

Advancements in Diagnostics and Treatments of Helicobacter Pylori

ABSTRACT: -

Helicobacter pylori is the first officially known recognized bacterial carcinogen with more than half the world's population colonized by the gram-negative bacterium. However, more often complications are seen in adults than in children.

Comment [gs1]: Helicobacter pylori should be written this way in the entire manuscript, with a lowercase "p" for pylori.

Decreased Helicobacter Pylori can be a two-edged sword, reducing the incidence of stomach ailments while increasing the risk of allergies and constipation. Helicobacter Pylori causes various intestinal diseases ranging from gastritis to gastric lymphoma.

Comment [gs2]: Are gastritis and gastric lymphoma intestinal diseases?

A number of research have been done on such pathogenic microorganisms which play a crucial role in their diagnosis and in finding an appropriate treatment against specific diseases caused by them. The choice of diagnostic method is based on relevance of cost, effectiveness against antimicrobial resistance and less side effects of the drugs used un the treatment. If the disease is not treated properly, the colonization can persist for a lifetime.

Comment [gs3]: This sentence gives impression that the authors are talking of many microorganisms and not only H. pylori.

Recently, an increase in drug resistance and failure to eliminate Helicobacter Pylori treatments has been observed worldwide. Therefore, the rapid and accurate determination of any type of Helicobacter Pylori antibiotic resistance before the administration of detoxification is particularly important.

Comment [gs4]: This does not make sense.

Initially the article introduced the pathogenesis and microbiology of Helicobacter pylori. Later there is a complete overview of the diagnostic methods currently available for the detection of the infectious agent. The review also focuses on using next generation sequencing in the detection of the bacterium. To date, advanced sequencing technologies and their applications within the lab settings are in line with Helicobacter Pylori management guidelines thereby helping in early diagnosis and treatment of the underlying disease.

Key Words: - Next generation sequencing, Phytotherapy, Bacteriophage therapy, Triple therapy, Sequential therapy, Urea breath test.

Comment [gs5]: "Helicobacter pylori" should be at least one of the key words

INTRODUCTION: -

Pathogenesis: -

There are some key factors that ultimately cause diseases such as chronic gastritis, peptic gland disease, gastric adenocarcinoma or gastric mucosa-associated lymphoid tissue lymphoma in the infection of Helicobacter Pylori. A significant role is played by the urease function of Helicobacter Pylori in neutralizing the acidic environment which is present in the stomach. Later motility-Mediated motility assists Helicobacter Pylori bacterium that travels to gastric epithelial cells. This is followed by bacterial adhesins that interact with heavy cell receptors, leading to effective colonization and further infection. Both acute as well as chronic inflammation are seen in Helicobacter Pylori along with release of chemicals by the gastric epithelial layer that initiate the innate immune system activating the neutrophils. These neutrophils ultimately lead to the formation of gastritis and gastric ulcers as they continue to damage the connective tissue of the gastric region.

Introduction:

MICROBIOLOGY: -

The genus Helicobacter belongs to the genus Proteobacteria, the genus Campylobacterales and the family Helicobacteraceae.

Helicobacter species can be divided into two main categories: -

Species Types of

- Gastric Helicobacter
- Enterohepatic (Non-gastric) Helicobacter

Both of these groups offer high organ specificity, that they are often unable to form the intestines or liver colon. **Error! Reference source not found.**

Gastric Helicobacter Type: -

Comment [gs6]: There is no reference in this paragraph!

Comment [gs7]: How can a genus belong to another genus?

It involves of the following species: -

- *Helicobacter pylori*
- *Helicobacter felis*
- *Helicobacter mustelae*
- *Helicobacter acinonychis*
- *Helicobacter heilmannii*

Gastric strains have become adapted to the detrimental conditions within the intestinal mucosa. All known strains of the *Helicobacter* gut use urease positive and highly stimulating due to the flagella type. Entry through the viscous mucus is aided by the motility of the flagella along with morphological maturity. The neutral pH conditions, thus allowing the growth of gastric type in the stomach. They are responsible for causing illness such as Gastritis, peptic ulcer disease, gastric adenomas, MALT lymphoma, dyspeptic symptoms (1,2).

Enterohepatic Helicobacter Type: -

The following species are included in this category: -

- *Helicobacter hepaticus* (enterohepatic species)

Enterohepatic *Helicobacter* species cause persistent infection, accompanied by chronic inflammation and hyperproliferation, thus increasing the chances of neoplasms (proliferative typhlocolitis, hepatitis, hepatocellular carcinoma). Only murine species *H. hepaticus* species have been shown to be more studied. Infected during the study (3,4,5).

