risk factors for *H. pylori* infection (8).

A basic prerequisite for *H. pylori* to colonize human gastric mucosa is to control the host cell-signaling program. In order to cope with these challenges, *H. pylori* has acquired virulence factors. Another interesting feature of *H. pylori* is the stealth effect, created by this pathogen, which disallows the host cells to eradicate *H. pylori* and allows these bacteria to survive in the human gastric mucosa for unlimited periods. *H. pylori* has the capacity to neutralize the harsh acidic environments of the stomach by actively metabolizing urea into ammonia with the aid of enzyme urease; this feature has given *H. pylori* an edge over other microbes for survival.

Genetic studies have clearly revealed that *H. pylori* is not a new phenomenon among human. In fact, this pathogen has coexisted with Homo sapiens over the past 58,000 years (2). In 1994, remarkable studies of gastric cancer at that time presented *H. pylori* as a type-1 carcinogen. Among infection-based cancers, *H. pylori* is a common etiological agent, and its contribution to the global cancer burden is nearly 5.5% (2). Current studies have revealed that risk of MALT lymphoma and gastric adenocarcinoma among *H. pylori*-infected individuals is 2 - 5 times higher than that of the normal population (Table 1) (9).

1.4. Pathogenic Elements in H. pylori

There are various pathogenic elements in *H. pylori*, which are involved in colonization and subsequent pathogenesis. There is a family of conserved molecular transporters in these bacteria, known as T4SSs, which have the unique ability to transport molecules in bacterial cells. Owing to their functions, T4SS families can be categorized into 3 main entities, i.e., conjugative family, protein translocators, and DNA uptake channels. In *H. pylori* strains, the bacterial chromosome has the capacity to produce 4 different T4SSs, including tfs3, tfs4, and cytotoxin-associated genes (cagPAI and ComB) (24).

Table 1. The Global Prevalence of *Helicobacter Pylori*

SR/NO	Country	Prevalence of *Helicobacter pylori*, %	References
1	The Netherlands	31.7	(10)
2	Portugal	84.2	(11)
3	Cyprus	39.8	(12)
4	Turkey	82.5	(13)
5	Canada	37.9	(14)
6	Mexico	52.2	(15)
7	Saudi Arabia	28.2	(16)
8	Korea	54.4	(17)
9	India	58	(18)
10	China	63.4	(19)
11	Bhutan	73.4	(20)
12	Ethiopia	65.7	(21)
13	Morocco	75.5	(22)
14	Nigeria	93.6	(23)

H. pylori contains an important pathogenic element, which is known as a cytotoxicity-associated gene (Cag). The size of this gene is 35 kb and includes 26 open reading frames (ORFs). Cag-A has been found as a prominent member of Cag-PAI, with a more potential virulence impact, compared to other products. There is evidence regarding the direct involvement of Cag-A in the development of gastric cancer. There are various cellular mechanisms, which are altered by the intervention of Cag-A. This pathogenic protein has been also found to interfere with the activation of cell cycle regulatory proteins, such as extracellular regulated kinase (ERK), Ras, SHP2, MAPK, and STAT3.

Similarly, Cag-A is involved in Src kinase activation, expansion of epithelial transcription, destruction of cellular polarity, and morphological changes in gastric epithelial cells (GECs) (Figure 2) (25). Although Cag-A plays a major role in the pathogenesis of peptic ulcer, studies have revealed another virulence determinant of *H. pylori*, known as the vacuolating toxin (Vac-A). Vac-A has a size of 3.9 Kb and is majorly smaller than the Cag-A island. Among different strains of *H. pylori*, the N-terminus of Vac-A has exhibited many variations, leading to the evolution of various Vac-A subtypes with diverse cytotoxic activities. The s1/m1 subtype of Vac-A has shown major contribution to the prognosis of peptic ulcer. Vac-A has been also found to be associated with vacuolation and detachment of cells, leading to apoptosis induction (25).

