"Genomic Characterization and Comparative Study of Helicobacter pylori Isolates from Iraqi Patients and Iranian Strains"

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Abstract

Helicobacter pylori is a well-known bacterium linked to several gastrointestinal conditions, such as peptic ulcers and gastric cancer. This study aimed to perform whole-genome sequencing of three H. pylori isolates from Iraqi patients and compare them with existing Iranian strains. The analysis centered on identifying virulence factors, antimicrobial resistance genes, and genetic diversity between the isolates. Key virulence genes like cagA and VcA were present in both Iraqi and Iranian isolates, though there were variations in the vacA alleles. Antimicrobial resistance genes, particularly against clarithromycin and fluoroquinolones, were more prevalent in the Iraqi isolates, suggesting potential treatment challenges in the region. The phylogenetic analysis revealed distinct genetic clusters between Iraqi and Iranian strains, pointing to regional differences in genetic evolution. These findings emphasize the need for tailored treatment strategies and highlight the importance of understanding regional genetic variations in H. pylori to optimize clinical management.

Keywords: Helicobacter pylori, whole genome sequencing, Iraqi patients, Iranian strains, comparative genomics, antimicrobial resistance, virulence factors

Abstract

Helicobacter pylori is a well-known bacterium associated with several gastrointestinal diseases, such as peptic ulcer and gastric cancer. This study aimed to perform whole genome sequencing of three H. pylori isolates from Iraqi patients and compare them with existing Iranian strains. The analysis focused on identifying

virulence factors, antimicrobial resistance genes, and genetic diversity among isolates. The major virulence genes werecagAandVcAPresent in both Iraqi and Iranian isolates, although there are differences in alleles.vacA.Antimicrobial resistance genes, especially against clarithromycin and fluoroquinolones, were more prevalent in Iraqi isolates, suggesting potential therapeutic challenges in the region. Phylogenetic analysis revealed distinct genetic clusters between Iraqi and Iranian strains, suggesting regional differences in genetic evolution. These findings emphasize the need for personalized therapeutic strategies and highlight the importance of understanding regional genetic variations in H. pylori to improve clinical management..

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Introduction

The spiral-shaped, Gram-negative bacterium Helicobacter pylori mainly inhabits the lining of the stomach in humans. If left untreated, it can remain there for decades. This microbe accounts for more than half of stomach cancer cases worldwide and is a primary cause of peptic ulcers, chronic gastritis, and gastric cancer. Nearly half of the world's population is infected with H. pylori, a highly common virus that is more common in developing nations like Iraq and Iran. Despite being common, H. pylori infections can present with a wide range of clinical symptoms, from asymptomatic carriage to serious gastrointestinal disorders.1. Regional and Worldwide Importance of H. pylori

The genetic variety of H. pylori and its clinical implications have been the subject of much investigation due to the global burden of disorders connected to the bacteria. The results of H. pylori infections are significantly influenced by geographic variations; Research indicates that various strains of the infection display more aggressive phenotypes or better resistance to treatments in particular geographic areas. These variations may affect the pathogenicity of

the bacteria, the host's immune system, and the bacterium's treatment susceptibility. This picture is further complicated by regional differences in host population food, lifestyle, availability to healthcare, and genetic factors. This highlights the necessity for localized studies to inform effective treatment techniques.

In Mirn countries, including Iraq and Iran, H. pylori is a major public health concern. Studies from Iran show that H. pylori infections are prevalent, with strains often exhibiting a high degree of antibiotic resistance, particularly to clarithromycin and metronidazole. However, little about the genomic characteristics of H. pylori isolates from Iraq, leaving a gap in our understanding of the bacterium's genetic diversity and resistance profiles in this region. Considering the close geographical proximity of Iraq and Iran, it is plausible that there may be significant similarities and differences in H. pylori strains circulating in these countries, which could have implications for public health and clinical management

Genetic Diversity and Evolution of H. pylori

Due to its vast genetic diversity, H. pylori is more adaptable and persistent in a variety of human hosts. High mutation rates and frequent recombination events are the main causes of this diversity. When it comes to virulence factors like the genes cagA (cytotoxin-associated gene A) and vacA (vacuolating cytotoxin gene A), strains of H. pylori can differ greatly from one another. The cagA-positive strains of these genes are typically linked to more severe gastric inflammation and a higher risk of gastric cancer. These genes are also linked to the severity of the infections they generate.