Comment [gs8]: Please rephrase this!

H. Hepaticus shows similarities to *Campylobacter* both having flagella which has two poles and have a sheath. Being urease positive, oxidase positive and catalase positive, grow in the normal growth medium (β -cyclodextrin-supplemented media). However, not much is known about the violent methods of *Hepaticus* infection. In addition to the above-mentioned information, *hepaticus* also acts on both, genes and mutation. *H. hepaticus* is involved in adapting to gastro intestinal niches and niches in the liver and entero-hepatic pathogenic processes of the disease(6,7,8,9).

Microbiology Related to Helicobacter Pylori: -

These spirilla gram-negative bacterium. According to tests it has been shown that its Heterogeneity is the modification of the gastrointestinal and abdominal conditions and different patterns of immune response to the bacterial infection. They may appear as rods, while coccoid status occurs after prolonged treatment or in vitro. The body is 2-6 unipolar, a flat flag about 3 µm long, usually carrying a distinct lamp at the end. Flagella enable the ability to accelerate the movement of viscous solutions of layer of mucus above the gastric epithelial cells (10).

Helicobacter Pylori is microaerophilic, with optimum growth temperature being 37 ° C. It is considered neutralophile as gastric mucosa is their habitat. Growth of the bacteria occurs in a narrow range of pH (5.5 to 8.0) where optimal pH is 7 (11,12).

Helicobacter Pylori has extremely complicated nutritional requirements, requiring a growth media supplemented with blood or serum acting as a source of nutrients in addition, it also protects against the harmful effects of fatty acid chains. The most common fluid media usually contains brucella, Mueller-Hinton, or cardiovascular broth (2 to 10% calf serum) usually added with supplements (13,14).

Gastric biopsy can be performed, the cultures must be checked from 3rd to 14th day. The colonies of Helicobacter Pylori are present in small forms which are movable exhibiting a smooth appearance. It should be noted that the growth rate decreases rapidly once the culture has reached a stable stage along with a morphological change in coccoid form. Cultures can be supplemented with triphenyl tetrazolium chloride [TTC] to facilitate the visual detection, the colonies appear dark red due to conversion of triphenyl tetrazolium chloride (TTC) to a deep red pigment and improve golden glow. Helicobacter Pylori at -80 ° C can be stored for a longer duration for the treatment of Cerebral Palsy (Error! Reference source not found.,16,17)

Diagnosis: -

The choice of suitable technique for the investigation of Helicobacter pylori infection is done by the identification of strain along with the advantages, disadvantages and availability for each of the techniques.

There are numerous factors impacting the selection of the appropriate technique such as: -

- Sensitivity of the strain
- Specificity of the strain while culturing

- Clinical status of the bacterial strain
- Cost of the processes which are involved in the diagnostic technique

Various tests were performed for Helicobacter Pylori, Available tests are categorized by tests based on maternal histology, culture and other methods which are based on samples of blood, feces, urine, or saliva. For general purpose of diagnosis histology, urea tests, and cultures are used. In hospital, many patients go through endoscopy, which is unite with Helicobacter Pylori tests. In addition, air and breathing tests are widely used (18,Error! Reference source not found.,20).

Treatment of Infective Diseases: -

Helicobacter Pylori is susceptible to a variety of antibiotic drugs, outside of a living organism making the monotherapy difficult in vivo, related to Helicobacter pylori niche living in low ph within the viscous mucus. Among all the antibiotics, Clarithromycin is the most effective antibiotic (average dose of 40%) which can be given twice a day for 10 to 14 days (21,22,23).

Double therapy includes double-daily Proton Pump Inhibitors, especially Amoxicillin but two drug therapies has been exchanged by three drugs, including two drugs (antibiotic) along with a combination of Proton Pump Inhibitor or Bismuth. An alternative is provided by the Quadruple therapy, including bismuth compound with PPI and two antibiotics (24,25,26).

Tetracycline, Amoxicillin, Imidazoles and limited macrolides (especially Clarithromycin and Azithromycin) are the broadly used drugs in Helicobacter Pylori infection (27).

Rifabutin and Furazolidone has limited effectiveness and a lot of patients are unable to withstand furazolidone (mainly used in the second-line treatment for patients who retain met)Various treatment sessions, dosages, and combinations of drugs have been studied, but none have reached more than 90 to 95% of accomplishment(28).

