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Intitulé

Development of Genomics Typing Webtool of *Helicobacter Pylori*

Soutenu par :

ELKAMILI FADOUA

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Devant le jury composé de :

Pr. Ibrahimi Azeddine, Faculté de Médecine et de Pharmacie de Rabat, Président

Pr. EL Allali Achraf, Université Mohammed VI Polytechnique de Benguerir, Encadrant

Dr. Laamarti Mariem, Université Mohammed VI Polytechnique de Benguerir, Co-Encadrante

Pr. Kandoussi Ilham, Faculté de Médecine et de Pharmacie de Rabat, Examinatrice

Dedicace:

To my family

I am extremely grateful to my parents **Hafida Ifran** and **Nour Eddin El kamili** for their love, prayers, caring, and for educating and preparing me for my future. I am very much thankful to my mother for her love, understanding, prayers, sacrifices, and always supporting me in my professional and personal life.

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In memory of my beloved grandfather

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Abstract

Title: Development of Genomics Typing Webtool of Helicobacter Pylori

Author: ELKAMILI FADOUA

Key words: Helicobacter pylori, website, Drug resistance, genomic Analysis.

Helicobacter pylori is present in all regions of the world with a higher prevalence in developing countries. Almost half of the world's population are infected with this bacterium. In this study, we present the first webtool for H pylori resistance, typing and virulence prediction based on the detection of SNPs and genes from WGS data of H pylori. The new webserver of *H.pylori* was made to make typing an easy approach by performing a detailed analysis of genetic variants of *H.pylori* strains, to provide information on genetic diversity and transmission of the bacteria in the whole world. The webserver also provides tools for virulence and resistance analysis which can play an important role in orienting the therapeutic approach and offer rapid identification and diagnosis for disease control and prevention.

Résumé

Titre : Développement d' Web de typage génomique d'Helicobacter Pylori

Auteur : ELKAMILI FADOUA

Mots clés : Hélicobactere pylori, Site, Résistance aux antibiotics, Analyse génomique.

Helicobacter pylori est un pathogène Gram négatif présent dans toutes les régions du monde avec une prévalence plus élevée dans les pays en développement. Près de la moitié de la population mondiale est infectée par cette bactérie. Dans cette étude, nous présentons le premier outil Web pour la résistance, le typage et la prédiction de virulence de H pylori basé sur la détection des mutations à partir des données de séquençage complet du génome, de H pylori. Le nouveau serveur web de H.pylori a été conçu pour faciliter le typage en effectuant une analyse détaillée des variantes génétiques des souches de H.pylori, afin de fournir des informations sur la diversité génétique et la transmission des bactéries dans le monde entier. Le site web fournit également des outils d'analyse de virulence et de résistance qui peuvent jouer un rôle important dans l'orientation de l'approche thérapeutique et offrir une identification et un diagnostic rapides pour le contrôle et la prévention des maladies.

ملخص

العنوان: تطوير موقع للتصنيف الجينومي لهيليكوباكتر بيلوري

الكاتب: الكاملي فدوى

الكلمات الرئيسية: هيليكوباكتر بيلوري، موقع، مقاومة المضادات الحيوية، التحليل الجينومي.

جرثومة المعدة أو هيليكوباكتر بيلوري هي عبارة عن جرثومة ممرضة تتميز بكونها سلبي الغرام وانتشارها في جميع أنحاء العالم مع انتشار أعلى في البلدان النامية. هناك ما يقرب من نصف سكان العالم مصابون بهذه البكتيريا. في دراستنا، أنشأنا موقعًا لتحليل بيانات هذه البكتيريا معتمدين على التسلسل الجينومي الكامل لجعل التصنيف الجينومي سهلا من خلال اجراء تحليل مفصل للطفرات الحاصلة داخل الجينات. تم انشاء هذا الموقع الجديد لتوفير معلومات حول التنوع الجيني لسلالات هذه البكتيريا وانتشارها في جميع أنحاء العالم بالإضافة الى النهج العلاجي وتوفير التعرف والتشخيص السريع لمكافحة الأمراض والوقاية منها.

Acronyms:

BWA: Burrows Wheeler Alignment

CD: Cluster of differentiation

ELISA: Enzyme-Linked Immunosorbent Assay

GERD: Gastroesophageal reflux disease

GML: Gastric MALT lymphoma

Hp: Helicobacter population

Hsp: Helicobacter Subpopulation

IFNγ: interferon γ

Ig: Immunoglobulin

IL: Interleukins

ITP: Idiopathic thrombocytic purpura

LPS: Lipopolysaccharide

MALT: mucosa-associated lymphoid tissue

MDR: Multi-Drug Resistance

MLST: Multilocus Sequence Typing

NF-Kb: nuclear factor-kappa B

NK: Natural killer

PAMP: Pathogen-Associated Molecular Patterns

PCR: Polymerase Chain Reaction

PNN: Polymorphonuclear Neutrophils

PPI: Proton Pump Inhibitor

PRR : Pattern Recognition Receptors

PUD: Peptic ulcer disease

RUT: The Rapid Urease Test

SAT: Stool Antigen Test

SnpEff: SNP Effect

SNPs: Single Nucleotide Polymorphism

SRA: Sequence Read Archive

TGFβ: Transforming growth factor β

Th1: Type 1 T helper

TNF-α: Tumor Necrosis Factor

Tregs: regulatory T lymphocytes

UBT: Urea Breath Test

VCF: Variant Call Format

WGS: Whole Genome Sequencing

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Introduction:

In 1982, the Australian doctors Robin Warren and Barry Marshall identified the bacterium *Helicobacter Pylori* as an etiological agent of gastritis and peptic ulcer disease. In order to this discovery, the two researchers won in 2005 the noble prize in physiopathology or medicine ("Marshall_Warren_Lancet, 1984) (Watts, 2005).

H.pylori is a Gram-negative, microaerophilic, curved bacterium that colonizes the human stomach permanently with a global prevalence of more than 50% and its prevalence varies by country and their level of development. In developing countries, such as Morocco, it ranges from 80 to 95 percent, while in industrialized countries; it varies between 15% and 30%. While its prevalence appears to be decreasing in developed countries, it continues to be a big issue (major problem) in developing countries (Mwangi et al., 2020) (Wroblewski et al., 2010) (Suerbaum & Michetti, 2002).

In 1994, *H. pylori* was declared a class I human carcinogen by the International Agency for Research on Cancer (IARC) of the World Health Organization (WHO), and this classification was confirmed in 2012. Gastric cancer is now considered a type of cancer that is associated with inflammation, and it is believed that the presence of H. pylori is necessary but not sufficient for this to happen.

H.pylori infection is associated with several pathologies of varying severity, including peptic ulcers, chronic gastritis, MALT lymphoma, and gastric cancer. But its pathogenic potential is profoundly shaped by the exceptional genetic diversification and geographical diversity of the species(Mwangi et al., 2020) (Wroblewski et al., 2010).

I.Bacteriological characters:

1-Taxonomy and Classification

Kingdom: Eubacteria

Branch: Proteobacteria

Class: Epsilon proteobacteria

Order: Campylobacterales

Family: Helicobacteraceae

Genesus: Helicobacter

Species: pylori

The genus *Helicobacter* belongs to the subdivision of the *Proteobacteria*, order *Campylobacterales*, family *Helicobacteraceae.* Members of the genus *Helicobacter* are all microaerophilic organisms and in most cases are catalase and oxidase positive, and many but not all species are urease positive.