1.5. Biofilm and Helicobacter

Biofilm is a complex multimicrobial community, which comprises of bacterial populations, attached to biotic or abiotic substrates. There are different varieties of materials used for attachment purposes, ranging from synthetic medical equipments to living tissues. Biofilm has a variety of advantages, including a mutual relationship among bacterial communities, enhanced tolerance against harsh environments, resistance against multiple drugs, and storage of nutrients.

Studies have revealed that microbes show greater potential to form biofilms on hydrophobic surfaces in comparison with hydrophilic surfaces. A major contributor to biofilm formation is the extracellular polymeric substance (EPS). The formation and composition of EPS mainly depend on the surrounding environment. Proteins and glycol-proteins have been found to constitute 75% - 89% of EPS, while other components include nucleic acids, lipids, phosphor lipids, and humic substances. An important and systematically efficient communication system, known as Quorum sensing based on small diffusible molecules, allows bacteria to survive inside the biofilm.

Recent studies have shown that many diseases associated with bacterial pathogens are involved in microbial biofilm at the infection site (26). Biofilm formation in *H. pylori* was reported by Stark et al. in 1999 (27). Formation of dense and mature *H. pylori* biofilm in the human gastric mucosa was reported in a previous study, and gastric ulcers were biofilm manifestations in the stomach (28). Using gas chromatography-mass spectrometry (GC-MS), researchers identified biofilm on the water-air interface of *H. pylori* growth. The biofilm of *H. pylori* has different EPS compositions with a greater content of naked extracellular DNA. This extracellular DNA plays 2 important roles, as it provides adhesiveness, as well as adequate genes for bacterial cells (i.e., transformation) (29).

1.6. Detection of H. pylori Infection

There are various methods to detect infection in peptic ulcer. These methods can be categorized into 2 groups, depending on biopsy. The biopsy-based tests include microscopy-based tissue analysis, microbial culture, polymerase chain reaction (PCR), and rapid urease test (RUT). On the other hand, noninvasive methods employ active detection of *H. pylori*, using urea breath test (UBT), serological analysis, and stool antigen test (SAT) (30). The microscopy-based tissue analysis provides useful knowledge about the impact of infection on stomach mucosa.

There are conflicts among pathologists about the number of required biopsy samples from a patient. However, a large group of gastric pathologists follow the Sydney classification system, which indicates sampling from at least 5