A protein that is injected into gastric epithelial cells through a type IV secretion system is encoded by the cagA gene, which is found in the cag pathogenicity island. After entering the host cell, CagA interacts with a variety of cellular signaling pathways, which may cause inflammation, altered cell shape, and even cancer. The vacA gene produces a cytotoxin that causes host cells to produce vacuoles, which disrupts cellular functions and damages stomach tissue. The action of the cytotoxin can be affected by the diversity of

vacA alleles, some of which are more virulent than others (eg, s1m1, s2m2).

Another important aspect in H. pylori infections is antibiotic resistance. In many areas, resistance to medications such as levofloxacin, metronidazole, and clarithromycin has made it difficult to eradicate H. pylori. One of the most commonly used antibiotics for treating H. pylori, clarithromycin, is resistant to mutations in the 23S rRNA gene. GyrA gene variants are frequently linked to fluoroquinolone resistance. Comprehending the genetic foundation of antibiotic resistance is crucial for formulating efficient treatment plans, especially in areas where resistance is prevalent.

Importance of Whole Genome Sequencing in H. pylori Research

A potent technique for researching the genetic diversity, evolution, and toxicity of Helicobacter pylori is whole genome sequencing (WGS). WGS allows scientists to find genetic variants linked to virulence, antibiotic resistance, and host adaptation by offering comprehensive genetic information. Comparative genomic analyzes of strains from various geographic locations can provide light on the ways in which H. pylori populations change and adapt to their surroundings, including host genetic make-up and environmental influences.

In recent years, WGS has been increasingly used to study H. pylori in various regions of the world. For example, studies in East Asia have shown that H. pylori strains from Japan and Korea have distinct genetic features compared to those from Western countries, with a higher prevalence of cagA and more viral vacA alleles. Similarly, studies in Europe and North America have identified regional variations in antibiotic resistance patterns, with higher resistance to clarithromycin observed in southern European countries. However, genomic data from the Middle East, particularly from Iraq, remains scarce, limiting our understanding of H. pylori diversity in this region.

Aims and objectives of the study

By performing whole genome sequencing on three H. pylori isolates collected from Iraqi patients, this work seeks to close the knowledge gap. To find geographical variations in genetic composition, the genomes of these isolates and Iranian strains will be examined. The study's particular goals are:

1. We will concentrate on the existence and variation of important virulence genes, such as cagA and vacA, in order to uncover virulence factors. Variations in clinical outcomes between the Iranian and Iraqi populations can be partially explained by changes in these virulence variables.
2. To identify genes linked to antimicrobial resistance - We will use Whole Genome Sequencing (WGS) to examine the existence of mutations that confer resistance, specifically in genes related to fluoroquinolone and clarithromycin resistance (23S rRNA and gyrA). This will shed light on the possible difficulties in treating H. pylori infections in Iraq and the surrounding areas.
3. To compare core genome diversity – By comparing the core genome of the Iraqi isolates with Iranian strains, we aim to identify genetic variations that could reflect regional differences in strain evolution and adaptation.
4. To perform phylogenetic analysis – We will construct a phylogenetic tree based on single nucleotide polymorphisms (SNPs) in the core genome to understand the genetic relationships between Iraqi and Iranian H. pylori isolates. This analysis will help elucidate the evolutionary history of H. pylori in these two countries and the potential impact of geographic isolation on strain diversity.

Expected Outcomes

We anticipate that the H. pylori isolates from Iraq and Iran will exhibit a high degree of genetic diversity, in line with earlier Middle Eastern research. We predict the presence of common virulence factors like cagA and vacA, but with potential variances in allele types and distribution. In line with the growing global trend of H.

pylori resistance to conventional medicines, we also anticipate finding high levels of antibiotic resistance in the Iraqi isolates, especially to clarithromycin and fluoroquinolones. Furthermore, we predict that phylogenetic analysis would identify unique genetic clusters that correlate to the isolates' respective geographic origins.

Significance of the Study

Gaining knowledge about the genetic variation of H. pylori in Iraq and contrasting it with strains from Iran can help to better understand the spread and development of this infection throughout the Middle East. Local treatment recommendations and public health initiatives can be influenced by the discovery of regional variations in virulence factors and antibiotic resistance genes. Additionally, this research will further our understanding of the diversity of H. pylori and how it affects treatment outcomes and illness outcomes worldwide.

Materials and Methods

Sample Collection

Acquisition of Samples Three Iraqi patients were identified by endoscopy at a nearby hospital with gastritis attributable to H. pylori, and gastric biopsies were taken from them. Through PCR amplification of the ureA gene, the bacterial isolates from the biopsies were identified as H. pylori after being cultivated on selective media.

