# Journal of Genetics and Genome Research

# Microevolutionary History of *Helicobacter pylori* During Infection: A Review

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## Abstract

*Helicobacter pylori* is a bacterium that has evolved with humans, is transmitted from person to person and persistently colonizes the stomach. During its long coexistence with humans, *H. pylori* has developed complex strategies to limit the degree and extent of inflammation and damage to the gastric mucosa. It is capable of altering the physiology and immune response of the host, thereby allowing it to persist throughout life. Infection with *H. pylori* has been linked to such diseases as severe gastritis, peptic ulcers and gastric cancer. Differences in the incidence of gastric cancer across the world can be attributed to regionally different *H. pylori* genotypes, dietary factors, environmental factors, and host factors, as well as virulence gene polymorphisms (particularly *cagA* and *vacA*) in different strains. The colonization of an individual by more than one strain of *H. pylori* is common, and a recurrent infection during childhood provides substrates for the acquisition or recombination of new genetic sequences. This review explores the evolutionary history of *H. pylori* during infection recurrence and the genetic diversity in humans as well as in an animal model.

## Keywords

*H. pylori*, Recurrence, Genetic diversity, Recombination

## Introduction

Humans are the definitive host of *H. pylori*. Phylogeographic studies have indicated that humans have been colonized by *H. pylori* for ≥ 58,000 years, since the first migration out of Africa [1]. *H. pylori* is a Gram-negative, pleomorphic bacillus that is classified as a non-invasive bacterium because it does not cross the epithelial barrier. *H. pylori* is motile owing to the presence of four to six sheathed polar flagella. It grows in microaerophilic conditions and requires between 3 and 5 days of growth at 37°C for primary isolation; its colonies are translucent and 1 to 2 mm in diameter [2]. Its genome size averages 1.7 Mbp, has a G+C content of 35 to 40% and encodes 1500 proteins [3]. Approximately 40% of *H. pylori* isolates contain a 23.3 kb plasmid that contains no known virulence factors. Additionally, the bacterium possesses at least two operons for each ribosomal ribonucleic acid (rRNA): 16S, 23S and 5S [4].

Infection with *H. pylori* is acquired within the first years of life and is related to geographic location [5]. The course of the infection is highly variable. Most infected individuals remain asymptomatic; only 10-20% develops atrophic gastritis, and of these, less than 3% develop gastric cancer [6]. In developed countries, the prevalence of *H. pylori* infection varies from 1.2% to 12.2%; it is low in children and adolescents and higher in adults and the elderly. The infection rate is higher (80%) in developing countries [7]. The main factor that predisposes individuals to infection is socioeconomic status, with greater infection prevalence observed among individuals living in overcrowded or impoverished conditions [8].

In contrast to many other bacterial pathogens, *H. pylori* does not exhibit clonal propagation in different human populations or within groups of patients. An exception to this rule is transmission among families, where clonal infectious strains have been observed more frequently among mothers and children [9].

Children are particularly vulnerable to infection by *H. pylori*, which is transmitted by infected parents, especially mothers. However, little is known about how and when maternal transmission occurs in childhood, particularly whether it occurs before or after weaning [10].

The colonization of an individual by more than one strain of *H. pylori* is common, and a recurrent infection during childhood provides substrates for the acquisition or recombination of new genetic sequences. This review explores the microevolutionary history