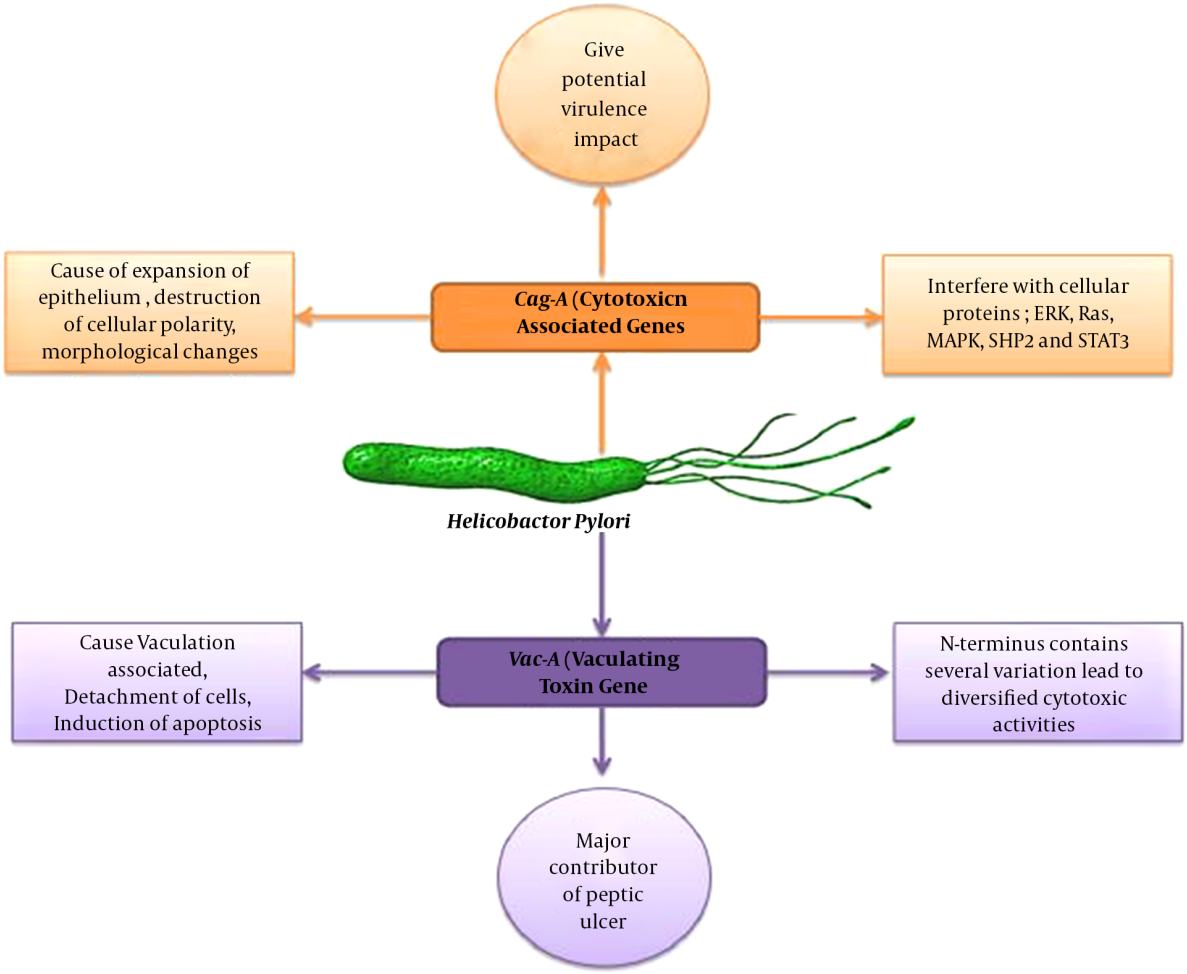

Figure 2. Two Important Genes of *Helicobacter pylori* (*Cag-A* and *Vac-A*)

biopsy sites: angulus, antrum, corpus, incisura angularis, and pylorus. Endoscopy is a painful process for patients, who insist on short periods of treatment. Due to such uncertainties, pathologists rarely follow these recommendations during biopsy collection (30).

1.7. Conventional Treatment Approaches Against H. pylori

Complete elimination of pathogens from the stomach mucosa is the ultimate goal in the treatment of *H. pylori* infection. Evidence suggests that durability of treatment regimens is fairly high, as the reoccurrence of infection is rare (4). Treatment of infection cannot be done alone by antibiotics. However, treatment is not confined to antibiotics only, as many antibiotic compounds are pH-sensitive and data suggest the problem of stomach acidity by antibiotics. A solution to this problem is application of proton pump inhibitors (PPI), which aid denatured antibiotics to resume activity (4). In current clinical procedures, triple therapy comprises of PPI, metronidazole, and amoxicillin to eliminate infection with *H. pylori*. Triple therapy drugs are administered for 10 days in the USA and 7 days in Europe (31).

A combination of antibiotics offers many advantages, including increased rate of infection elimination and reduced development of resistance. Resistance against antibiotics can be eliminated by application of multiple antibiotics, which confer rapid elimination of pathogens. Considering the presence or absence of antisecretory drugs, therapeutic agents can be divided into 2 categories, i.e., first-line and second-line therapies. Examples of antisecretory agents include bismuth, PPI, and ranitidine bismuth citrate (2, 4).