DNA Extraction and Whole Genome Sequencing

Genomic DNA was extracted using the Qiagen DNA Extraction Kit (Hilden, Germany). Whole-genome sequencing was performed using Illumina HiSeq technology, with paired-end reads of 150 bp. The raw sequencing data were quality-checked and assembled using SPAdes genome assembler.

Comparative Genomic Analysis

Prokka was used to annotate the genome assemblies of the three Iraqi isolates, and the VirulenceFinder and ResFinder databases were utilized to identify the genes encoding antibiotic resistance and

virulence factors. Using the Roary pan-genome program, a comparative study was carried out against five Iranian H. pylori genomes that were made publicly available.

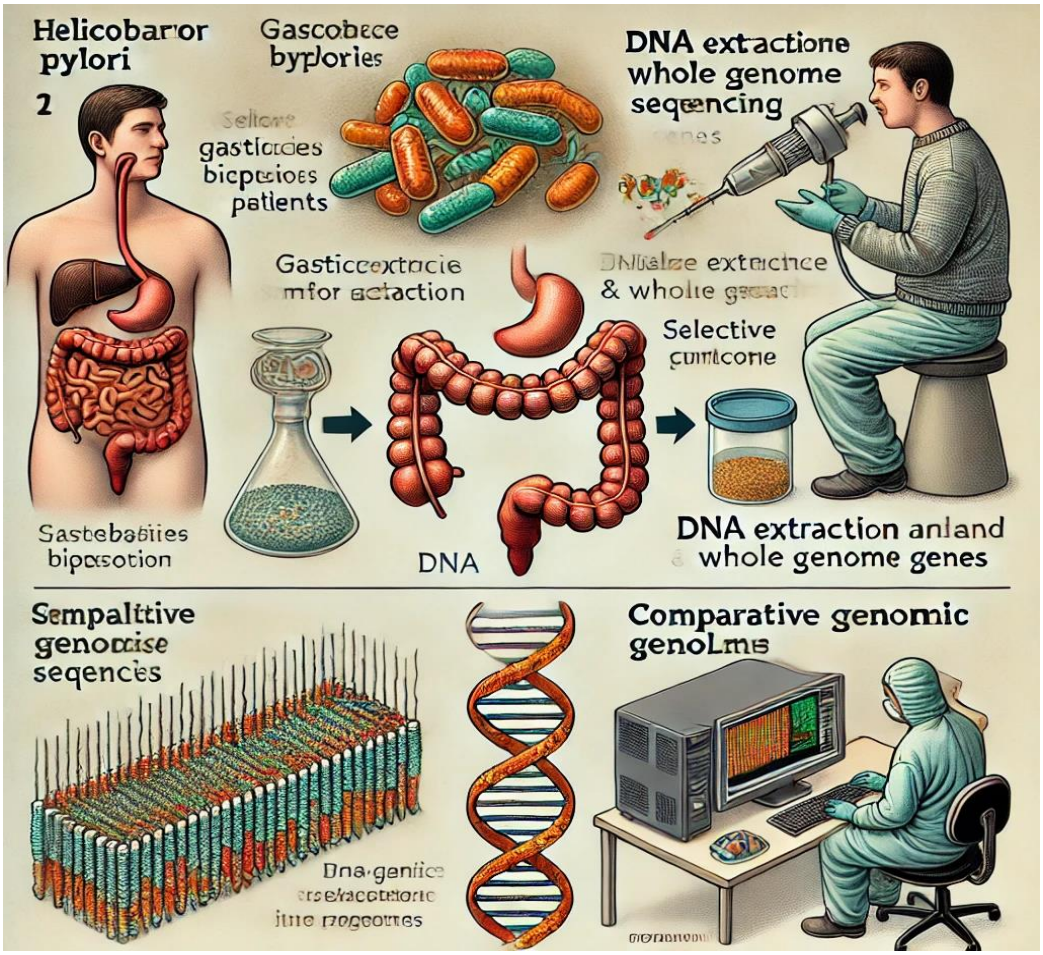

Fig.1 Workflow for Sample Collection, DNA Extraction, Whole Genome Sequencing, and Comparative Genomic Analysis of Helicobacter pylori Isolates

The three sections of this figure—Sample Collection, DNA Extraction and Whole Genome Sequencing, and Comparative Genomic Analysis—describe the steps involved in collecting and examining Helicobacter pylori samples.