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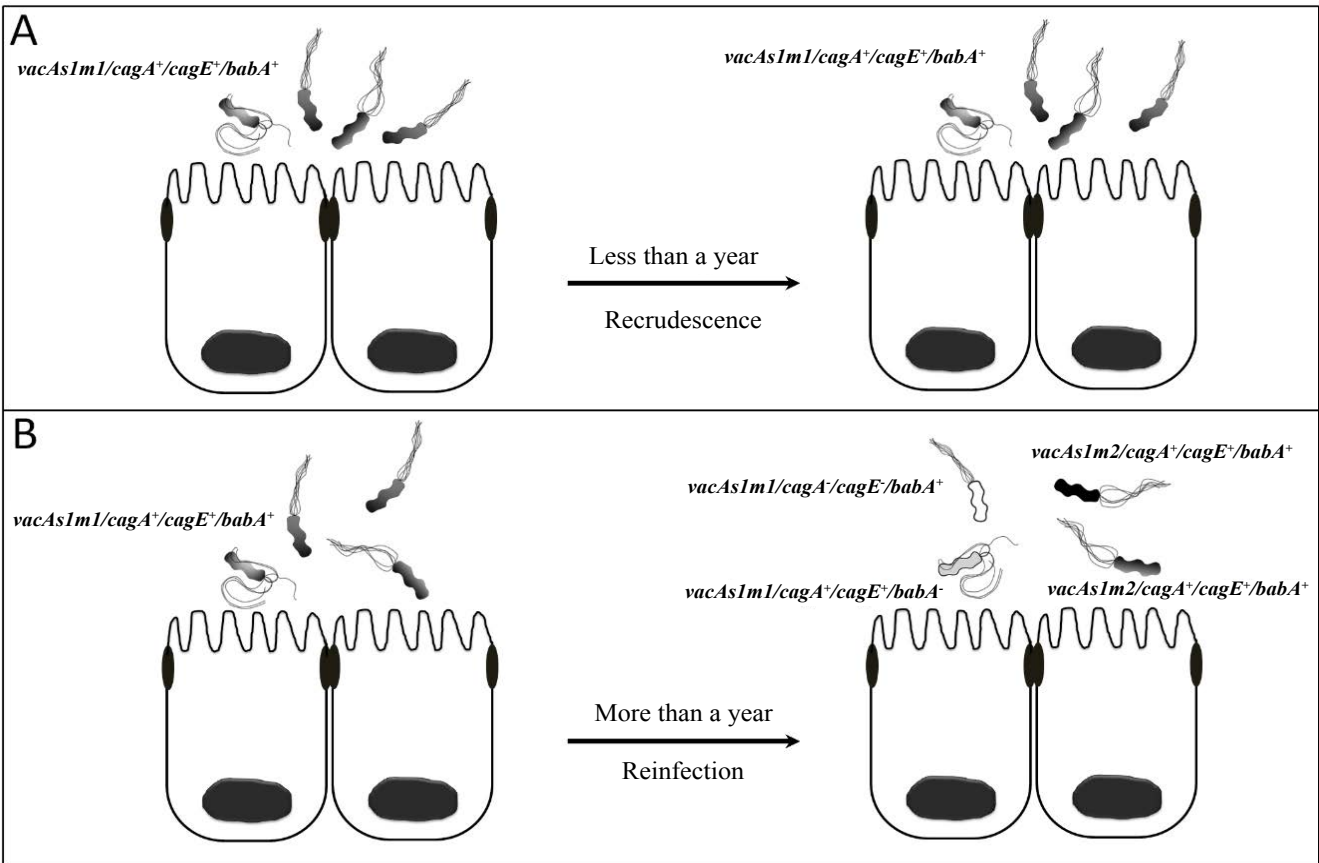
**Figure 1:** Recurrence of *H. pylori* infection in children and adults has been proposed to have two mechanisms. 1) Recrudescence: the apparent elimination of infection due to suppression but not successful eradication of the bacteria (infection with the same genotype strain by lack of compliance with treatment or antibiotic resistance), and 2) Reinfection: occurs after successful eradication, when a patient is re-infected with a new strain or with the same strain at least one year after eradication (infection due to strains with different genotype and antimicrobial susceptibility).

of *H. pylori* during infection recurrence and the genetic diversity in humans as well as in an animal model.

## Recurrence of *H. pylori* Infection

Recurrence of *H. pylori* infection in children and adults has been proposed to have two mechanisms: recrudescence and reinfection. Recrudescence is defined as the apparent elimination of infection due to suppression but not successful eradication of the bacteria [11,12]. By contrast, reinfection occurs after successful eradication, when a patient is re-infected with a new strain or with the same strain at least one year after eradication (Figure 1) [13,14].

The effectiveness of antimicrobial treatment for *H. pylori* infection may be limited due to patient compliance with the treatment regimen, selective pressure for strains with high levels of resistance to antimicrobials of choice and high rates of recrudescence and reinfection. Furthermore, *H. pylori* infection recurrence is common in patients with low-efficiency treatments. Thus, treatment has been proposed to only temporarily suppress the bacteria and to be incapable of completely eradicating the infection from the host [14].