Triple therapy is considered as the first-line approach, comprising of PPI, amoxicillin/metronidazole, and clarithromycin. Evidence suggests that part of the population exposed to amoxicillin have allergic problems. In order to tackle this problem, metronidazole has been recommended as an alternative for amoxicillin (Tables 2 and 3) (32).

1.8. Antimicrobial Resistance

Antimicrobial resistance emerged about 70 years ago when antibiotics were used as clinical agents for combating infections. Multiple antibiotics are administered to treat *H. pylori* infections. In about 20% - 30% of patients, antibiotic therapy is not effective because of antibiotic resistance. This increase in resistance patterns necessitates the development of advanced drugs and novel therapeutic approaches (34).

Recent studies indicate a remarkable increase in antibiotic resistance among clinical isolates of *H. pylori*. A European study indicated that the overall resistance to metronidazole had increased up to 34.1% (followed by clarithromycin; 17.5%); the lowest resistance was found against levofloxacin (14.1%) (35). Apart from antibiotic potential, many fundamental factors contribute to the effectiveness of antibiotics, including side effects, cost of drugs, duration of therapy, tolerability of regimen, resistance of pathogens, and use of antibiotics in local populations (32).

The latest developments in molecular biology techniques have allowed scientists to establish the genetics behind the emergence of drug resistance. Such approaches have enabled remarkable progress in this area. For instance, it has been revealed that the mode of action in clarithromycin includes inhibition of amino acid synthesis via binding to bacterial ribosomes.

Research has revealed that a gene in *H. pylori*, known as *rrl*, encodes 23S ribosomal subunit. This gene undermines various point mutations, which enable *H. pylori* to resist clarithromycin. Population-based analyses in Western countries have provided insights into these mutations of *rrl* gene. These mutations were not rare and could be found in clinically isolated *H. pylori* at positions, including A2146G, A2147G, and A2146C. These mutations account for 90% of resistance to clarithromycin (35).

The *gyrA* gene is an important regulatory element, which contributes to the topology of DNA in *H. pylori* during DNA replication. This gene has been found to confer resistance to Quinolones by developing mutations at Asn-87 and Asp-91 positions (35). In *gyrA* gene, there is a region, called the quinolone-resistance determining region (QRDR), which is prone to mutation. Studies have revealed the role of this region in the cleavage and rearrangement of DNA. Moreover, this region extends to the action site of fluoroquinolones.

According to molecular studies of QRDR, hyperresistant mutants have rearrangements in *gyrA* gene at N87K and D91G points (36). In *H. pylori*, the pattern of metronidazole resistance is complex, compared to other drugs. Redox-based genes, including *frxA* and *rdxA*, play important roles in the development of resistance to metronidazole. Various point mutations have been reported to inactivate redox genes, leading to the deregulation of membrane potential and loss of cell integrity (36).

There is a class of antibiotics, known as rifabutin, which affect DNA-directed RNA polymerase enzymes. This class of antibiotics is a derivative of spiro-piperidyl-rifamycins and has a tendency to bind to the B-subunit of RNA polymerase (RPO). Due to its unique structural properties, rifamycin confers an advantage over other antibiotics, as it is highly effective against gastric pathogens. The clinical strains of *H. pylori* were analyzed for mutations in *rpoB* gene, and mutations conferring resistance were located at positions 524, 525, and 585 (36).

Tetracycline is a bactericidal drug, which acts on the 30S subunit of ribosome and blocks protein synthesis. Tetracycline-resistant strains have developed mutations in 16S RNA gene, which is responsible for the formation of 30S subunit. The molecular analysis revealed that various positions of 16S RNA gene had substitutions, such as AG-926/927-GT and A926G/A928C. Apart from mutations in the ribosomal RNA gene, the pathogen confers resistance to tetracycline using exclusion pumps, which do not allow the drug to accumulate inside the cell. Such exclusion pumps work by proton motive force (PMF) to exclude drugs from the inner bacterial compartments (Figure 3) (36).