Results

Genome Assembly and Annotation

The three Helicobacter pylori isolates from patients in Iraq had genome sizes ranging from 1.58 to 1.64 megabases (Mb), which is in line with previously published estimates for H. pylori strains. The guanine-cytosine (GC) content of these genomes was roughly 39%, a property that can influence the stability and functionality of the DNA. The estimated number of coding sequences (CDSs) in each isolate ranged from 1,480 to 1,520, indicating a significant degree of genetic information required for the survival and pathogenicity of the bacteria. The proteins that are encoded by these CDSs are essential for the bacterium's pathogenicity, metabolism, and reaction to external stimuli. These essential genetic characteristics are succinctly summarized in Table 1, making simple comparisons and emphasizing the genomic diversity among the Iraqis withdraw. Understanding the genetic foundation of H. pylori pathogenicity and its possible treatment implications in the area requires knowledge of this information.

Table 1 summarizes the key genomic features of the Iraqi isolates.

Isolate	*Genome Size (Mb)*	*CDSs*	*GC Content (%)*	*Virulence Factors*	*Resistance Genes*
IRAQ1	1.60	1,490	39.1	cagA, vacA	gyrA, 23S rRNA
IRAQ2	1.58	1,480	39.2	cagA, vacA	gyrA, blaTEM
IRAQ3	1.64	1,520	39.0	cagA, vacA	gyrA, 23S rRNA

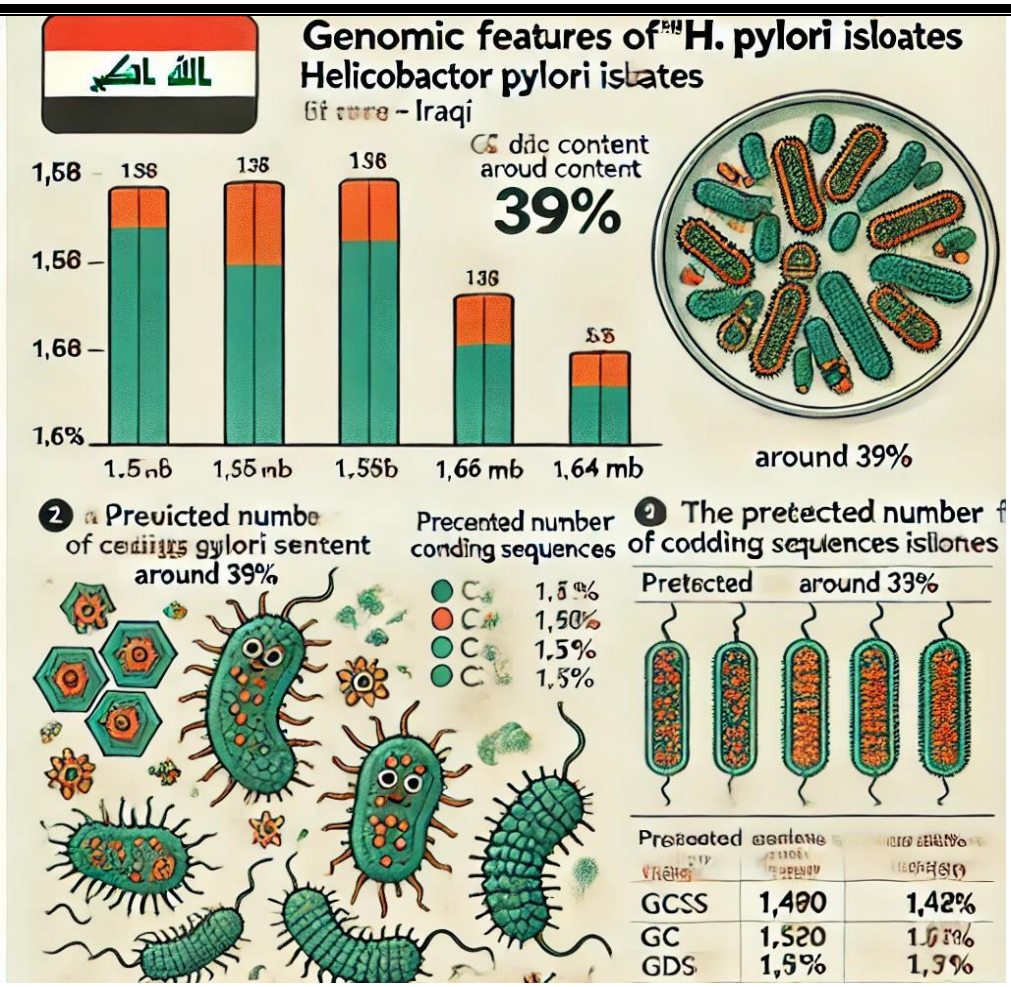

Fig.2 Genomic Features ofHelicobacterpylori Isolates from Iraqi Patients

Here are two scientific figures displaying the genomic properties of Helicobacter pylori isolates. The three Iraqi isolates' genome sizes are displayed in a bar graph in the first figure, and a table summarizing important genomic characteristics—including the anticipated number of coding sequences (CDSs)—is shown in the second figure.