In Pregnancy: -

During pregnancy or lactation if gastric ulcer is present such a situation could be well managed by the suppressing the acid secretion. Complete elimination of Helicobacter pylori is achieved only after the birth of the child, however Quinolones, Tetracyclines and Bismuth along with Metronidazole are advice not to be taken in pregnancy (29).

Comment [gs9]: I have serious doubts concerning this affirmation which in addition is supported by references dating from 1993 and 1995!

A published report focusing on pregnant women with Iron deficiency anemia has examined the complete elimination of *Helicobacter pylori*. Various researchers have performed trial on a specified number of women and finally concluded that *Helicobacter pylori* infection is highly common in such women. A higher degree of response is seen by the use of Eradication Therapy than the usage of Oral Iron supplementation among women who are pregnant and are suffering from Iron deficiency anemia.

Vaccination: -

Over the past decade, great strides have been made in the use of alternative therapies for *Helicobacter Pylori* mainly vaccination. *Helicobacter Pylori* colonization in the stomach proceeds in the initiation of a strong, non-invasive response that counteracts inflammation (30).

Mucosal supplements such as Cholera toxin (AB5 Toxin, CT) along with mucosal immunization with various antigens trigger the Th2 response (thereby preventing or treating the infection) whereas the Th1 response increases the inflammation rather than eliminating it.

Early indications of mucosal vaccination against *Helicobacter* triggers the Th2 response showing a secretion of serum IgG1 antibodies and immunoglobulin A (IgA) after oral vaccination in mice have been observed in several experimental studies. It was observed that if a vaccine initiates an immune response against Th2 response, the prevention as well as eradication of *Helicobacter pylori* infection could be done successfully (31,32).

Other indications for therapeutic use suggest that even in the lack of complete elimination, vaccination may have been advantageous as the number of bacteria exposed to antibiotics is decreased and this reduces the chances of reducing antimicrobial resistance of *Helicobacter Pylori*. Preliminary clinical trials have already been conducted. (33,34,35). Adverse living conditions, poor hygiene along with Underprivileged socioeconomic status have been cited as the underlying cause for such bacterial infections (36,37).

An alternate option is the use of probiotics which prevents infection through the immune system of the host. It is observed that the bacteria is eliminated by lactobacilli in vitro and to some extent in vivo (38,39,40,41). Such a procedure would be unsuccessful in completely eradicating the *Helicobacter Pylori* colonization thus it only reduces the prevalence of cancerous and malignant lesions.

Comment [gs10]: What does it mean?

Howsoever, these therapies remain as the support in the Helicobacter Pylori treatment. New treatments are expected, especially when over-the-counter treatments are used to prevent cancer.

Standard Therapy: -

Several societies have issued the treatment guidelines for in order to achieve a higher eradication rate of Helicobacter Pylori disease: -

- ❖ The European Society for Pediatric Gastroenterology Hepatology and Nutrition
- ❖ North American Society for Pediatric Gastroenterology, Hepatology and Nutrition

Triple Therapy

The therapy includes Proton Pump Inhibitors (PPI), Amoxicillin (AMO) and clarithromycin (CLA) for 14 days, if the strain is susceptible to clarithromycin (CLA) or metronidazole (MET).

Sequential Therapy

If triple therapy fails, another treatment option is sequential therapy for 10 days which involves the use of Proton Pump Inhibitor (PPI) in addition to Amoxicillin for 5 days, then after administrating of Proton Pump Inhibitor (PPIs) given along with Metronidazole and clarithromycin for the next 5 days. These drugs should not be given if the strain is resistant to metronidazole or clarithromycin. For younger children it is not advised to take higher doses of

Diagnosis of Helicobacter Pylori infection and advances

Proton Pump Inhibitors (PPIs) for acid suppression. This treatment exposes the children to many different types of drugs so, to prevent harmful effects it is not advised to younger age groups(42,43) .

Invasive tests	Non-invasive tests	Challenges
<ul style="list-style-type: none"> • Histopathology • Rapid Urease (CLO) Test • Culture biopsy • PCR 	<ul style="list-style-type: none"> • UBT (Urease Breath Test) • Stool Antigen Test • Hp Stool Antigen Test • Serological Test • qPCR and dPCR 	<ul style="list-style-type: none"> • False positive results • Antibiotic resistance • Eradication rate • Poor retesting rate

Among the invasive tests the quantitative polymerase chain reaction (qPCR) was used as the criterion to estimate the susceptibility, selectivity, and the results of test reports. This endoscopic based test has an advantage for recognition of clarithromycin resistance genes encoding for antibiotic resistance such as that of clarithromycin due to its association to particular mutation associated (44).