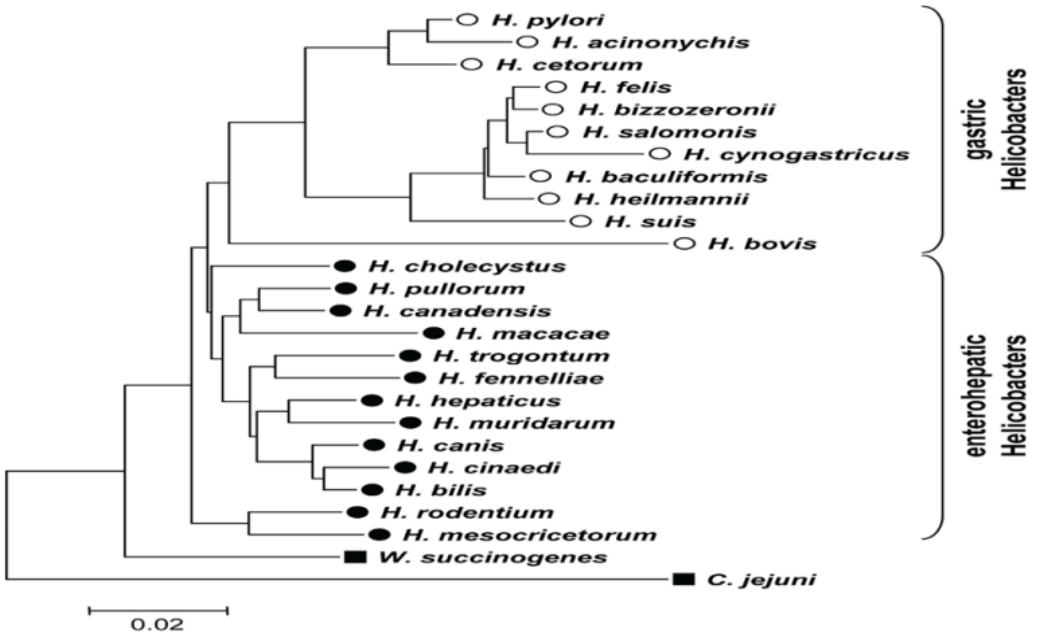

Figure 1: Phylogenetic tree of bacteria of the genus *Helicobacter*. (McColl, 2010)

The presence of spiral-shaped bacteria in the human stomach has been suspected for more than a century. But it was during 1982 that two Australian researchers, J.R. Warren and B.J.Marshall, confirmed the existence of a bacterium in the stomach environment. Because of the similarities

between the gastric spiral bacteria and the campylobacter due to its spiral morphology and the presence of flagella this bacteria was given the name **Campylobacter pyloridis** in 1983 for the first time. In 1987, the name was corrected to Campylobacter pylori. Subsequently, the study of its phenotypic and genotypic properties led to the definition of a new genus called Helicobacter. The bacterium was renamed H.pylori in 1989. (Thagard, 1998) (Suerbaum & Michetti, 2002) (Humans, 2012).

2-Morphology:

H. pylori organisms are 2.5-5.0 μm long and 0.5-1.0 μm wide. This bacterium has 4 to 6 flagella which give it great mobility and penetration in the mucus and allow it to colonize chronically the gastric mucosa of half of humanity, it also has the ability to switch from the bacillary structure to coccoid, this morphological variability helps the bacteria to adapt in stressful environmental conditions and increased tolerance to antimicrobial substances (Charitos et al., 2021) (Krzyżek & Grande, 2020).

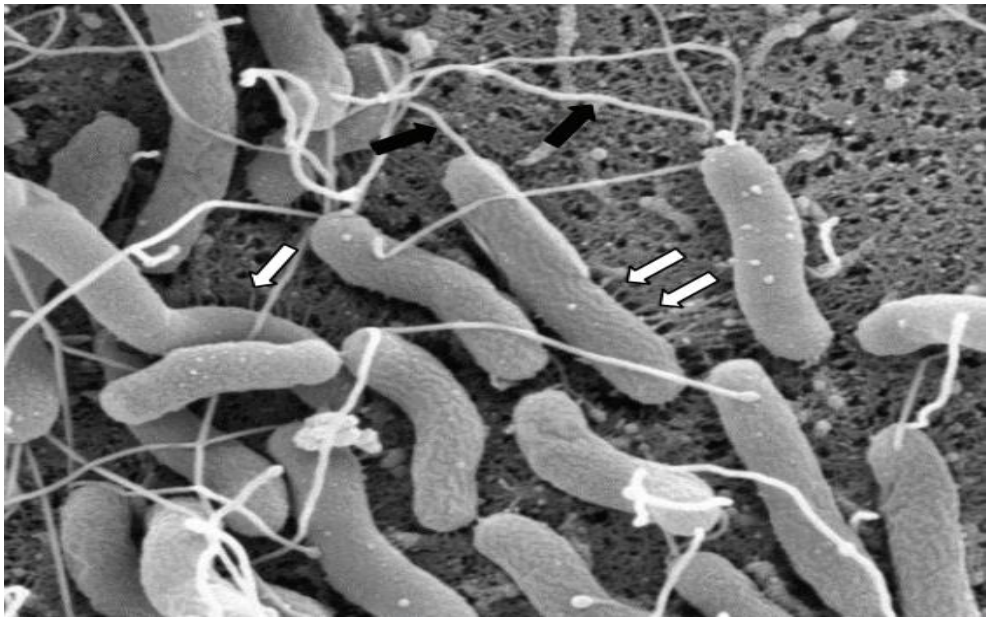

Figure 2: *H. pylori* in human gastric epithelial cells taken by high-resolution election scanning. The white arrows show pili, black arrows show flagella (Haley & Gaddy, 2015).

3-Genetic characteristics:

a. Genome:

The complete genome of *H.pylori* strain 26695 was first sequenced in 1997 in a patient with chronic gastric disease, followed by the genome of the J99 strain in a patient in the United States with duodenal ulcer. Subsequently, other strains of various pathological and geographical origins have been found and sequenced to provide many elements on the explanation of the more or less pathogenic character of the bacterium. *H.pylori* is composed of a single circular chromosome with a variable number of base pairs between 1.58 Mb and 1.67 Mb depending on the strains. The body of the genome contains about 1200 genes common to all strains and 200 to 400 genes present in various strains. (Tomb et al., 1997) (Alm et al., 1999) (Thiberge et al., 2010)

b.Genetic Diversity:

H.*pylori* is one of the bacterial species with the highest genetic polymorphism. This genetic diversity allows *H.pylori* to adapt its genotype to the conditions of its host and it is probably responsible for the variability of its pathogenicity. *H pylori* populations are very genetically diverse due to point mutations, substitutions, insertions or deletions that may involve one or more multigenic genes or segments. Chromosomal rearrangements (mainly inversions) are also considered as a source of this diversity. (Giannakis et al., 2008) (Blaser & Berg, 2001) (Reuse and Bereswill, 2007).

4-Virulence Factors of *Helicobacter pylori*

The high diversity of the *H.pylori* genome can demonstrates the pathogenesis of H.pylori by several virulence factors that facilitate colonization, induce inflammation, and damage host cells. These virulence factors have been linked to the risk of developing severe gastric diseases, they include: the cag pathogenicity island, vacuolating cytotoxin, Urease, Flagella, Peptidoglycan, and Lipopolysaccharide.

a.CagPAI:

The cag Pathogenicity Island is the most studied virulence factor of *H. pylori*; it is a 40 kb region of chromosomal DNA composed of about 27 to 31 genes. GagPAI codes for a type IV secretion

system (SSTIV) that permits the translocation of the CagA protein, peptidoglycan, and other components inside the epithelial cell. The presence of this virulence factor in *H.pylori* strains can increase the risk of higher grades of inflammation, which can lead to the most severe gastrointestinal diseases, such as atrophic gastritis, and gastric cancer (Roesler et al., 2014) (Shiota et al., 2013).

b.VacA

The vacuolating cytotoxin VacA is encoded by the VacA gene present in the majority of *H. pylori* strains, it is considered the second most extensively studied *H. pylori* virulence factor. VacA is a major determinant of *H. pylori*-associated gastric disease for its ability to induce many large vacuoles in cells in culture. More likely, VacA can induce vacuolation and multiple cellular activities, including membrane channel formation, cytochrome c release from mitochondria, which leads to apoptosis, and binding to cell membrane receptors followed by the initiation of pro-inflammatory response. (Lu et al., 2005) (Shiota et al., 2013) (Roesler et al., 2014)

c.Urease:

All clinical isolates of *H. pylori* express urease. This enzyme is important for bacterial colonization and it is considered as an indirect marker for the presence of *H.pylori*. The bacterium produces this important enzyme in order to counteract the acidic environment of the stomach by hydrolyzing urea into NH3 (ammonia) and CO2 (carbon dioxide). It has been demonstrated that urease-deficient bacteria cannot survive in the stomach but *H.pylori* express and produce urease in abundant quantities which gives it a unique ability to resist and colonize the gastric acidic environment (Roesler et al., 2014).

d.Flagella:

H. pylori is a mobile bacterium armed with four to six unipolar flagella. Each flagellum measures around 30 nm in diameter and 12–15 nm in length. The flagella allows *H.pylori* to move faster, penetrate, colonize and induce inflammation in the host cells of the gastric mucosa very easily. These flagella are protected by a protein sheath resistant to acidity. Each flagella is made up of two types of flagelline necessary for mobility; a major flagelline FlaA and a minor flagelline FlaB. (Cheok et al., 2021) (Sharndama & Mba, 2022).

e.Peptidoglycan:

Bacterial peptidoglycan (PG), one of the main protective barriers in the bacterial cell wall, it has been also shown that the presence of PG in *H.pylori* is an important inducer of gastric inflammation. In addition, PG can also be delivered into host cells through the T4SS and outer membrane vesicles (Kalali et al., 2014b)

f.Lipopolysaccharide

Lipopolysaccharide is an important cellular component of the outer membrane of Gram-negative bacteria, including Helicobacter pylori. LPS plays a key role in the pathogenicity of the *H.pylori* by contributing to colonization and persistence in the stomach and therefore, *H. pylori* LPS is one of the factors that could potentially influence local gastric inflammation and the clinical outcome during an *H. pylori* infection (Innocenti et al., 2001) (Leker et al., 2017).

II.Epidemiology:

1-Transmission:

The exact mode of transmission of *H.pylori* remain poorly understood and not clear identified. However, epidemiological studies recognize certain modes of transmission. Person-to-person contamination (through, saliva, vomit, or stool) by direct contact is considered the most likely route of infection in various ways: oral-oral, fecal-oral. Although indirect transmission via water and food sources are also mentioned and remains possible.(Stefano et al., 2018)

a.Gastro-oral transmission:

The transmission route could be via gastric fluid, vomit from infected subjects as well as air samples during vomiting or gastroesophageal reflux (GERD) in childhood, in association with poor hygienic conditions.(Stefano et al., 2018)

b.Oral-Oral Transmission:

The oral-oral transmission is also a possible source of *H.pylori* since the oral cavity was considered an appropriate reservoir for the sustenance of *H.pylori*, and it was, therefore,

suggested that oral-oral transmission involves especially the mother-child transmission infection (Stefano et al., 2018).

c.Fecal-Oral Transmission:

H.pylori was detected from the feces of infected human, but the culture of *H.pylori* from feces has been rare due to the bacterium persists there predominantly in a non-culturable (coccoid) form (Stefano et al., 2018).

d.Transmission by water:

Some studies hypothesize that water plays an important role as a source of transmission of *H.pylori*. it was demonstrated that children living in rural areas and swim in rivers or consume water have a higher prevalence of *H.pylori* infection (Lu et al., 2002).

e.Transmission by Food:

The transmission by food product may also happen due to poor hygienic conditions. Some authors suggested that infection caused by vegetables, meat and mainly by milk because milk mostly consumed during childhood which explain the high infection by *H.pylori* in this period (Stefano et al., 2018).

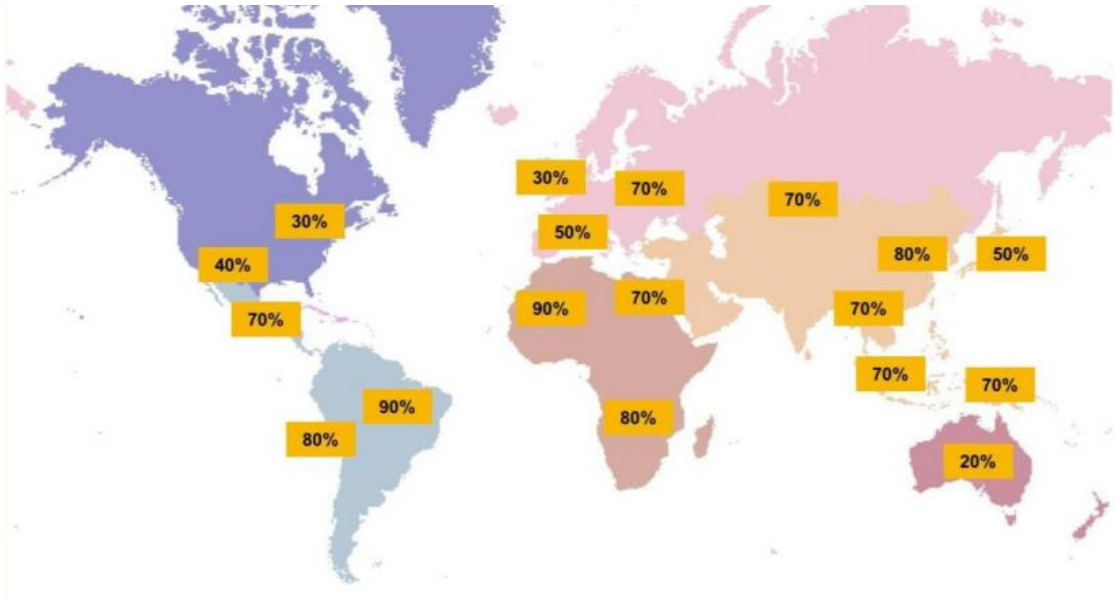

Figure 3: *Helicobacter pylori* prevalence in populations worldwide (Azevedo et al. 2009).

III.Physiopathology:

1-Inflammation response:

H. pylori induces a strong innate in the first place and then adaptive immune response in second place, stimulating the expression of cytokines and pro-inflammatory chemokines of gastric epithelial cells, which leads to the attraction of polymorphonuclear neutrophils (PNN), macrophages, dendritic cells, natural killer (NK), B and T lymphocytes, and induce the release of reactive oxygen species (ROS). This leads to chronic active inflammation characteristic of *H. pylori* infection, which may lead to alteration of the epithelium. (Smith, 2014) (Kalali et al., 2014)

The innate immune response is the first line of defense against *H.pylori* where the body sets up different defense mechanisms for eliminating the pathogen. Colonization of the gastric mucosa by *H.pylori* triggers the activation of innate defense mechanisms in the host in particular by the interaction with epithelial cells and the activation of the molecular patterns (signaling pathways), which include Toll-like receptors and Nod-Like Receptors (NLR),that recognize the molecular patterns associated with pathogens PAMP such as LPS, flagelline and PG. On the other hand, thanks to the pathogenicity island Cag, *H. pylori* causes a pro-inflammatory response of epithelial cells via the activation of the NF-κB pathway, resulting in the production of chemokines, responsible for the recruitment and activation of PNN at the site of infection, which amplifies the pro-inflammatory response by secreting pro-inflammatory cytokines in order to recruit and activate macrophages, dendritic cells and lymphocytes to have a more specific response and induction of an adaptive response. Macrophages are present in the infected gastric mucosa and secrete pro-inflammatory cytokines, and therefore have a powerful bactericidal activity. Finally, NK and lymphocytes are abundant in the infected gastric mucosa. Granzymes and perforine derived from NK lymphocytes can damage host cells. NK cells respond to *H. pylori* infection by secreting proinflammatory cytokines such as interferon γ (IFN) and TNF-α. (Kalali et al., 2014)

