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Advances in Research Progress of *H. pylori*

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Helicobacter pylori infection is a global public health problem. It can lead to chronic gastritis, stomach & duodenal ulcer, mucosa-associated lymphoid tissue lymphoma and gastric adenocarcinoma. Globally a lot of research has been conducted on *H. pylori*. This review is focused on its biological characteristics, pathogenic mechanisms and epidemiological characteristics.

Keywords: Helicobacter pylori; biological characteristics; pathogenic mechanism; epidemiological characteristics.

1. INTRODUCTION

Helicobacter pylori (*H. pylori)* has been a mainstay of infection in humans for more than 58000 years [1]. However it largely escaped notice until it was cultured by Marshall and Warren[2]. Studies conducted on *H. pylori* have largely changed the paradigms regarding disease causation.

Physicians previously attributed ulcers to stress or anxiety and did not believe that bacteria could cause ulcers [3]. It was discovered in 1983 that the stomach could be colonized by bacteria [4]. Increasing evidence emerged of *H. pylori* as a

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pathogen closely related to a variety of gastric conditions. These conditions vary from benign stomach diseases such as chronic gastritis, duodenal peptic ulcers and gastric peptic ulcers to malignant diseases such as gastric cancer [5],and gastric mucosa-associated lymphoid tissue [MALT] lymphoma [6].

2. BIOLOGICAL CHARACTERISTICS

H. pylori is a curved gram-negative bacillus with a bundle of unipolar flagella. *H. pylori* is a microaerophilic, gram negative, spiral shaped rod, between 2.5 and 4 μm in size, and under certain conditions it can be U- shaped or coccoid. *H. pylori* is actively motile using 4- 6 unipolar, sheathed flagella [7]. It is naturally present in the gastrointestinal tract of humans and nonhuman primates.It is also reported that it can infect pigs, cats, sheep and pups [8,9]. A large population of *H. pylori* is present in the gastric mucosa; however a few are found adhered to the gastric mucosal epithelium. The *bacterium* is able to survive in the hostile environment of the stomach where few other organisms can survive. Although *H. pylori* is considered to be an extracellular bacteria, there is strong evidence suggesting that the bacteria has a mechanism for intracellular invasion [10]. *H. pylori* is the best known member of the *Helicobacter* genus, which includes dozens of species that primarily colonize the gastrointestinal tract of a variety of animals [8].

Biochemical identification of *H. pylori* relies on the activities of the urease, catalase and oxidase enzymes. The *bacterium* is slow- growing and requires a rich medium and a microaerophilic atmosphere for in vitro culture. After starvation through prolonged culturing, a coccoid form can be found in cultures and it has been debated whether this form represents dormant or degenerated, non-viable bacteria [11]. Latest studies show that *H. pylori* can grow in deep ground water as well as in sea water (Saline water) and in culture medium at laboratory level (Brucella broth culture liquid medium), it survived in spiral shape only while in sea water and deep ground water it was found in both spiral as well as coccoid shape. This study also shows it can effectively grow at 37°C as well as at 4°C [12].

3. PATHOGENIC MECHANISM

A virulence factor imparts some function that renders the microorganism more pathogenic, that is, increases the likelihood for disease development. *H. pylori* infection is usually lifelong and asymptomatic and disease may be attributed to the host response towards colonization. Thus, some of the factors commonly designated as virulence factors in *H. pylori*, for instance the flagella, may rather be regarded as “colonization factors” [3]. Helicobacter species exist in the stomach as *H. pylori*, the intestinal tract as *Helicobacter canadensis*, in the liver as *Helicobacter hepaticus*, and in the gall bladder as *Helicobacter bilis*. The natural habitat of *H. pylori* is the gastric mucus and the mucus producing epithelium. In the duodenum of *H. pylori* infected patients, the *bacterium* is always found closely associated with gastric metaplastic cells, which is a precancerous condition and relatively common in the upper Gastrointestinal tract (GI) tract [13]. The formation of gastric-type epithelium in the duodenum is related to increase gastric acid output. This new habitat could be essential for colonization of *H. pylori* when gastric changes such as chronic active atrophic gastritis or cancer induced by bacteria take place in the natural habitat [13]. The differences in protein expression level and activity caused by gene polymorphism at the same locus have gradually become a new explanation for the clinical outcome of *H. pylori*-infected hosts. With the differences in the detection of disease-related genes and gene expression levels, a series of new pathogenic genes (BabA, Saba, OipA, DupA, etc.) were gradually explored.These were found to be helpful to elucidate the pathogenesis of *H. pylori*, and to investigate the mechanism of *H. pylori.*

4. PROGNOSIS OF INFECTION AND CLINICAL TREATMENT [14]

H. pylori infection has been clearly linked to peptic ulcer disease and some gastrointestinal malignancies. Increasing evidence demonstrates possible associations to disease states in other organ systems, known as the extraintestinal manifestations of *H. pylori*. Different conditions associated with *H. pylori* infection include those from hematologic, cardiopulmonary, metabolic, neurologic, and dermatologic systems [15].