The annual recurrence rate of *H. pylori* is 2.67% in developed countries and 13% in developing countries such as Peru, Brazil, Chile, Vietnam and Bangladesh [11]. Many cases of recurrence in developed countries are due to late recrudescence within the first year (46%), while in developing countries the rate of reinfection is more pronounced and continues at the same pace as the eradication rate [13]. The rates of reinfection are extremely low in Europe and the USA; indeed, their rates are similar to that of South Korea, where the annual reported rate is approximately 1% [14].
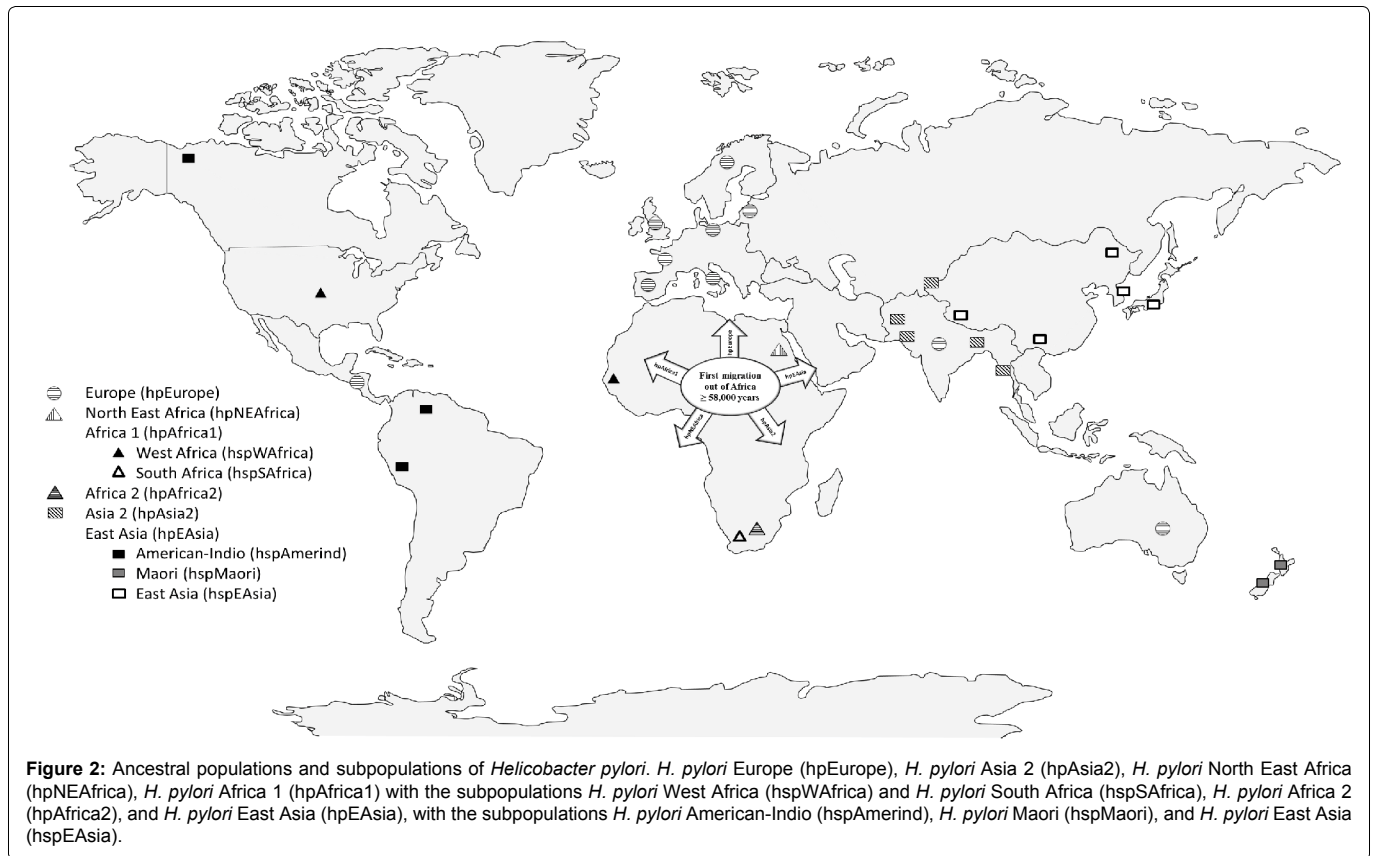
Molecular biology techniques are very useful tools for the diagnosis of *H. pylori* infection. These techniques can identify the genotype of the bacteria before and after treatment and determine whether strains are genetically identical; thus, it has been confirmed

that the recurrence of infection is due to recrudescence in 80% of cases [13]. However, the possibility of reinfection with an identical community strain that is shared among family members cannot be dismissed. Furthermore, different strains may be isolated from the same host, representing a broad diversification due to micro evolutionary changes in the host itself. This microevolution is driven by changes in the genetic background of a bacterial species that results in changes in various genes over time and can result in the presence of several existing genotypes in a single *H. pylori* population.

