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HELICOBACTER PYLORI IN WATER SOURCES AND FOOD PRODUCTS: A CONSTANT PUBLIC HEALTH PROBLEM

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Abstract

Helicobacter pylori is a curved, microaerophilic, multi-flaked, Gram-negative bacillus associated with different gastrointestinal disorders. It´s still a relevant issue in public health due to several scientific investigations that are being carried out in different subjects as, virulence factors, ecosystem interactions, immune response, eradication strategies and Pathogen Transmission Routes in food and water sources. The present review included the association between food, water sources and *H. pylori,* also, the importance of promoting preventive measures for this pathogen transmission, such as the improvement of the living conditions of families and good hygienic practices in the preparation of food, to prevent the infection by this pathogen, considering, that food and drinking water has always played a very important role as vectors of this pathogen.

Keywords: *Helicobacter pylori, Biofilm, Foodborne Illness, Waterborne Illness, Pathogen Transmission Routes, Public health.*

Introduction

Helicobacter pylori is a pathogen associated with gastrointestinal diseases ranging from gastritis, gastroesophageal reflux, iron-deficiency anemia, recurrent abdominal pain, gastric and duodenal ulcers to neoplastic diseases such as MALT lymphoma and gastric cancer. It has been classified as a type I carcinogen [1, 2, 3, 4, 5, 6, 7, 8, 9, 10].

H. pylori colonization increases the relative risk of developing several disorders in the upper gastrointestinal tract and the hepatobiliary system. This microorganism colonizes the whole gastric epithelium and adapts to the mucosa; it stays within the mucus allowing the attack to epithelial cells, evading the immune response and, as a result, the colonization and transmission thereof. The bacterium is associated with a range of extra-gastric alterations like heart diseases, migraine, thrombocytopenic purpura, diabetes mellitus, hepatic encephalopathy, chronic urticaria, Hashimoto's thyroiditis, among other pathologies probably generated by autoimmune disorders derived from the microorganism-caused infection [2, 11, 12, 13, 14, 15, 16, 17, 18].

An association with eating habits and custom influencing the emergence of symptoms has been identified [6, 19, 20, 21, 22, 23, 24, 25, 26]. Infection patterns and prevalence rate vary according to the geographical zone and it´s ethnic groups, the prevalence is the highest in Africa (79.1%), followed by Latin America and the Caribbean (63.4%), and Asia (54.7%). In contrast, it´s prevalence is lowest in Northern America (37.1%) and Oceania (24.4%) [27]. It colonizes the gastric mucosa in 70 to 90 % of individuals in underdeveloped countries, they acquire the organism at early ages, but the majority remain asymptomatic. The prevalence of infection in the adult population may be up to 80% [17, 24, 27, 28, 29].

Over the years the prevalence of peptic ulcer has decreased parallels to the decrease rate in the infection prevalence of *H. pylori* [30] With the improvement of general hygienic conditions, water safety and antibiotics, the acquisition of infection was delated until the immune system and inflammatory responses are more mature [26, 30]. Also, the widespread use of antisecretory medications hamper *H. pylori* management since the increasing antimicrobial resistance and the expanded use of antithrombotic therapy in adult population [17, 31, 32]. Likewise, changes in its epidemiology, often associated with the decline in gastric cancer and peptic ulcers, can impact the epidemiology of other diseases such as: gastrointestinal reflux, Barrett's esophagus, allergies and asthma [2, 29, 31].

Microbiological aspects

The microbiology of ulcerative diseases was first established in the XIX century and described by Professor Walery Jaworsky, however his research received no attention. In the seventies, Robin Warren and Barry Marshall reported the presence of a "curve-shaped bacterium" in the mucosa of gastric biopsy specimens that was subsequently isolated and called *Campylobacter pylori*. In 1989, after having corroborated its taxonomic classification, the name changed to *Helicobacter pylori*. They noticed that *H. pylori* infection was associated with duodenal ulcer and gastric ulcer. These groundbreaking reports were met with skepticism by the scientific community. Nevertheless, Warren and Marshall were awarded the 2005 Nobel Prize in Medicine for this crucial discovery [7, 10, 15, 17, 19].