Current evidence most supports extraintestinal manifestations with *H. pylori* in immune thrombocytopenic purpura [16], asthma, [17] iron deficiency anemia [18], urticaria, [19] Parkins on's [20], Alzeheimer disease [21] migraines [22]; however, there is still a plausible link with other diseases that requires further research [23].

H. pylori infection rate is different in different places. The clinical outcome after *H. pylori* infection suggests the complexity of pathogenesis. *H. pylori* pathogenesis includes: *H. pylori* colonization, toxin-induced gastric mucosal damage, host immune response,mediated gastric mucosal injury and *H. pylori* infection after gastrin and somatostatin regulation imbalance caused by abnormal gastric acid secretion.Inflammation, immune system, acid, oxidation and other aspects, virulence factors, cytokines, free radicals, virulence genes and other *H. pylori* pathogenic factors are involved [24,25].

5. EPIDEMIOLOGICAL CHARACTER-ISTICS

H. pylori infection is one of the commonest infections worldwide, occurring in all regions and infecting at least half of the world's population [26]. The exact routes of transmission are not definitely known due to the inability to clinically detect acute *H. pylori* infection along with technical difficulties in isolating the microorganism from sources other than the gastric mucosa. A reason might be that the transmission of the infection occurs in multiple pathways, which may differ in different societies and age groups. Childhood is a period of high risk for *H. pylori* acquisition, so a good understanding of the modes of transmission in children is required to identify how to break the chain of transmission of the infection.The minimum infectious dose of *H. pylori* for humans is not yet established.In human volunteers, ingestion of 104–1010 CFUs of *H. pylori* after administration of famotidine resulted in infection in 18 out of 20 subjects. For non-human primates, the established minimum infectious dose of *H. pylori* is 104 CFUs. The most important reservoir of *H. pylori* is the human stomach; and potentially *H. pylori* may pass from the stomach into the external environment by feces, vomitus or gastric regurgitation [27]. *H. pylori* transmission pathways are fecal - mouth, mouth - mouth, close contact and zoonotic transmission, infection also has a family aggregation phenomenon. Drinking contaminated water, close contact with *H. pylori* and family members, meals, kindergarten school children, students and eating from roadside stalls etc. can cause the spread of *H. pylori*. After the human body is infected with *H. pylori*, the bacteria will be long latent in the stomach, without any symptoms, some patients will experience recurrent abdominal pain, vomiting, iron deficiency anemia, chronic gastritis, duodenal ulcer. *H. pylori* hospital infection caused by contaminated endoscopy has been reported. This gram-negative *bacterium* infects more than half the world's population and its prevalence has been shown to correlate with poor socio-economic conditions. In many underdeveloped nations, more than 80% of the population is infected with this pathogen.

The prevalence of *H. pylori* infection worldwide is approximately 50%, as high as 80%–90% in developing countries, and ≈35%–40% in the United States [28]. Whereas within countries, the prevalence is higher among group with lower socioeconomic status [29,30].*H. pylori* prevalence is generally found to increase with age, reaching 20-50% in adult populations in Europe and North America. *H. pylori*-positivity in adults is more closely associated with living conditions and with the parents' socioeconomic status in childhood than with current living conditions and socioeconomic status.The infection is also associated with low Socio Economic Status (SES) within countries. In the United States, for instance, a significantly lower prevalence was found in Caucasians [26%] compared to Hispanics [65%] and Afro-Americans [66%]. This dissimilarity was interpreted to reflect the different socioeconomic backgrounds. In a follow-up study,it was found that the difference in prevalence between Afro- Americans and Caucasians resulted from different seroconversion rates, although the rate of seroreversion could also have played a role.