## Genetic Diversity of *H. pylori*

Genetic diversity is the sum of the genetic information that exists among the members of a species. *H. pylori* is a bacterium with high genetic diversity. Typically, high genetic diversity in bacteria is attributed to the ability to easily incorporate exogenous DNA, to the possession of genes with a high mutation rate, to the ability to horizontally transfer genes and to recombination events [6,15]. Several studies have suggested that the acquisition and loss of genes are mechanisms that facilitate the adaptation of the bacterium to new host environments beyond the changes that occur in the gastric environment [16-23]. Likewise, specificity, inflammatory responses, and the availability and distribution of adhesion molecules in the human host are factors that may select for divergence between strains [24].

Possible scenarios have been proposed to account for the generation of variability in *H. pylori*: monoclonal and polyclonal transmission. In the first scenario, a single clone may diverge rapidly within the microniches of the stomach to adapt to the new environment; however, as soon as the niches are occupied, the genetic exchange rate is reduced. Thus, genetic changes can occur in a stepwise manner when there is variation in selection pressures. According to this scenario, most of the genetic variability of the clone was generated during the infancy of the patient (assuming that he or

**Figure 2:** Ancestral populations and subpopulations of *Helicobacter pylori*. *H. pylori* Europe (hpEurope), *H. pylori* Asia 2 (hpAsia2), *H. pylori* North East Africa (hpNEAfrica), *H. pylori* Africa 1 (hpAfrica1) with the subpopulations *H. pylori* West Africa (hspWAfrica) and *H. pylori* South Africa (hspSAfrica), *H. pylori* Africa 2 (hpAfrica2), and *H. pylori* East Asia (hpEAsia), with the subpopulations *H. pylori* American-Indio (hspAmerind), *H. pylori* Maori (hspMaori), and *H. pylori* East Asia (hspEAsia).

she was infected early in life), and therefore the clone was present at the initial medical examination [9]. In polyclonal transmission, clonal variants are gradually developed over several generations in the host as a divergent population; consequently, the genetic variability of the clones observed in any host is the sum of all of the accumulated variations in all transmissions from previous infections [9].

Recombination events between genes from the same strain or between different alleles in the genome of *H. pylori* have been suggested to contribute to the high diversity of strains in the host. Therefore, sub clones of the bacterium may differ both in genotype and phenotype despite having been isolated from the same biopsy [9]. Israel et al. (2001) [25] demonstrated that all the sub clones in an individual are unique within the biopsies and between biopsies from different parts of the stomach. Mendoza-Elizalde et al. (2015) [26] found that 27 of 32 Mexican paediatric patients presented sub clones of *H. pylori* with different genotypes, thereby confirming the extensive allelic diversity of the bacteria within a single patient.

### Diversity between Nucleotide Sequences of *H. pylori*

The diversity of *H. pylori* nucleotide sequences exceeds that of other studied bacteria. The most unusual feature of this diversity is the high number of unique nucleotide sequences for each gene studied. Kansau et al. (1996) [27] analysed the nucleotide sequences of the *glmM* gene (encoding glutamate racemase) in 29 strains of *H. pylori* and found only two strains with identical *glmM* sequences.

The DNA sequences of virulence and housekeeping genes in *H. pylori* have different genotypes that may predominate in different human populations. Global studies have shown that high degrees of polymorphism in housekeeping genes is associated with changes in the third nucleotide position of codons; therefore, much of the observed variation is synonymous [28-30]. Mendoza-Elizalde (2015b) [31] observed a high degree of polymorphism in the housekeeping genes of 80 *H. pylori* strains from two Mexican paediatric patients, revealing 26 new alleles for the *atpA, efp, mutY, ppa, trpC, ureI* and *yphC* genes.

Phylogenetic analysis based on housekeeping gene sequences provides an overview of the evolutionary dynamics of infectious *H. pylori* strains within individual families [32]. These studies have revealed the presence of five ancestral populations and subpopulations of *H. pylori*: *H. pylori* Europe (hpEurope), *H. pylori* Asia 2 (hpAsia2), *H. pylori* North East Africa (hpNEAfrica), *H. pylori* Africa 1 (hpAfrica1) and *H. pylori* East Asia (hpEAsia) (Figure 2) [1,33-35].