1.9. Natural Compounds with Anti-Helicobacter Activity

In order to reduce the morbidity and mortality of cancer, there is a need for the development of novel modes of chemoprevention. Introduction of novel therapeutic agents is a relatively new domain, which encompasses the formation and assessment of biological or synthetic compounds to prevent propagation of carcinogenic cells and result in tumor destruction.

Nature is the greatest source of medicinal agents, as it provides a vast diversity of plants (both multicellular and unicellular), from which different compounds with antimicrobial and antitumor effects can be extracted (37). Plants have been analyzed for centuries in the development of novel drugs as sources of metabolites. Different studies have assessed a variety of plants and have confirmed that plants contain compounds with anti-*Helicobacter* activities (38).

Table 2. Routine Tests for *Helicobacter pylori* Detection (30)

SR/NO	Nonendoscopic TESTS	Advantages	Disadvantages
1	Serological tests	Inexpensive and easily available	False positive results
2	Urea breath test	Highly predictive results	False negative results
3	Fecal antigen test	Highly predictive results	False negative results
		Endoscopic Test	
1	Urease test	Inexpensive, rapid, and accurate	False negative results
2	Histological analysis	Highly specific and sensitive	High cost and need for properly trained staff and facilities
3	Microbiological culture	Highly specific and indicative of antibiotic resistance profile	Need for biosafety level-3 facilities

Table 3. Foods and Products with Anti-*Helicobacter pylori* Potential (33)

SR/NO	Food	Putative Active Compounds	Experimentation Level
1	Bovine milk	Lactoferrin	*In vitro* and *in vivo* (animals), *In vivo* (humans)
2	Green tea	Catechin compounds	*In vitro* and *in vivo* (animals)
3	Ginger	6-gingerol, 8-gingerol, 10-gingerol, 6-shogaol, and phenolic compounds	*In vitro*
4	*Curcuma amada*	Cinnamic, caffeic, ferulic, and gallic acids	*In vitro*
5	Turmeric	Curcumin	*In vitro*
6	Apple peel	Quercetin glycosides	*In vitro*
7	Garlic	Allicin and di-allyl sulfur components	*In vitro*