Chronicity of *H. pylori* infection is associated with induction of a response adaptive following the innate response. *H. pylori* infection causes a strong T-cell response, which includes both T CD4+ and T CD8+ (cytotoxic T) lymphocytes, which contribute to inflammation. CD4+ T lymphocytes can be differentiated into two main subtypes: Th1 secreting IL-2 and IFNγ, promoting the macrophagic response, and Th2 secreting IL-4, IL-5, and IL-13, but also regulatory T lymphocytes

(Tregs) via the production of IL-10. Tregs lymphocytes participate in the regulation of the Th1 response by the production of IL-10 and TGFβ (Transforming growth factor β) and thus allow the persistence of the infection via their anti-inflammatory action. They protect the gastric mucosa from excessive inflammation and tissue damage.(Kalali et al., 2014a) (Niu et al., 2020)

H. pylori infection also induces a humoral response, characterized primarily by the production of immunoglobulin M (IgM), IgG and IgA. The strong humoral response to IgG, especially in the chronic stage of infection, is used as an indirect marker of infection. (Kalali et al., 2014)

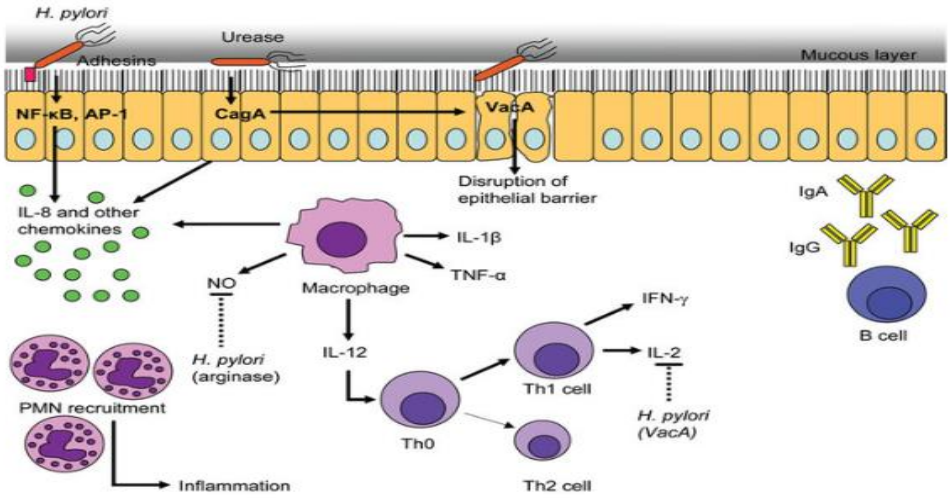

Figure 4: *H. pylori* pathogenesis and the inflammatory response (Cynthia Portal-Celhay, 2006)

2-Complications associated with H. pylori infection:

The majority of people, who have *H. pylori* colonization, remain asymptomatic; the likelihood of developing the disease may be influenced by host traits, *H.pylori* genotype, innate host physiopathology, genetic predisposition, and environmental factors. This gastric colonization by *H.pylori* may induce severe diverse human pathologies including chronic gastritis, dyspepsia, peptic ulcer disease, gastroesophageal reflux disease, MALT lymphoma, Gastric adenocarcinoma, and extragastric diseases. This clinical outcome of *H. pylori* infection is highly variable and depends on several factors. (Israel et al., 2001) (Kandulski et al., 2008).

a.Dyspepsia:

Dyspepsia also known as indigestion, is a set of symptoms that include chronic or recurrent pain or discomfort that occurs in the upper abdomen, Discomfort may be a feeling of epigastric fullness, early satiety, bloating or nausea, eructation, or burning of the epigastric gland.

b.Peptic ulcer disease:

Peptic ulcer disease is a condition in which painful sores or ulcers develop in the lining of the stomach or the first part of the small duodenum. *H.pylori* considered from the most common cause of PUD since the strong correlation between the colonization of *H. pylori* and PUD is well established by an abundance of studies (Ahmed & Belayneh, 2019) (Narayanan et al., 2018).

c.Gastroesophageal Reflux Disease:

Gastroesophageal reflux disease is a common chronic disease worldwide and is defined as a disease that develops due to a chronic retrograde flow of gastric contents from the stomach into the esophagus, oral cavity or lungs. Some studies demonstrated that *H.pylori*-positive was frequently seen in patients with GERD. A statistically significant relationship was found between *H.pylori* positivity and the grade of GERD (Polat & Polat, 2012).

d.MALT lymphoma

Gastric MALT lymphoma (GML) is the most common marginal zone lymphoma of the digestive tract, it is characterized by massive infiltration of lymphoid cells into the lamina propria resulting in destruction of the gastric glands and formation of lymphoepithelial lesions. The involvement of *H.pylori* as a pre-MALT lymphoma condition is now well established based on epidemiological, pathological, clinical, and bacteriological evidence and since it was demonstrated that more than 90% of cases, MALT lymphoma are infected with *H.pylori* (Floch et al., 2017) (Stolte et al., 2002).

e.Gastric cancer

Gastric cancer is considered from the leading common cause of cancer-related death in the world. The association of *H. pylori* and gastric cancer has been confirmed by large-scale epidemiological studies, meta-analysis of case control studies and experimental studies. And also since been classified as a class I carcinogen by the World Health Organization (Ishaq & Nunn, 2015).

f.Extragastric diseases

As of today, among th long list of possible associations, *H. pylori* infection is confirmed to play an important role in three extragastroduodenal diseases.

f-1 Iron deficiency anaemia

Iron deficiency anaemia (IDA) occurs in 2-5% of adult men and postmenopausal women in the developed countries, with blood loss from the gastrointestinal tract being the most common cause. The relationship between *H.pylori* infection and Iron-deficiency anaemia (IDA) has been proved over the past years (Monzón et al., 2013) (Kandulski et al., 2008).

f-2. Idiopathic thrombocytic purpura (ITP)

Idiopathic thrombocytopenic purpura, also known as Immune thrombocytopenic purpura (ITP), an autoimmune hematological disorder characterized by a decrease in the number of platelets in the blood. It has become clear that *H. pylori* infection is actively involved in the pathogenic process of ITP (Kuwana, 2014) (Kandulski et al., 2008).

f-3.Vitamin B12 deficiency

H.pylori infection is responsible for hypochlorhydria which limits the intestinal absorption capacities of iron and vitamin B12, and may lead to Vitamin B12 deficiency that can lead to hematologic and neurological symptoms (de Korwin, s. d.).

IV.Diagnostic:

The diagnosis methods of *H.pylori* infections is based on many techniques with varied performances grouped according to whether they are invasive or not. Invasive methods consist of endoscopic evaluation, the rapid urease test (RUT), histology, bacterial culture, and PCR. Non-invasive tests include the urea respiratory test (UBT), stool antigen test (SAT), and serology. The choice refers to the clinical circumstances, the sensitivity, and specificity of the test but also to its cost (Sabbagh, Mohammadnia-Afrouzi, et al., 2019).

1-Invasive methods

These methods usually require gastric biopsies performed during a gastroduodenal endoscopy.

a.Rapid Urease Test:

Rapid urease test (RUT) is the popular invasive for the detection of *H. pylori infection*. This test is based on the production of urease enzyme by *H. pylori* bacteria and the presence of this enzyme in the gastric mucosa. After the biopsy, the specimen is transferred to the solution comprising urea and a pH indicator. If *H. pylori* exist, urease will convert the urea into ammonia and CO2, which leads to a change in color of the indicator due to an increase in ph. The RUT is a very fast, inexpensive, reliable, and simple technique that provides results in a few hours (Sabbagh, Mohammadnia-Afrouzi, et al., 2019).

b.Histology

Histology is reviewed and considered as the gold standard in the direct diagnosis, it is recommended to obtain biopsy samples from multiple locations in the stomach due to the wide distributions of bacteria. This method can give essential information about the different types of gastritis, atrophy, dysplasia, and metaplasia. (Sabbagh, Mohammadnia-Afrouzi, et al., 2019)

c.Bacterial Culture:

The culture of *H. pylori* is performed on the gastric biopsy samples to confirm the H. pylori infection. The culture like the other invasive tests requires biopsy obtained by endoscopy of

the upper gastrointestinal tract. Culture allows the isolation of *H. pylori* for phenotypic and genotypic studies.

d.Polymerase chain reaction

Polymerase chain reaction (PCR) is one of the best molecular methods used in a wide range of clinical applications. This method is accurate, fast, and can detect very small amounts of DNA and thus can detect the presence of *H. pylori* even at a low bacterial load.