### Bacterial Strain-Host Interactions

Humans can be colonized by a single strain or multiple strains of *H. pylori*. Over time, the evolutionary dynamics of the organism itself can give rise to multiple subpopulations of *H. pylori*. Processes such as mutation or genetic recombination play a fundamental role in this diversity [30], thereby allowing the emergence of different genotypes such as genotype transitions (presence of chimaeras), virulent or toxigenic genotypes and non-virulent or non-toxigenic genotypes.

It has been observed that recombination of *H. pylori* over long periods of time may give rise to new clones. For example, strains with the *cagA* gene, which belongs to the pathogenicity island (*cag*PAI+), can pass *cagA*+ to *cagA*- strains through the acquisition of genetic material from *cagA*- strains (*cag*PAI-) [36,37]. These arrangements have not been well studied and are detectable only during the transition phase, after passage of an *in vitro* strain (culture) to an *in vivo* environment (animal) or during colonization of an uninfected host [36].

A model has been proposed to explain the evolution and transmission of *H. pylori*. This model assumes that strains containing the virulence genes encoding the oncogenic protein CagA, the vacuolating cytotoxin VacA, and the adhesin BabA represent a "state of maximum welfare" and that the activation of any of these genes reduces the physical state of the bacterium. *H. pylori* lives in the stomach of an individual for many years, where it continually produces strains that are *ccag, vacA or babA* positive or -negative [38]. The emergence of multiple defective derivatives in some cases may overcome the wild-type bacterium, allowing the isolation of *cag*+ and *cag*- strains from the same patient. However, these defective derivatives do not survive for long times and thus represent the loss of some branches of the evolutionary tree. Therefore, only strains that are efficient in long-term colonization and can participate in person-

to-person transmission will endure and direct the evolution of the species [37,38].

## Animal Model of *H. pylori* Infection

As mentioned above, *H. pylori* displays a high level of genetic diversity due to high rates of mutation and recombination that allow the generation of variability between different strains. The use of animal models that are informative and manageable allows control over the presence or absence of bacterial antigens during different stages of infection. Therefore, animal models are routinely used to study the relationships between the immune response or basic genetics and susceptibility to bacterial infection and to identify factors related to survival and growth [23,39].

Microbial populations in infected or colonized hosts are subjected to selective forces and therefore are dynamic. The population dynamics of *H. pylori* during colonization of an infected animal offers an experimental model in which phenotypic and genotypic evolution are quantifiable *in vivo*, from the initial inoculation until the definitive establishment of a quasi species [40]. The spectrum of organisms used to study the pathogenesis of *H. pylori* includes rhesus monkeys, mice, and gerbils [41,42]. Since Hirayama et al. (1996) [43] reported the establishment of a persistent *H. pylori* infection in Mongolian gerbils (*Meriones unguiculatus*), research has been conducted on the association between *H. pylori* and gastric diseases. Some findings have suggested that *H. pylori* is directly related to gastric carcinogenesis [42].

The Mongolian gerbil infection model is considered useful because the changes observed in the gastric mucosa in this model are similar to those seen in human stomachs after infection with *H. pylori* [44]. Nakagawa et al. (2005) [42] reported that gastric colonization by *H. pylori* in Mongolian gerbils is related to several bacterial factors, including urease production and motility. Iwao et al. (1999) [45] reported that *H. pylori* motility was an important factor for the gastric colonization of Mongolian gerbils and that there were differences in gastric colonization due to phenotypic differences between the strains in the gerbils. Karita et al. (1991) [46] and Ohkusa et al. (2003) [47] observed that different strains induced different levels of infection severity in Mongolian gerbils. Furthermore, the *cag* PAI has been reported to play an essential role in the inflammatory process and formation of ulcers *in vivo* in Mongolian gerbils [48].

The microevolutionary history of *H. pylori* infection in humans reveals a remarkably high level of genetic diversity in this bacterium that is mainly generated by point mutations and by recombination (intra genomic or inter genomic) [49,50]. In turn, this high level of diversity has allowed the identification of different strains, sub clones or genotypes in the same individual that may occupy different microniches in the stomach. These strains may have selective advantages at different times during colonization and may allow the emergence of new bacterial populations [51]. Additionally, the use of an animal model has allowed the analysis of microevolutionary changes in the bacterium that occur during colonization, persistence and induction of disease in animals.

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Mendoza-Elizalde et al. J Genet Genome Res 2016, 3:1

ISSN: 2378-3648 • Page 5 of 5 •