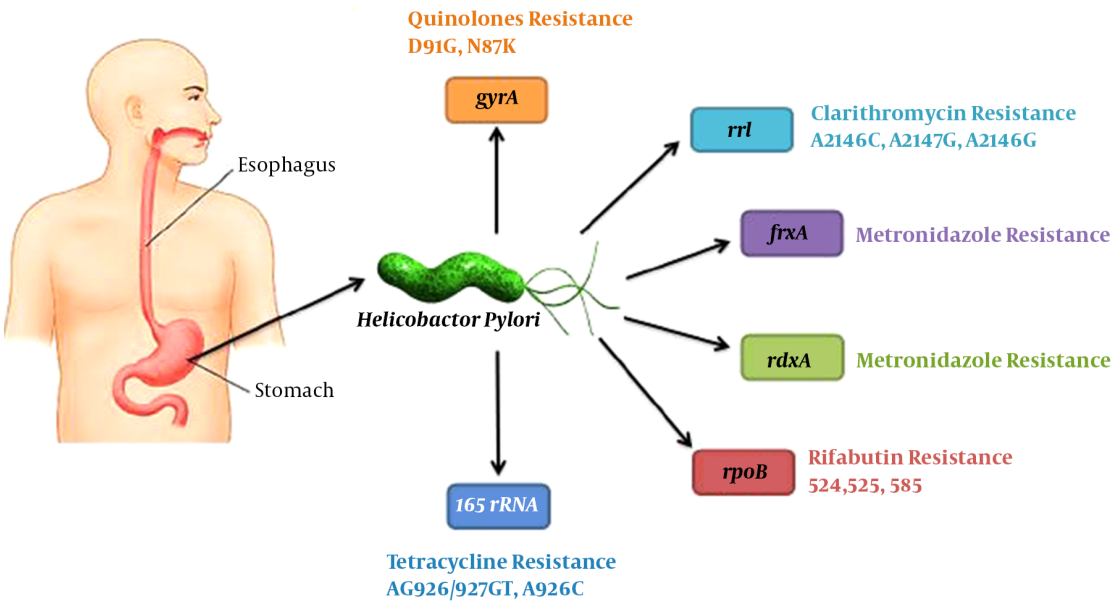

Figure 3. *Helicobacter pylori* Genes and Their Mutations Conferring Antibiotic Resistance

Herbal therapy has been employed for hundreds of years, involving whole plants or parts of plants to cure diseases. In whole plant-based therapeutics, a mixture of leaves, roots, and stem parts is applied to the infected area.

This approach was only employed for external wounds, as internal diseases required targeted compounds with greater purity. Since the introduction of modern therapeutic regimens, phytochemicals have been developed for therapeutic purposes both *in vivo* and *in vitro*. Many studies used dried plant materials in form of powdered extracts and dissolved them in methanol, ethanol, acetone, and dimethyl sulfoxide (DMSO). This therapeutic syrup was successfully used to eliminate *H. pylori* infection (39).

Apart from herbs, various natural fluids, such as saliva, milk, and serum, contain a multidimensional protein, which is glycated by various amino acids. This protein is known as lactoferrin, owing to its ability to bind to iron. Lactoferrin is a glycated protein with an anti-*Helicobacter* potential, which has been assessed *in vitro* and *in vivo* via animal models. Such a remarkable potential was immediately tested in a study on gastric ulcer patients, who were divided in 2 groups: with antibiotics and without antibiotics. The findings indicated 100% elimination in both groups (33).

Among commonly used plants, ginger is of exclusive importance and has been analyzed for its antiulcerative potential, specifically against *H. pylori*. This plant has been a promising source of various compounds, such as 8-gingerol, 6-shagol, 10-gingerol, and various phenolic acids, all of which have anti-*Helicobacter* potentials. Caffeic, ferulic acid, and cinnamic compounds, isolated from another variant of ginger, called mango ginger, have also shown significant *in vitro* effects against *H. pylori* (33).

Similar to ginger, turmeric has been also used as a source of compounds, which are known to have antiulcerative potential. Curcumin has the capacity to inhibit shikimate dehydrogenase enzymes in *H. pylori*. The shikimate pathway plays an important role in bacterial propagation, as shikimate dehydrogenase catalyzes the formation of various metabolites. Another mechanism of curcumin activity is NF-κB inactivation, which leads to a reduction in inflammation. Metalloproteinases play a significant role in the development of *H. pylori* infection. Curcumin has been found to inactivate and destroy metalloproteinases, associated with cancer progress (39).

There are diverse mechanisms in phytochemicals to inhibit microbial pathogens. Three factors play an important role in the survival of *H. pylori*, including DNA gyrase, urease, and vacuolation toxin. Flavonoids have shown significant inhibition of all 3 enzymes, as mentioned earlier. The mechanism of action in *H. pylori* involves initiation of apoptosis in gastric epithelia through releasing lipopolysaccharides (LPS) and vacuolating cytotoxin VacA, which leads to the destruction of parietal cells and compromises their acid production potential.

Under the mentioned circumstances, flavonoids play their modifying role by reducing apoptosis, as they inhibit the activities of vacuolation cytotoxins. A class of flavonoids found in grapes and wine, known as resveratrol, is a primary example of antivacuolation agents, inhibiting pathogen colonization. The survival of *H. pylori* at low pH is circumvented by ATPases, which are another target of resveratrol. Previous research suggests that these remarkable developments can help eliminate *H. pylori* infection (39).