6. GLOBAL PREVALENCE OF INFECTION

Infection with *H. pylori* occurs worldwide, but there are substantial geographic differences in the prevalence of infection both within and between countries. [31] Multiple studies have demonstrated that low socioeconomic status is associated with increased risk of *H. pylori* infection [32] Additionally, an age-related cohort effect has been observed with prevalence of infection increasing with age. [33] Within Europe, *H. pylori* prevalence rates range from 11% in Sweden to 60.3% in Spain [34] In China, *H. pylori* prevalence has been reported as high as 83.4%. [35]. Additionally, many countries such as China, Japan and Bulgaria have

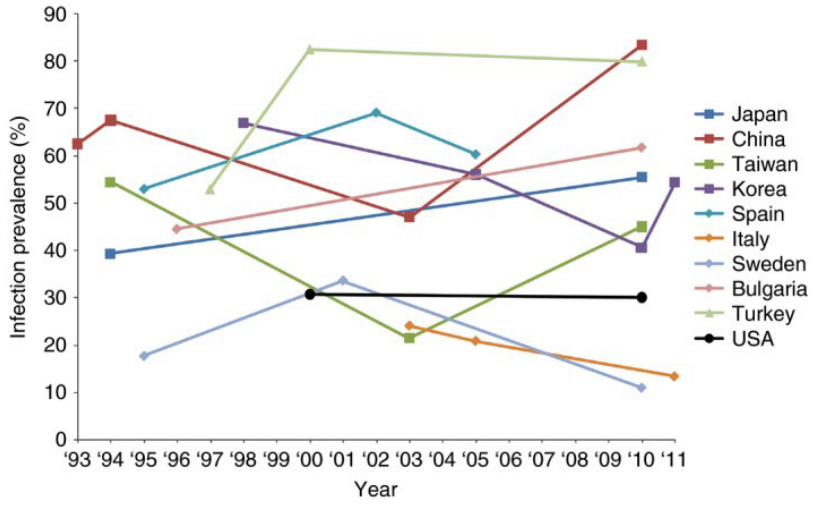

Fig. 1. Global prevalence of the *H. pylori* Infection is shown in graph [39]

experienced an overall increase in the prevalence of *H. pylori* infection over the last 20 years [36] In Canada, the prevalence of *H. pylori* is approximately 30%; however, within the Aboriginal populations living in Canada, the prevalence of *H. pylori* has been reported as high as 95%. [37] In the USA, cross-sectional studies of the participants in the National Health and Nutrition Examination Survey (NHANES) III and NHANES 1999–2000 demonstrate an overall seropositivity rate of approximately 30% [38]. In populations with high infection rates, it is likely that patients are infected with more than one strain of *H. pylori*.

7. DETECTION METHODS

There will be a high probability of positive serology or other test when using the test-and-treat strategy in populations with high prevalence of *H. pylori* regardless of symptomatology [40]. *H. pylori* laboratory diagnostic methods are divided into two categories. One is an invasive test: done through the endoscope to obtain gastric mucosal tissue as a test material; a conventional endoscopic exam is usually performed to diagnose *H. pylori*-associated diseases. Culturing of *H. pylori* from gastric biopsy specimen is a highly specific but less sensitive method.

7.1 Histopathological Examination

Histology is usually considered to be the gold standard in the direct detection of. *H. pylori* infection and is also the first method used for the detection of *H. pylori*, Rapid urease test (RUT) is the most useful invasive test for the diagnosis of *H. pylori* infection because it is inexpensive, rapid, easy to perform, highly specific and widely available [41] .

7.2 Rapid Urinary Enzyme Test and Genetic Diagnosis

The other is non-invasive test: no need for gastric mucosal tissue, the use of gastric juice, blood, saliva, feces and other specimens, methods are fecal *H. pylori* antigen detection, serum *H. pylori* antibody detection, urea breath test and feces and other specimens of *H. pylori* gene determination. Stool antigen test (SAT) uses an enzyme immunoassay to detect the presence of antigens against *H. pylori* in stool samples. It is a reliable method to diagnose an active infection and to confirm an effective treatment of infection [42]. Detection of *H. pylori* includes morphological, biochemical, molecular biology detection methods. The main methods were *H. pylori* culture, urease test, smear Gram stain, tissue sections of various staining, *H. pylori* antibody ELISA detection and immune blotting and *H. pylori* specific gene PCR diagnosis and other methods. In addition, the application of *H. pylori* expression microarray can study the effect of different stimulating conditions on its gene expression profile. The expression of *H. pylori* growth metabolism and virulence-related genes under different growth conditions was explored, which could help reveal the mechanism of growth and metabolism Closely related genotypes help guide the diagnosis of clinically relevant diseases.