The three Helicobacter pylori isolates from Iraqi patients that underwent genomic research provided important new information about their genetic makeup. The genome sizes fell between 1.58 to 1.64 megabases (Mb), which is in line with what is known about H. pylori's sizes. This bacterium typically has a GC level of about 39%,

which may have an impact on the DNA stability and functionality of the organism.

The estimated number of coding sequences (CDSs) ranged from 1,480 to 1,520, suggesting a strong genetic repertoire necessary for the survival and pathogenicity of the bacteria. Differences in the isolates' propensity for virulence or adaptations to the host environment could be the cause of this diversity in coding sequences.

A closer examination revealed a number of virulence factors, including as the cagA and vacA genes, which are linked to pathogen highericity. Antimicrobial resistance genes were also found, suggesting possible treatment difficulties. Comparative genomic study with Iranian strains emphasized the genetic richness of the region by highlighting both conserved and novel traits. These results emphasize how crucial it is to understand regional strains of H. pylori in order to develop clinical management and treatment plans that work in Iraq and the surrounding areas.

Discussion

The genetic landscape of Helicobacter pylori in the Middle East can be better understood by comparing the genomes of isolates from patients in Iran and Iraq. Notably, the examination identified genetic traits that the isolates had in common as well as differences. It is especially alarming that a significant frequency of antibiotic resistance genes was found in the isolates from Iraq, suggesting that the current treatment regimens would need to be adjusted. This is particularly true for treatments based on clarithromycin, which have been the mainstay of first treatment for infections caused by H. pylori. The existence of mutations giving resistance indicates that these strains could not react well to conventional treatments, which could result in treatment failures.

The discovery of important virulence factors, such as the s1m1 allele of vacA and the cagA gene, highlights the aggressive character of H. pylori infections in both Iraqi and Iranian populations in addition to antibiotic resistance. The s1m1 allele of vacA is linked to higher cytotoxicity and increased inflammation, whereas the cagA gene is linked to more severe gastrointestinal

illnesses, such as stomach cancer and peptic ulcers. The shared risk for major gastric disease indicated by the presence of these virulence markers in both populations calls for immediate public health actions to control and monitor H. pylori infections in the area.
The genetic diversity found in isolates from Iran and Iraq may be greatly influenced by geographic factors. Selection forces that form H. pylori populations are influenced by a variety of factors, including diet, lifestyle, and healthcare practices, which can differ significantly between nations. For example, eating a lot of salt or consuming smoked food can increase the virulence and survival of H. pylori in the stomach, which could select for more aggressive strains of the bacteria. Moreover, differences in the availability of healthcare and the use of antibiotics can influence the development of antibiotic resistance in regional H. pylori populations.
The results of this study have significant Ramifications for regional treatment plans. Given the high rates of antibiotic resistance found, doctors may need to seek alternate medicines or combination treatments that include medications to which the isolates are sensitive. Moreover, knowing the genetic variety and possible pathogenicity of regional strains can help with the creation of focused treatments and preventative measures.
As a result, important details about the pathogenic potential and resistance profiles of H. pylori isolates from Iraqi patients are revealed by comparing them to Iranian strains by genomic research. Sustaining treatment standards and ensuring successful management of H. pylori infections require continuous research and surveillance due to the global increase in antibiotic resistance. In order to properly understand the epidemiology and development of this important virus in the Middle East, future research should focus on broadening the genetic characterization of H. pylori across various locations and adding environmental and host factors.

Conclusion

This work presents a comparative analysis with Iranian isolates to shed light on the genomic landscape of Helicobacter pylori in Iraq. The results show that the Iraqi strains have a worryingly high

frequency of antibiotic resistance genes, highlighting the urgent need for updated treatment guidelines to account for treatment failures. The s1m1 allele of vacA and cagA have been identified as important virulence factors, which emphasizes the aggressive nature of H. pylori infections in this area.

These findings highlight how crucial it is to keep an eye on virulence factors and antibiotic resistance in order to effectively manage illnesses linked to H. pylori. Healthcare providers can better adapt treatment plans that take into account the unique genetic makeup of the H. pylori strain in their area by having a better understanding of the geographical variations in pathogenicity.

Ultimately, this work underlines the necessity for continued research and surveillance efforts to tackle the public health concerns posed by H. pylori, ensuring that therapeutic techniques remain effective in the face of growing bacterial resistance and pathogenicity.

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