2-Non-invasive methods:

These methods are based on the presence of bacterial enzymes, antigens, antibodies, or DNA sequences. They include 13C or 14C urea breath test, stool antigen test (SAT), and serology.(Sabbagh, Mohammadnia-Afrouzi, et al., 2019)

a.Serology:

H. pylori infection causes immunological responses and antibody productions (IgA, IgG, and IgM). In this method, antibodies against *H. pylori* are detected by ELISA technique, which detect antibodies, produced against the bacterium and only the IgG antibody test is reliable. (Sabbagh, Javanian, et al., 2019) (Sabbagh, Mohammadnia-Afrouzi, et al., 2019)

b.Stool antigen test

The *H. pylori* stool antigen test is a non-invasive and easy method that can be used for the clinical and epidemiological studies. There are two types of SAT methods developed for detecting H. pylori antigens in the stool. These are enzyme immunoassay (EIA) and immunochromatography assay (ICA)-based methods and utilize either polyclonal or monoclonal antibodies. Monoclonal antibody–based tests show better results compared to polyclonal-based tests mainly because of the difficulty in obtaining polyclonal antibodies of consistent quality every time. EIA-based tests provide more accurate and reliable results than ICA-based tests although both tests can be performed with monoclonal antibodies**.**

c.Urea breathe test:

The urea breath test is a reliable and non-invasive technique, which does not need an endoscopy. The UBT is a valuable method to determine whether the pathogen has been eradicated or not. To perform this test, isotopically labeled urea (12 C, 13C, or 14C) is administered into the patient and bacterial urease produced by *H. pylori* into the stomach will be measured. If the urease enzyme is present in the stomach, the urea will be transformed into carbon dioxide and ammonia. CO2 . (Sabbagh, Javanian, et al., 2019)

V.Drug Resistance:

Antibiotic resistance is a natural phenomenal of bacterial adaptation. However, today, it is well known that the major negative impact on the results of *H.pylori* eradication therapies has increased because of multidrug resistance. This bacterium arises antibiotic resistance as a consequence of point mutations in bacterial DNA in a certain position. This MDR mainly concerns antibiotics used in the eradication protocols of the bacterium, such as clarithromycin, metronidazole, ciprofloxacin, rifabutin, and tetracycline. The antibiotic Data vary from country to country due to different prescribing and antibiotic use policies. It seems that this resistance is linked mainly to the use of these antibiotics in indications other than the eradication of *H.pylori* and poor compliance. Therefore, it is essential to have demonstrated the presence of *H. pylori* infection before any eradication treatment (Jaka et al., 2019) (Buran et al., 2022).

VI.Treatment:

The eradication of *H. pylori* requires a polydrug treatment, based on a combination of many antibiotics. However, this association is questioned because of the increasing prevalence of antibiotics resistant strains in the world. The standard treatment protocol for the eradication of *H. pylori* is triple therapy. It consists of a proton pump inhibitor (PPI) such as omeprazole and two antibiotics, usually amoxicillin, clarithromycin, or metronidazole. It has been shown that this treatment eradicated approximately 80% of *H. pylori* at the beginning of the 1990s. But, later, the eradication success rate dropped below 60%, this is related to the increase of clarithromycin and metronidazole resistance in the world. Fluoroquinolones, such as levofloxacin and moxifloxacin, are often used as second-line treatment rescuers (De Francesco et al., 2017) (Kipritci et al., 2020).

Table l: The summary of *H.pylori* treatment

Therapy	Dose	Duration	Recommendation
Non-bismuth based	PPI 2x/d Amoxicillin 1g 2x/d Clarithromycin 500mg 2x/d Metronidazole 500mg 2x/d	10-14 Days	**First-line therapy**
Quadritherapy with bismuth	PPI 2x/d Bismuth subcitrate 120-300mg Bismuth subsalicylate 300mg 4x/d Metronidazole 250 or 500mg 4x/d Tetracycline 500mg 4x/d	10-14 Days	**First-line therapy**
Triple Therapy	PPI 2x/d Clarithromycin 500 mg 2x/d Amoxicillin 1g 2x/d or Metronidazole 3x/d 500mg	14 Days	**First-line therapy**
Sequential	PPI and amoxicillin 1g 2/d PPI 2/d Clarithromycin 500mg2/d Metronidazole 500mg 2/d	5-7 then 5-7 Days	**Alternative**
Hybrid	PPI + amoxicillin 1g 2/d PPI + clarithromycin 500mg 2/d + amoxicillin 1g 2x/ +metronidazole 500mg 2x/d	7 then 7 Days	**Alternative**
Levofloxacin based	PPI 2x/d Levofloxacin 500mg 4x/d Amoxicillin 1g 2x/d	10-14 Days	**Levofloxacin containig**
Levofloxacin sequential	PPI 2x/d + amoxicillin 1g 2x/d then PPI 2x/d +Levofloxacin 500mg 4x/d + metronidazole 500mg 2x/d	5-7 then 5-7 Days	**Levofloxacin containig**

VII. The importance of genomics in epidemiology:

The importance of genomics as a tool in the epidemiological investigation has already been proven since genomics is having a profound impact on the practice of epidemiology, particularly in the area of infectious diseases. Sequencing of the genome of the bacteria was instrumental in tracing its phylogenetic lineage, and a combination of genomic and epidemiological information allowed tracing the genotypic variation of the transmission paths.

H. pylori strains can be divided into seven population types based on geographical associations: hpEurope, hpEastAsia, hpAfrica1, hpAfrica2, hpAsia2, hpNEAfrica, and hpSahul. This genomic diversity within *H. pylori* populations was examined by employing the Multilocus sequence typing analysis (MLST) using seven housekeeping genes (atpA, efp, mutY, ppa, trpC, ureI, and yphC). Population structure analysis based on MLST can also be useful as a tool to track and predict the history of human migrations utilizing the high genetic diversity and frequent recombination between different *H. pylori* strains. (Suzuki et al., 2012)

Phylogenetic tree constructed by using MLST data (fig5). Branch colors represent *H. pylori* populations determined by using the Bayesian clustering method. HspSAfrica and hspWAfrica are subpopulations of hpAfrica1 and hspAmerind, hspMaori, and hspEAsia are subpopulation of hpEastAsia.