8. MOLECULAR METHOD

The gold standard methods of antibiotic resistance are based on phenotypic methods performed by the agar dilution method.[43] These methods, however, can take up to 2 weeks to be completed. In addition, molecular techniques can often use either fresh or formalin-fixed samples. Real-time PCR has been used to successfully determine *H. pylori* susceptibility to *Clarithromycin* [44] Additionally, PCR using formalin-fixed paraffin-embedded samples has been shown to reliably detect the *H. pylori* 23S rRNA mutations associated with *Clarithromycin* resistance[45]. Another advantage of PCR is the potential to gather complete antimicrobial resistance data in patients infected with multiple strains of *H. pylori*. Although the use of PCR-based methods provides rapid detection of micro-organisms, these techniques can be affected by DNA contamination or degradation since the high sensitivity of these methods often result in the detection of dead or nonculturable microorganisms.[46]

Fluorescence in situ hybridisation (FISH) is a time-saving, accurate and cost-effective method for the detection of antibiotic resistance in cultured *H. pylori* colonies. This method can be used directly on biopsy specimens procured for histopathological and microbiological examination, allowing for rapid detection of *H. pylori* resistance without requiring DNA preparation. [47] The results can theoretically be available within 3 hours after an endoscopy by utilising frozen tissue sections.[46] The limitations of this method include the degradation of the probe by proteases and nucleases present in the sample and poor accessibility of the microbial cell wall for the probes.

Recently, peptide nucleic acid (PNA) probes using FISH have been used for the detection of several bacteria in lieu of the typical DNA molecular probes. [48] PNA molecules are DNA mimics with high affinity for DNA or RNA complementary sequences. [49] PNA probes are normally relatively small (13–18 nucleotides), increasing their ability to penetrate the bacterial cell wall. Moreover, the PNA molecules are more resistant to nucleases and proteases than DNA molecules.

9. DRUG RESISTANCE

H. pylori drug resistance is becoming increasingly prevalent. Optimal treatment for *H. pylori* has yet to be defined for all patients. Furthermore, rates of antibiotic resistance vary by region, and local resistance data should be used to guide treatment where available [3]. The treatment options for *H. pylori* infection are drugs such as imidazoles for instance *Metronidazole* (MTZ), macrolides such as *Clarithromycin* (CLR), β-lactams such as amoxicillin (AMO), quinolones, tetracycline and nitrofurans. Amoxicillin resistance is below 3% in America and Europe, but over 60% in Africa. Africa also has the highest rates of resistance to *Metronidazole* (92.4%) and tetracycline 43.9% [50]. *Metronidazole* resistance is above 50% in much of the world but there are indications that *Metronidazole* resistance may be dropping in Northern Europe [51]. Within Europe, resistance patterns vary by country and even within a country. For example, the reported *Clarithromycin* resistance rate is 1.5% in Sweden, but 7.5% in Germany and *Clarithromycin* resistance in Italy is lower in the north than in the south [51]. Increasing resistance to *Clarithromycin* and *Levofloxacin* has been attributed to widespread use of these antibiotics for respiratory tract and urinary tract infections, respectively [50]. In the *H. pylori* Antimicrobial Resistance Monitoring Program, the resistance pattern showed 29.1% of United States isolates were resistant to one antimicrobial agent and 5% were resistant to two or more antimicrobial agents [52]. Multidrug resistance remains low worldwide, offering hope that rescue therapy will work in most patients. Previous treatment for *H. pylori* is the single largest risk factor for drug resistance [52]. Success rates of antimicrobial therapy do not always mirror in vitro susceptibility data. This could be partially due to variability in antibiotic resistance testing protocols and poor patient compliance [51]. From The Japanese National Surveillance Study, it was found that from 3707 *H. pylori* isolates from 2002 to 2005, *Clarithromycin* resistance rates increased from 18.9% to 27.7% between this 3-year interval. *Metronidazole* resistance remained fairly consistent, ranging from 3.3% to 5.3%. Amoxicillin resistance rates were negligible [53]. The proportion of people infected with *H. pylori* strains is getting higher and higher, resistance is often the main cause of failure to eradicate treatment. Studies have shown that *H. pylori* strains isolated from different countries and regions can produce varying degrees of resistance.Modern microbiology suggests two reasons for the origin of *H. pylori* resistance: (1) the spontaneous mutation theory, that postulates

that resistance is due to the spontaneous mutation of bacteria beads. According to Darwin's theory of evolution, with the application of antibiotics, antibiotic-sensitive bacteria gradually reduced and resistant bacteria gradually increased; (2) resistance to genetic information transmission produced a new drug-resistant strain. Chromosomal DNA or plasmid genes are resistant to genetic information, *H. pylori* resistance gene in the bacterial DNA recombination into the sensitive strains, so that resistant strains increased. At present, many scholars have been committed to the study of *H. pylori* vaccine, to aid in its development, and for the prevention and eradication of *H. pylori* infection.

10. CONCLUSION

H. pylori is a versatile organism capable of persevering under a wide variety of environments, and rapidly acquiring resistance to the most common agents which may lead to serious effects in the near future. There is a need to establish new therapeutic goals and treatment that can aid us in the fight to treat the disease.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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