Side effects of drugs are a primary concern in treatment. Phytomedicine has helped protect patients against the potential side effects of drugs. Antibiotics, if taken in excess doses, can pose serious damage to the liver and kidneys. Due to the biodegradability of phytochemicals, larger doses are safe for human use. A comparative study of herbal extracts and antibiotics revealed that cinnamon, flavonoids, and Nigella sativa had rare side effects, compared to the potent consequences of triple therapy (Table 4) (39).

1.10. Algae: Promising Protection Against Helicobacter

Algae are remarkable organisms, which have the capacity to survive extreme conditions, owing to the diverse nature of their metabolites. Algae are present in different sites, ranging from fresh water lakes to highly salted water bodies, contributing to the global ecosystem. The primary factor, which helps algae survive harsh conditions, is their ability to produce metabolites as required. Such production requires external influences, such as stress from the surrounding environment. Different algal metabolites have been isolated, purified, and analyzed so far, including tannins, carotenoids, phenolic acids, and flavonoids, all of which exhibiting various antimicrobial and anticancer activities. As tumor progresses via certain enzyme activities, angiogenesis, cell cycle arrest, apoptotic deregulation, and immune changes, these compounds target these mechanisms and prevent tumorigenesis (37).

A unique compound, derived from the microalgae, is astaxanthin, which is originally a carotenoid. Analysis of astaxanthin confirmed that this compound has antitumor activities. Targets of astaxanthin in the prevention of cancer progress include cell cycle arrest in the G0/G1 phase, regulation of ERKs, and increased expression of p27. Following the success of *in vitro* studies, this compound was applied in vivo, resulting in the apoptotic reduction of tumor mass (37).

A more detailed genetic study of astaxanthin found that this compound targets various enzymes, which play key roles in tumor progression. For instance, expression of cyclooxygenase-2 (COX-2), NF-kB, AKT, matrix metalloproteinase-9 (MMP-9), ERK-2, and MMP-2 was regulated by astaxanthin. Another remarkable compound,

Table 4. Assessment of Herbs for Their Anti-*Helicobacter pylori* Activity

SR/NO	Herb	Study Design	Outcomes	References
1	Garlic oil	Blind nonrandomized trial	Negative for histology and urease	(40)
2	Jalapeno pepper	Open nonrandomized trial	Reduction on urea breath test count	(41)
3	Cinnamon	Blind placebo control	Reduction on urea breath test count	(42)
4	Lycopene	Quasi control trial	Increased eradication rate	(39)
5	*Nigella sativa*	Randomized trial	Less significant eradication	(43)
6	Green propolis	Nonrandomized trial	Eradication of *H. pylori* after 40 days	(44)
7	*Glycyrrhiza glabra*	Randomized, double-blind, placebo trial	Greater effectiveness with GutGard	(39)
8	*Pelargonium sidoides* roots	*In vitro* studies using AGS cells	Inhibition of *H. pylori* cell growth and adhesion	(45, 46)
9	Cranberry juice	*In vitro* analysis using immobilized human mucus and erythrocytes	Inhibition of *H. pylori* cell adhesion	(47, 48)
10	Oregano and cranberry	*In vitro* agar diffusion assay	Zone of inhibition on agar plates	(49)
11	*Magnolia officinalis*	Compounds tested against urease	Urease inhibition	(50)
12	*Camellia sinensis*	*In vitro* analysis against *H. pylori* and anti-urease assay	Inhibition of urease and *H. pylori* population	(51)
13	Apple peel polyphenols	Compounds tested against Jack-bean urease; *in vitro* test against *H.pylori*; *in vitro* test using HeLa cells; and *in vivo* test on C57BL6/J mice	Inhibition of urease; prevention of vacuolation in HeLa cells; antiadhesive effects; and antiinflammatory effects	(52, 53)

found excessively in algae, is docosahexaenoic acid (DHA). Its primary role is lipid oxidation, which renders its toxicity against multiple cells. Molecular studies indicate that DHA targets mitochondria, which can lead to cell death after changes (37).