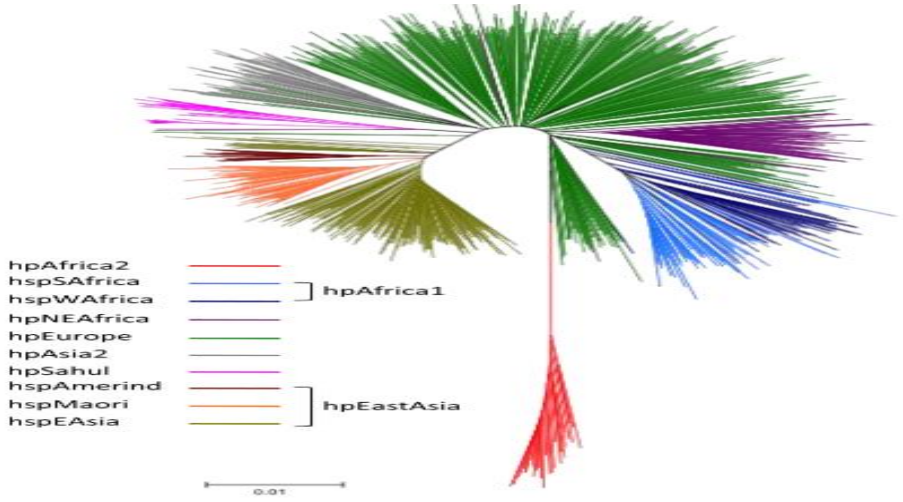

Fig 5: *Helicobacter pylori* populations and phylogenetic tree (Suzuki et al., 2012)

VIII.Materials and Methods

1-Data collection

The collected database contains several information about the genomes of the *H.pylori* samples represented in a metadata table that include BioSample Accession, BioSample Strain, BioSample Collection Date, Assembly Accession, BioProject Accession, BioSample Geographic Location, and most importantly the BioSample Host Disease information. This information were collected from NCBI by using *H.pylori* as keyword. Data with missing information in NCBI were completed using the literature review articles in PubMed containing the same accession number.

Besides the metadata database, we downloaded more than 4000 assembled *H.pylori* genomes from the NCBI Refseq database using the ncbi-genome-download tool and 1000 Fastq files from the NCBI Sequence Read Archive (SRA) database using Fastq-dump. After the metadata filtering and curation, 3352 genomes were selected (Table 2).

Table II: Summary of 3352 *H.pylori* genomes by origin.

Continent	Country	Number of isolates
Africa	Angola	1
	Morocco	6
	Nigeria	1
	South Africa	3
Asia	Bangladesh	20
	Bhutan	1
	Cambodia	53
	China	849
	India	64
	Indonesia	1
	Israel	48
	Japan	201
	Korea	3
	Kuwait	3
	Malaysia	102
	Nepal	1
	Singapore	18
	Taiwan	12
	Thailand	1
	Turkey	2
	Viet Nam	1
	Belarus	2
	Belgium	6
	France	50

Europe	Germany	516
	Ireland	1
	Poland	2
	Portugal	15
	Russia	3
	Spain	73
	Sweden	25
	Switzerland	131
	United Kingdom	10
North America	Canada	24
	El Salvador	2
	Guatemala	2
	Honduras	31
	Mexico	247
	Nicaragua	56
	United States of America	183
South America	Argentina	8
	Brazil	10
	Chile	3
	Colombia	256
	Peru	51
	Venezuela	12
Oceania	Australia	237
	New Zealand	4
	Papua New Guinea	1

Another database was collected based on the literature review articles was the antibiotics resistance database that contained the resistant gene with the position of the mutation in the sequences, the amino acid changing and the antibiotics names .

2-Variant calling analysis:

The reads generated by ILLUMINA were mapped to the reference genome *H.pylori 26695* (Sample name: AE000511), Using BWA-MEM algorithm version 0.7.17-r1188. To use BWA (alignment via burrows wheeler transformation), we first index the genome with “bwa index”, and then we use the alignment algorithms in BWA: “mem” (Li & Durbin, 2009) . The reads generated by PacBio or Oxford Nanopore were mapped to the same reference *H.pylori 26695*, using Minimap2 version 2.24 (Li, 2018). After the generation of SAM files, they were converted to BAM files using SAMtools view, and then BAM files were sorted using SAMtools sort. SAMtools and BCFtools version 1.15.1 are widely used programs for processing and analyzing high-throughput sequencing data (Danecek et al., 2021). The sorted files are used to call the genetic variants in

variant call format (VCF) by BCFtools using bcftools mpileup and bcftools call commands. VCF is a text file format, it contains meta-information lines, a header line, and then data lines each containing information about a position in the genome. The format also has the ability to contain genotype information on samples for each position. (Danecek et al., 2011)

VCF files are annotated using SnpEFF, which uses the variant call format (VCF) as an input format. SnpEff, an abbreviation of SNP effect that can annotate and predicts the coding effects of genetic variants such as SNPs, insertion, and deletions (INDELS. After using SnpEFF, we use SnpSift to filter large genomic datasets in order to find the most significant variants and classify SNPs based on gene annotation. (Cingolani et al., 2012)

3-Antibacterial resistance

All publications reporting mutations were carefully reviewed for the consistency of the information about the mutated nucleotide and amino acid. We used the codon numbering given by the annotation of the *H.pylori* whole genome sequence published in all studies reporting an association between specific mutations in clinical isolates of *H. pylori* and phenotypic resistance to antibiocs.

4-Virulence database

The virulence genes were taken from The Virulence Factors of Pathogenic Bacteria (VFDB) database (http://www.mgc.ac.cn/VFs/main.htm). All the genomic fasta files were blasted to retrieve pathogenicity-associated genes (Chen et al., 2005).

5-Helicobacter pylori typing

The VCF files were merged into one big VCF file to which a serie of Augur commands and other standard bioinformatics tools were applied, a phylogeny was created and can be viewed in Auspice, the web application that serves Nextstrain offer. Nextstrain is an open source project consists of a database of genomes, a bioinformatics pipeline for phylodynamic analysis, and an interactive visualization platform (Hadfield et al., 2018).

6-The creation of the Helicobacter pylori website:

The website of *H.pylori* was established based on three analysis: First analysis was an antibiotic resistance mutation analysis provides a database of SNPs based on variant files as input; second analysis was a typing analysis provides a phylogenetic and geographic visualization based on the Nextstrain framework, and the Third analysis was a virulence analysis provides a database for virulence genes of *H.pylori* using an online resource for curating information about virulence factors of bacterial pathogens. The website was created based on bootstrap (a free front-end framework for faster and easier web development), Css (one of the main languages of the Open Web), html (used to create and represent the content of a web page and its structure), php (is a free programming language, mainly used to produce web pages), javascripts (a programming language that execute a complex mechanisms on a web page), python (programming language that promotes structured, functional and oriented imperative programming), and MySQL(database management system).

IX.Results and Discussion:

1-Fist analysis: Antibiotic Resistance

All the Fasta and Fastq genomes with the available information from NCBI and articles were downloaded and analyzed for creating the Helicobacter website. All the genomes' sequences were mapped to the reference *H. pylori* 26695 in order to find the significant mutations based on the comparison between the reference and the sample using the VCF format that summarizes all the differences and the changes between them. The significant found mutations were compared to our antibiotic resistance database in order to identify the impact of mutations by identifying their positions and the change in the encoded amino acid in order to find which antibiotic the strain is resistance to.

In the website, the resistance analysis appears in the home page. The user can click on this analyze and submit his file to analyze it in order to give it the result of the detecting mutation and the antibiotics resistance database summarized in a table.