Helicobacter pylori is a Gram-negative, microaerophilic bacterium, measuring 2 to 4 µm in length and 0,5 to 1 µm in with, having 4 to 6 unipolar flagella. It exists in three different forms: spiral, coccoid and degenerative, of which only coccoid forms and spiral forms are virulent. It is a motile organism producing cytokines that are responsible for causing gastritis, cancer, gastric and duodenal ulcer. *H. pylori* infection is supposed to induce autophagosome formation, and these autophagic vesicles are adapted for the multiplication of *H. pylori* in the host. It may affect autophagy in a host cell/bacterial strain dependent manner. The surface of *H. pylori* is covered with urease aggregates and a heat shock protein presented in the form of a ring of 12.15 nm [7, 10, 15, 17]. *Helicobacter pylori* is genetically heterogenous, possibly as an adaptation of the bacteria to the gastric conditions of its host [5, 26]. In culture, there are colonies formed by the Gram-negative bacteria, they are urease, oxidase and catalase-positive, and they can catabolize glucose but no other sugars. Given its metabolism it can be

grown only in chemically define medium with the addition of several antibiotics and the following amino acids, arginine, histidine, isoleucine, leucine, methionine, phenylalanine and valine, it requires incubation at 37 °C for 48 hours in an anaerobic jar with a gas generator system [7, 26, 31, 33]**.** Although, their natural habitat is the gastric mucosa, *H. pylori* is considered to be a neutralophile. It can survive a brief exposure to pHs of <4, but the growth only occurs at a pH range of 5.5 to 8.0, with optimal growth at neutral pH [26].

The spiral form of *H. pylori* becomes a degenerative coccoid form divided into three types: a degenerative coccoid form composed of agonizing bacteria of pyknotic structure; a living coccoidal bacteria that can be cultured in the agar medium; and a viable but non-culturable state (VBNC). Owing to the complexity of the structure and various physic-chemical conditions acting on biofilm colonies, it has been reported that all bacteria forms, including the coccoid form and the coexisting spiral form, are indeed present in these biofilms. The transformation into grains and a possible return to the spiral forms opens up new prospects in the comprehension of *H. pylori* infection, transmission and reinfection processes given the fact that, once the microorganism has evolved, it produces bacterial DNA and RNA condensation but without fundamental changes in the expression of virulent genes. Considering these facts, a coccoid form found in drinking water or sea-water tanks accessible to human beings might influence the transmission of infection. In this sense, coccoid forms are surviving organisms under adverse growth conditions of *H. pylori* [3].

Virulence factors

The pathogenesis and disease outcomes are determined by a complex interaction between the host, environmental and several virulence factors that facilitate colonization, induce inflammation, and damage the host cells. These virulence factors have been intimate linked to the risk of developing severe gastric diseases [33, 34].

The first step for an effective and persistent infection, is the colonization and the survival in the acidic host stomach [33], the response of *H. pylori* to the acid pH levels found in the stomach is called acid acclimation, defined as the ability to maintain periplasmic pH at physiological levels in the presence of extra bacterial acidity. Several acid acclimation genes are required for the colonization, including the urease gene cluster that is composed of seven genes, those involved in its biosynthesis, and UreI, a proton gated urea channel essential for adequate access of medium urea to intrabacterial urease and periplasmic α-carbonic anhydrase [26]. The virulence depends directly on its capacity to colonize the stomach relying on the activity of the nickel-containing urease, this enzyme catalyzes hydrolysis of urea into ammonia and bicarbonate, two buffering compounds that helps the bacterium to maintain its cytoplasmic pH close to neutrality [35].

Afterwards, the penetration of the gastric epithelium cells is mediated by the flagella motility, *H. pylori* moves through the gastric mucosa epithelium layer to the basal layer where the pH is close to neutrality, it could invade the epithelial cells and multiply within the double-layer vesicles either on the plasma membrane or in the cytoplasm. The flagella are composed by the basal body, a hook and flagellar filament. The flagella may not be directly participating in the cell adhesion, but the regulators controlling flagellar related genes are thought to affect adhesin expression, therefore, there is a complex association between the flagella formation process and the pathogeniesis, which needs further investigation [33]. The attachment to the host cells is mediated by the interaction between host cellular receptors and bacterial adhesins, including blood-antigen binding protein (BabA, BabB), sialic acid-binding adhesin (SabA), which are the most well-characterirized adhesin, but not all the *H. pylori* strains express these two adhesins; also, there are other outer membrane proteins interacting with receptors on the host epithelium cells, like the outer inflammatory protein (OipA) [28, 32, 33], the neutrophil-activating protein (NAP), heat shock protein 60 (Hsp60), adherence associated proteins (AlpA and AlpB), *H. pylori* outer membrane proteins (HopZ and HopQ), and lacdiNAc-binding adhesin (LabA); this interaction lead to a successful colonization of the gastric mucosa, and then bacteria get metabolic substrates and nutrients to improve growth through releasing several effector/toxins (including the vacuolating cytotoxin A (VacA) and

cytotoxin-associated gene A (CagA)) which are involved in the damage of host tissue and intracellular replication, the gastric epithelium layer secretes chemokines trying to initiate the innate immunity and activate neutrophils, leading to the formation of the aforementioned clinical diseases [28, 33, 34].