1.11. Bioinformatics Studies

Bioinformatics studies are of great value in biological sciences, as these computer-assisted analyses have facilitated the analysis of biological compounds and molecules. Different tools in this field help scientists design and test drugs against specific targets, without the involvement of wet laboratory space. In peptic ulcer, urease enzyme is a major enzyme, which renders *H. pylori* to colonize gastric mucosa. Urea is the substrate for urease enzyme, which is converted into ammonia with alkaline pH of the surrounding pathogen. This significant enzyme is a major target for gastroenterologists to eradicate infection.

Since its discovery, many studies have been conducted on urease enzyme. The current data suggest that *H. pylori* without active urease cannot cause stomach infections (54). In 1990, Hue and Mobley purified urease enzyme from *H. pylori* and elucidated its molecular structure (55). Structural analysis of the active sites of *H. pylori* urease revealed that it consists of dinuclear nickel atoms, which are associated with carbamylated lysine residues. Furthermore, the catalytic potential has a strong association with cysteine residues containing sulfhydryl groups, located on the mobile flap in close proximity to the active site (56).

Similar to all other enzymes, urease has a metallic cofactor in form of nickel. *H. pylori* has developed a composite system to acquire, traffic, and regulate the level of nickel within the cell, owing to its importance in urease activity. A multistep process, which yields urease, begins with the formation of urease apoenzyme. Further events add 4 key proteins, known as UreF, UreG, UreE, and UreD, to form an active enzyme. A significant step, which activates the enzyme, is the establishment of the link between accessory proteins and apoenzyme. This step involves carbamylation of lysine residues on the mobile flap. Such a reaction requires a GTPase, which is present in form of UreG, while nickel transport towards the active site is achieved by a metallochaperone, known as UreE (57).

An important step in urea catalysis is the substrate access to the active site of enzyme, which requires a region encompassing α313 - α346 on the mobile flap. When this mobile flap is open, it allows urea to enter the active site. Once inside the active site, the urea molecule causes various structural changes in urease, which lead to the closing of the mobile flap due to α365 alanine rearrangement. This change of shape leads to catalytic activity and breaks the C-N bond in urea. This bond lysis leads to the release of nickel carbamate and ammonia, which change the surrounding pH. Once formed, ammonia is released by the movement of the mobile flap, aided by histidine α322 (58).

Molecular biologists have targeted this urease enzyme in order to eliminate stomach infections. Various compounds have been analyzed for antiurease activity using

bioinformatics tools (59). There are 2 broad categories of urease inhibitors. One group has substrate analogs, such as hydroxyurea and hydroxamic acid, which inactivate urease by binding to its active site instead of urea. The other group acts on the metal cofactor and prevents enzyme activity by sequestration of nickel. The current data suggest that both categories contain various biological molecules, which have been successfully assessed both *in vitro* and computationally (55).

2. Discussion and Conclusion

Peptic ulcer is becoming a threat to public health worldwide. Although treatment is available, continuous development of resistance is gradually limiting the therapeutic options. The major contributing factors of the rapid increase in antibiotic resistance are lack of public awareness and economic burden. On the other hand, there are no current data in third-world countries regarding the emerging antibiotic resistance against current regimens in local populations. This not only raises questions about the survival of therapeutic agents, but also encourages physicians to give blind prescriptions to patients. Although *H. pylori* is a major contributor of peptic ulcer, the importance of associated microbiota cannot be neglected. Novel therapeutics can be developed by the assessment of various plants and microalgae, which contain various metabolites and compounds. Similarly, bioinformatics studies have opened new windows to various naturally occurring structures as potent drugs. Time is needed to determine the natural curative potential of plants against major disorders through combining scientific bioinformatics tools and molecular biology to develop novel therapeutics.

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Footnote

Conflicts of Interest: There are no conflicts of interest in the current study.

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