Fig 6: Home page of the website showing the antibiotic resistance analysis

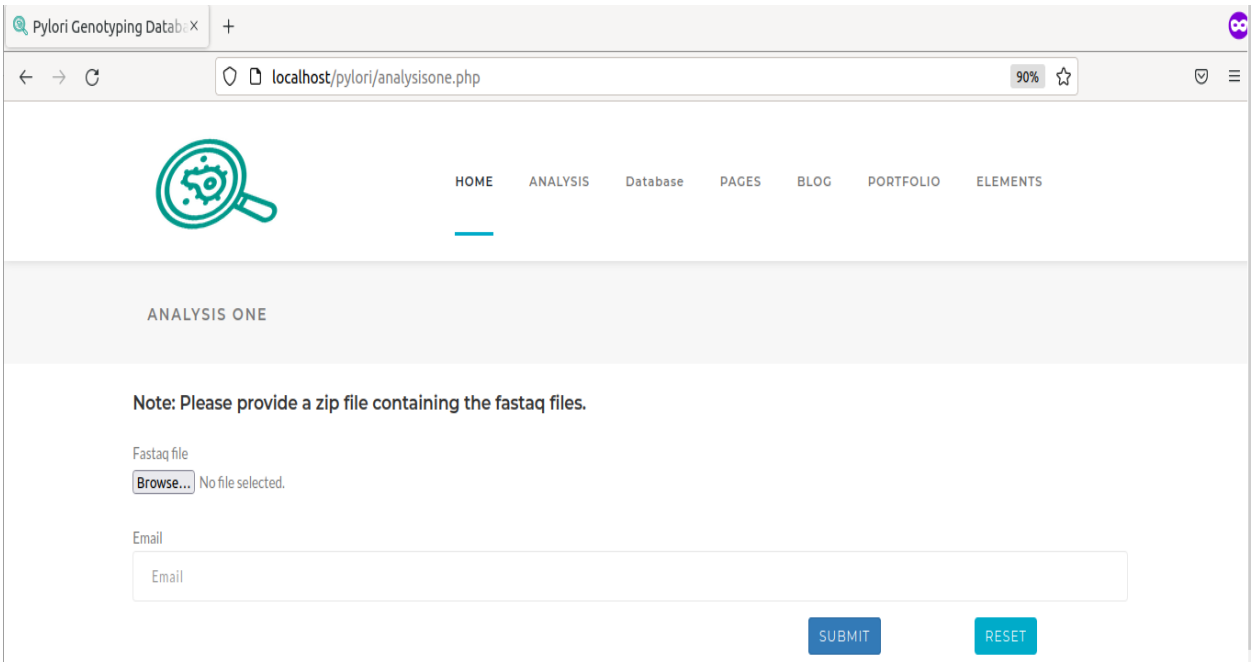

Fig 7: The window of the submission of the FASTq files

For the validation of this first analysis, we did run a Fastq file as an example in order to detect mutations in the genome sequence. The results of the analysis of the website were summarized in the following table.

Table III: The results of the finding mutation in the Fasta sequence genome with the antibiotics to which it is resistant (Metronidazole)

Gene	Locus	Variation or complete gene	Antibiotic
frxA	HP0642	Phe72Ser	Metronidazole
frxA	HP0642	Gly73Ser	Metronidazole
frxA	HP0642	Phe72Ser	Metronidazole
frxA	HP0642	Gly73Ser	Metronidazole
frxA	HP0642	Asn111His	Metronidazole
frxA	HP0642	Cys193Ser	Metronidazole
frxA	HP0642	Phe72Ser	Metronidazole
frxA	HP0642	Gly73Ser	Metronidazole
frxA	HP0642	Phe72Ser	Metronidazole
frxA	HP0642	Gly73Ser	Metronidazole

2-Second analysis: Typing Analysis:

All the genomes with the available information from NCBI and articles with their available geographic information were downloaded from the NCBI database in order to support *H.pylori* geographic typing. The website was established to be compatible with any whole-genome sequencing (WGS) with metadata based on Nextstrain framework that provides a phylogenetic and geographic visualization. The typing analysis (Fig 8) was developed in our website; the user can analyze his genome by clicking on the typing analysis and submit his file (Fig 9) in order to provide it with the phylogenetic tree with the corresponding continent and country, geographic transmissions and the genetic diversity across the genome.

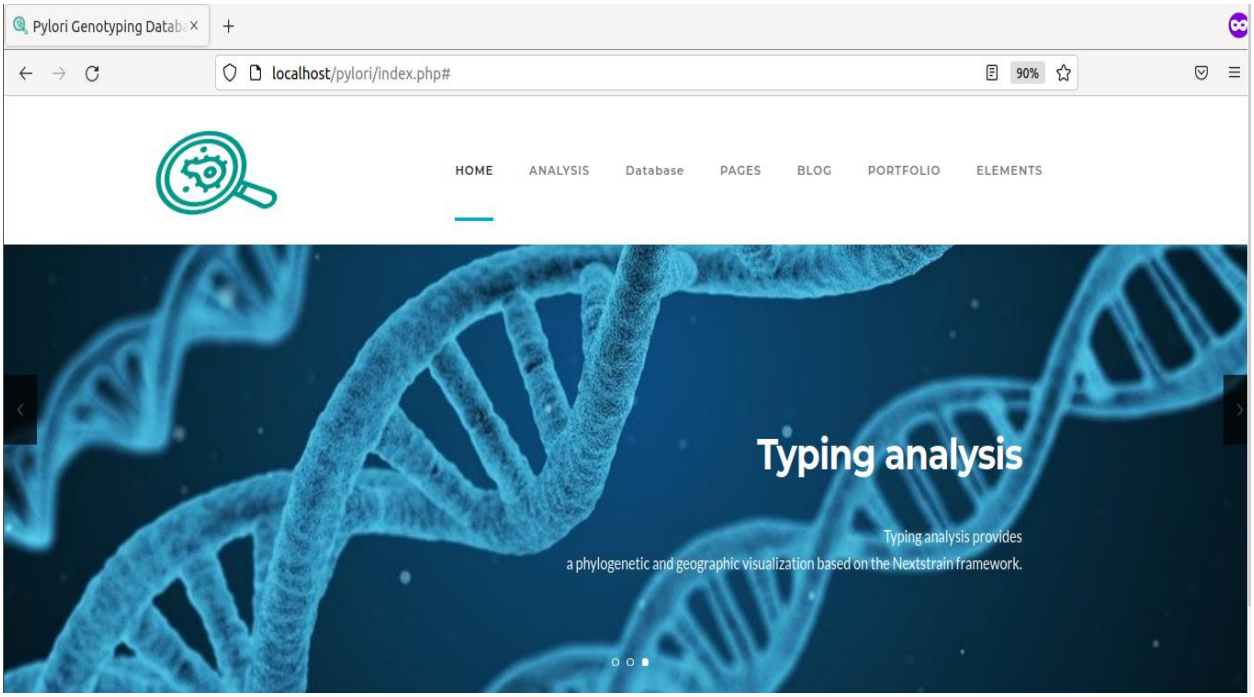

Fig 8: Home page of the website showing the typing analysis

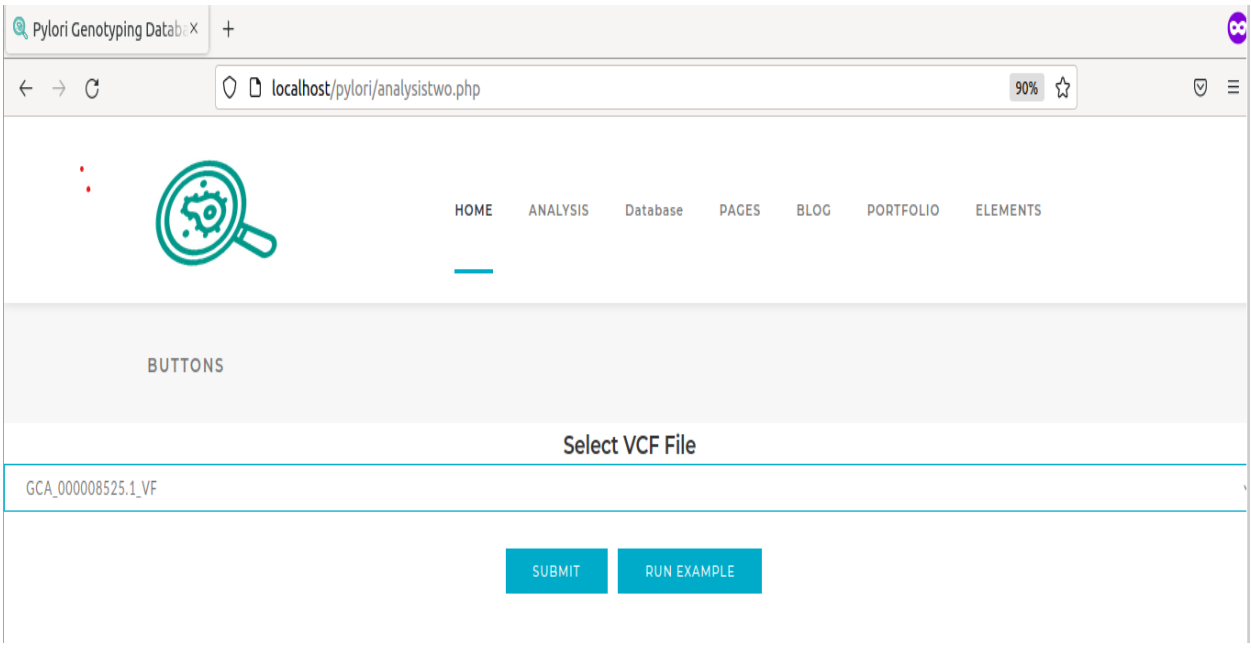

Fig 9: The window of the submission of the files

If the user want to generate like this phylogenetic tree for his sample. He can upload his fasta or VCF file and choose from our data another strains in order to establish the phylogenetic tree which be can visualized in the Nextstrain webtool and the user can choose the structure of the his phylogenetic tree by clicking on tree option and he can choose if he wants the tree to be rectangular, radial, unrooted or in format scatter (Fig 10).