The vacuolating cytotoxin A (VacA) is an exotoxin that induces vacuolation of eukaryotic cells [7, 34], thus altering anions composition in endosomes and producing osmotic swelling, which could have an effect on the gastric epithelium resulting in apoptosis, this toxin causes severe acute superficial gastric mucosal injuries [7]. It has been described as a multi-receptor protein that has pleiotropic effects, including membrane depolarization, mitochondrial dysfunction, autophagy, activation of mitogen-activated protein kinases, inhibition of T cell function, also inducing apoptosis, contributing to *H. pylori* colonization and its pathogenesis in the upper digestive track diseases [34]**.** All strains carry this *VacA* gene, which presents specific allelic diversity within its domains: a signal sequence region (s1a, s1b, s1c and s2), intermediate region (i1, i2 and i3) and a mid region (m1a, m1b, and m2). Recent molecular epidemiological studies have revealed two novel polymorphic sites, the deletion (d1 and d2 variants) and c-region (c1 and c2 variants) [25, 33, 34]. The genotype can be divided into different subtypes, according to the combinations of the diversity of these three regions. For example, in the s1/m1 genotype the expression of VacA is highly active damaging cells in a more acute manner, also, vacA s1 and m1 strains are associated with high levels of inflammation in the gastric mucosa increasing the risk for gastric atrophy and carcinoma, compared with the less virulent vacA s2 and m2 strains [33].

The *cag* pathogenicity island (*cag*PAI) is a chromosomic region of approximately 40 kb containing more than 30 genes, it encodes the cag type IV secretion system (*cag*-T4SS), about 18 genes identified within the PAI are responsible for encoding for this type IV secretion system in *H. pylori*, including the cytotoxin associated gene A (cagA). CagA is the antigen associated with the cytotoxin, a 120–140-kDa cellular effector that is considered the most relevant virulence factor in *H. pylori* [29, 34]. In turn, the *cag*-T4SS translocates CagA protein to the gastric epithelial cells. After translocation, CagA is probably phosphorylated by known oncogenes that rearrange the host cytoskeleton, affecting the proliferation of this cells and stimulating the gastric epithelium cells to secrete interleukin-8 (IL-8), alter cell signaling, and consequently disrupt the cell cycle, highlighting an important pathogenic mechanism of gastroduodenal diseases [7, 25, 28, 33]. The *cag*PAI can be used as a predictive marker for bacterial pathogenesis; the strains are classified into cagA negative and cagA positive, the cagA gene positive has been found in approximately 90% of all patients infected with the microorganism whose presence has been associated statistically with duodenal ulcer, gastric mucosa atrophy and gastric cancer [28, 32, 34]**.**

The prevalence of CagA-positive *H. pylori* infection in western countries is nearly 60%, and about 90% in Asian countries. Several studies indicated that the CagA-positive strains are directly associated with acute gastritis, gastric ulcer, and gastric cancer development [33]. The CagA protein can be divided into the Western-type CagA and East Asian-type CagA, by the repeat sequence Glu-Pro-Ile-Tyr-Ala (EPIYA) at the N-terminus of CagA, the East Asian-type CagA induces more cytoskeleton changes and is more likely to be associated with gastric cancer [33].

Co-evolution

Helicobacter pylori and humans have co-evolved for at least 100.000 years, long before human ancestors left Africa, developing a wide range of strategies to persist in adapt to the changing conditions inside the host and in the surrounding environments, exhibiting phylogeographic patterns that consistently correlate with that of their human hosts [30]. Despite of their high genetic diversity, it seems that the strains are genetically structured showing a phylogeographic patterns that can correlate with that of their human hosts [26, 34]**.** Therefore, *H. pylori* genetic diversity can be used as a maker for historical human migration and its currently used as a tool track in human demographic history [30, 34]. This microorganism's distribution pattern seems to follow human distribution and geographic diversity suggesting a co-evolution between this bacterium and mankind [26].

With the lack of gene allele clonality resulting from frequent genetic recombination they exhibit a close relationship with the ethno-geographical distribution of its human host. There had been distinguished three groups of bacterial populations, 1) Africa also having 3 bacterial populations (hpNEAfrica, hpAfrica1, and hpAfrica2), one from Europe (hpEurope), and three from Asia (hpEAsia, hpAsia2, and hpSahul), different subpopulations have been shown in EastAsia (hspAmerind, hspEAsia, and hspMaori), hpNEAfrica (hspEastNEAfrica and hspCentralNEAfrica), and hpAfrica1 (hspSAfrica, hspWAfrica, and hspCAfrica) [34, 36].