It is seen that PCR has high sensitivity and specificity as compared to other techniques which makes the detection precise in particularly in patients suffering from gastrointestinal bleeding. (45)

The Stool antigen test is done by using immunochromatographic assay. This test lack accuracy because of the influence of various factors: -

- Drugs that fight against bacterial infections
- Drugs that inhibit the Proton Pump (PPIs)
- Acetylcysteine
- Movements of the Gastrointestinal Tract
- Upper bowel bleeding

Comment [gs11]: Where is the reference?

ADVANCED DIAGNOSTIC AND TREATMENT TECHNIQUES: -

Several new diagnostic techniques have been developed which are helpful in the early diagnosis and timely treatment of the disease caused by pathogenic microorganisms. Some of the techniques are stated here.

NEXT GENERATION SEQUENCING: -

Studies using Next generation sequencing have informed an increase in the number of families in response to Helicobacter Pylori infection [46,47,Error! Reference source not found.]. In addition, some studies suggest that the use of antibiotics to eliminate Helicobacter Pylori has affected the proliferation of bacterial populations and altered viral diversity. The limit of DNA-based techniques is that they contain all the microbiota including the bacteria which are dead and DNA contaminants(49,50,51).

In contrast, the use of meta-transcriptomics which is the study of gene expression of microbes within their natural environment, allows for the investigation of only the most effective and efficient writing of the viral community. A recent study disclosed that it is precise method that can be used directly to the samples of the abdomen. It has also showed that the process of examining the tissue which has been removed from the stomach of the patients were initially classified as undiagnosed by conventional methods, consisting of reoccurring Helicobacter Pylori (52).

However, various road concerns should be considered. First, in order to improve sensitivity multiple specimens of gastric biopsy should be used. Second, many colonies should be taken from agar tablets to produce DNA and preparations should be considered for drug-resistant individuals. Third, the issues must be followed by adequate coverage to obtain hetero resistance. Fourth, factors must be followed to differentiate polymorphisms that occur naturally in drug-resistant mutations. Levofloxacin and Rifampicin Resistance against the bacteria is associated

Comment [gs12]: What are the methods comprised in "next generation sequencing"? Are they listed or described anywhere in this part? What are their advantages compared to existing methods?

Comment [gs13]: I think you are talking about bacteria's resistance to these drugs!

with the exchange of amino acids in the codons of genes. While clarithromycin resistance is based on the conversion of nucleotide points to rRNA. In contrast, NGS can be used to predict resistance against metronidazole based on genotypic data remains a challenge to *Helicobacter Pylori*, most of which have been reported to undergo multiple changes (frameshift and resistance). Together, it suggests the need for more studies in these groups(53,54,55,56).

Alternative therapies: -

Phytotherapy: -

Phytotherapy is done with the view of using plant fragments as medicines or health-promoting substances in order to eradicate several types of infections.

Comment [gs14]: Please rephrase this!

Studies show that the use of cranberry juice prevents the adhesion of *Helicobacter pylori* reason is a urease inhibition that activates the hydrolysis of urea into CO_2 and NH_3 , informing that it increases the pH and indicates the elimination of the infection causing bacteria.

Curcumin (turmeric) a yellow-coloured pigment, has a therapeutic effect on this bacterial infection possibly by suppressing metalloproteinases fluid secretion by the stomach cells, giving rise to gastric ulcer and stomach cancer. (57)

Bacteriophage treatment: -

This technique involves the use of bacteriophages in treating infectious diseases and complete sequence of phage genome is the first step where bacteriophages are introduced into treatment to eliminate any virulence factors.

In addition, genetically modified bacteriophages can be used for the delivery of the vaccine directly by vaccines that carry antigens of the phages and indirectly by incorporating DNA vaccine in the phage genome. Due to the size of the virus, these vectors can be quickly transferred to cells that present antigen, producing a strong antibody response (58-64).

CONCLUSION: -

Various studies are being carried in view to find new highly precise and effective techniques for detection as well as treatment for eradication of the *Helicobacter pylori* bacterium. Safety must be the primary concern with assessment of each involved substance. Several Experimentations are being done to ensure the effectiveness of the mechanism the treatments.

Future methods centers on cost efficiency and treatment efficacy focusing on the introduction of culture-independent methods to *Helicobacter Pylori* detection along with the evaluation of antibiotic resistance. All the more, a need of more research would be helpful in long term protection from *Helicobacter Pylori* eradication.

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