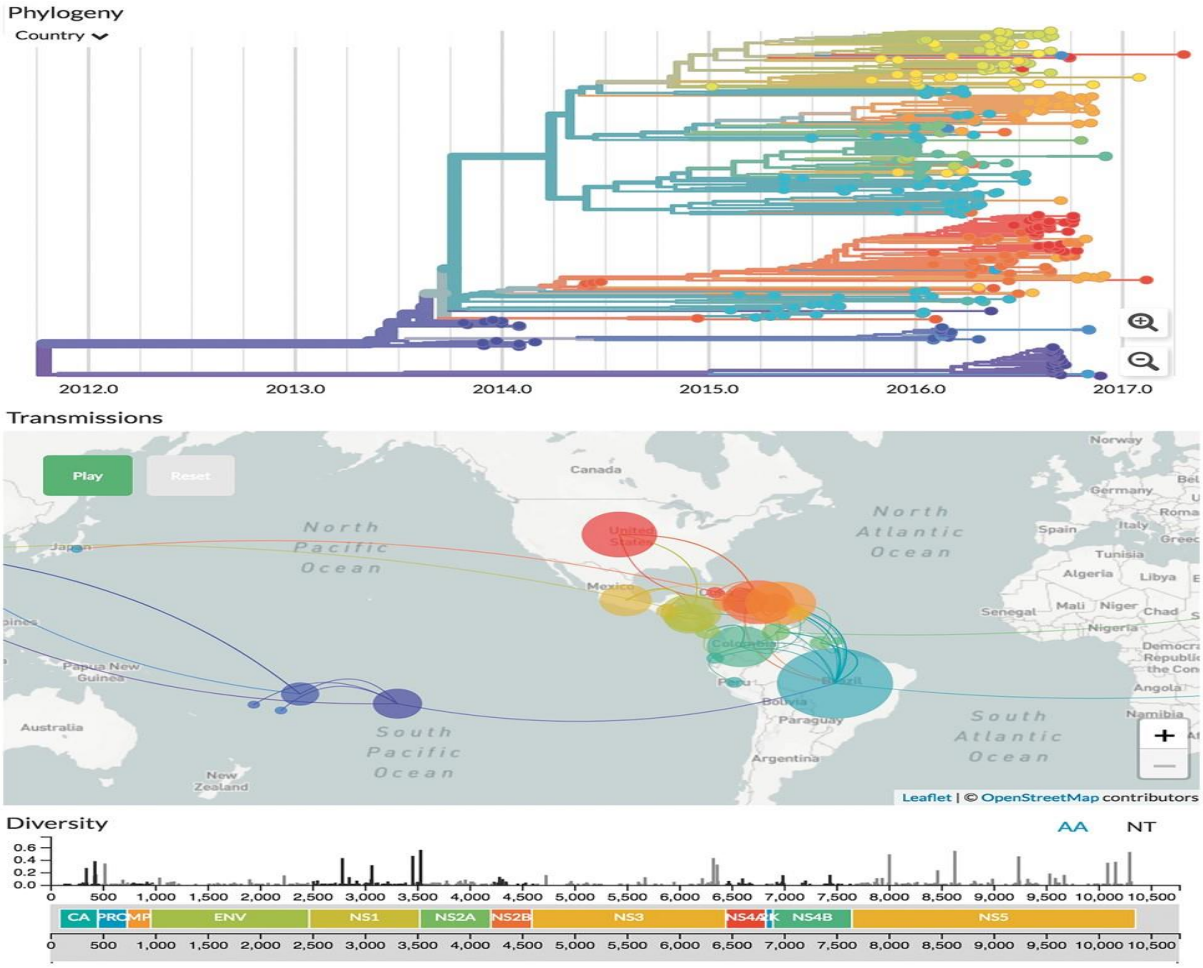

Fig 10: The main interface of Genomic epidemiology

Discussion:

Helicobacter pylori is responsible for various pathologies of the gastroduodenal mucosa. Half of the world's population is affected by this infection. *H.pylori* is present in all regions of the world with a higher prevalence in developing countries. The eradication of *H. pylori* can treat the most of *H.pylori* disease such as gastroduodenal ulcerative disease, MALT gastric lymphomas, and probably prevent from gastric adenocarcinoma. The combination of two or three antibiotics is necessary to ensure an optimal eradication of *H.pylori* and thus limit the emergence of resistant mutants. They can be combined differently depending on the treatment regimen. Clinical impact of antibiotic resistance is the main cause of therapeutic failures. Resistance to antibiotics is acquired following various genetic events by point chromosomal mutation.

Since the MDR strains showed higher genomic variability engendered by the genetic mutations that have an impact on virulence factors, pathogenicity and drug resistance. Our work help to determine the prevalence of *H. pylori* infection and study the mutations of the isolated strains involved in the resistance to antibiotics in purpose to allow confer drug resistance by the adaptation of the treatment. The administration of an adapted treatment will reduce the rate of eradication failures and the emergence of the bacteria.

A similar study to our project launched *H.pylori* typing website tool (https://db.cngb.org/HPTT/), whose purpose was to evaluate the genome typing resolutions and predict the possible origin of the epidemic *H. pylori* isolates, enabling the global surveillance of *H. pylori*.

In this study, we have combined the power of whole-genome sequencing in association with genotyping and phenotyping to get insight into *H.pylori* drug resistance. The phylogenetic analysis was carried out using 3332 genomes from different countries belonging to the six continents (Supplementary Material Table 3). The new website of H.pylori was made to make typing an easy approach by performing a detailed analysis of genetic variants of *H.pylori* strains, to provide information on genetic diversity and transmission of the bacteria in the whole world. The website is an easy way for users to visualize the results that include the genetic epidemiological surveillance of *H.pylori* and the antibiotic resistance by several mutations within the genes.

This information makes the website a powerful and useful platform that associates genomic typing with geographic information and phenotypes that plays an important role in orienting the therapeutic approach and offer rapid identification and diagnosis for disease control and prevention.

In the future, we aim to continue the project and the development of the website. The analysis and the detection of the responsible mutations for the antibiotics resistance going to be based on the molecular docking analysis as technique that predicts the strength of binding affinity between a drug target and ligand molecule using scoring functions. This Combination of computational methods provides a way in understanding the impact of mutations in altering the protein drug targets and disrupt the interactions sites that contribute to weaker interaction with the drug, primarily due to loss of interactions of the drug with surrounding residues.

Conclusion:

The results of this study provide valuable information on the diversity and impact of genetic variants of the *H.pylori* strains and their possible origins. This website have the ability to contribute to further in-depth investigations combining genomic data and clinical epidemiology of *H.pylori* tracing its phylogenetic lineage, and a combination of genomic and epidemiological information allowed tracing the genotypic variation of the transmission paths.

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