When modern humans left Africa 60.000 years ago they were already infected [26, 36], leading the subsequently bacterial diversification in parallel with their human hosts. This association began in Africa, where two discrete super-lineages differentiated, one of these super-lineages was predominantly associated with the San hunter-gatherers ethnicity of southern Africa (hpAfrica2) and large felines (Hac), whereas the second is widespread throughout Africa (hpAfrica1, hpNEAfrica). With the human migrations out of Africa the Asian and oceanic lineages emerge (hpAsia2, hpAsia and hpSahul). The only European lineage hpEurope is a hybrid population that came from the migration roots of the African hpNEAfrica and/or the Asian hpAsia2 populations which became predominant in Europe, the middle East and western Asia [36].

Epidemiology

The reservoirs of this microorganism are the antrum of the human stomach, domestic animals like cats and dogs, and water which can be both reservoir and vehicle of transmission. For its part, contaminated food is considered only to be a vehicle for the *H. pylori* transmission. The transmission modes are still not entirely clarified, but human-to-human spread through oral–oral, fecal–oral, gastro-oral and sexual routes are the most plausible ones suggesting its proliferation [2, 3, 4, 7, 10, 19, 21, 29]. Additionally, Person-to-person transmission can be subdivided in two main categories: vertical and horizontal transmission. In the vertical transmission the infection is spread to descendants within the same family, while horizontal transmission involves contact with individuals outside the family but does not exclude environment contamination [7, 10, 21, 26, 37].

This transmission may occur by four possible pathways: oral–oral, fecal–oral, gastro-oral and sexual, described below.

1) Oral–oral transmission: saliva is a possible source of *H. pylori* transmission since the gastric flora can reach and colonize the mouth after regurgitation or vomiting [26]. Recent studies have demonstrated the presence of *H. pylori* in the oral cavity, saliva, subgingival and supragingival biofilms, tonsillar tissue, and in the esophagus, corroborating this theory [3, 4, 10, 21, 26].

2) Gastro–oral transmission: bacteria are acquired in early life and the vomiting of chlorhydric mucus may serve as a vehicle for transmission. The transmission route could be by gastric juice, especially as a result of epidemic vomiting in childhood [26]. Gastroenterology Professionals who perform endoscopies can be infected when they are in contact with contaminated surfaces [3, 26]

3) Fecal–oral transmission: *H. pylori* DNA has been frequently detected in human faeces, which is mainly found in the nonculturable form. The bacterium presence in the drinking water reflects the socio-economic conditions of the population, which confirms that water is an important source of transmission and reaffirms the fecal-oral route [4, 10, 21] given that, the interior surfaces of drinking water pipes contain feces-contaminated water and *H. pylori* biofilms [3].

4) Sexual transmission: some studies support the hypothesis that *H. pylori* may be transmitted via the act of fellatio in the urethra, and it could be a causative agent of non–gonococcal urethritis [38, 39, 40].

Treatment

Once acquired, the infection is usually lifelong, unless treated. The Maastricht III Consensus report indicates that *H. pylori* should be eradicated in case of presence of associated pathologies [26, 41], It should also be sought for and eradicated in first degree relatives of patients with gastric cancer, and also in the presence of unexplained iron-deficiency anaemia and idiopathic thrombocytopenic purpura [26].

Nowadays, the worldwide eradication of *H. pylori* is very complex due to its resistance to antibiotics. The treatment of the infection usually consists of a 7-day to 14-day twice-daily triple therapy regimen based on two antibiotics (usually clarithromycin and metronidazole), there are records of employment of other antibiotics, the most commonly used are amoxicillin, tetracycline, amoxicillin, imidazole (metronidazole and tinidazol) and macrolids (clarithromycin and azithromycin), and a proton-pump inhibitor (PPI) or ranitidine bismuth [2, 7, 17, 26, 41, 42, 43]. The eradication success rate is between 80% and 90%, mainly due to the antibiotic resistance that are most frequently used in its treatment is increasing every day, which is partly due to the exposure of the population to these antibiotics as monotherapy for various infectious diseases [26, 43, 44,]. In some industrialized countries, a screening is used to detect the presence of *H. pylori* as a gastric cancer pre-detection method. The importance of eradicating this pathogen lies in the fact that it prevents the aforementioned cancer and it decreases discomfort associated with dyspepsia and peptic ulcer [21].

The Maastricht III Consensus report it was recommended to continue using the standard triple therapy for seven days in populations with clarithromycin resistance of less than 15-20% and, when greater than 20%, to prolong it to 14 days or use a quadruple therapy with bismuth for 10 to 14 days, and the employment of metronidazole in triple therapy when the resistance is less than 40% [44].

A study in Bogotá, Colombia in 2010 shows that the resistance to metronidazole in this population was 81.01% and to clarithromycin of 17.72%, the resistance to metronidazole reduces efficacy in 50% of triple and quadruple therapies [44]. In Colombia the first line of treatment is triple therapy containing metronidazole, clarithromycin or levofloxacin in addition to amoxicillin and proton pump inhibitors for seven days. Gastroenterologists prefer to prescribe clarithromycin in regions where resistance to this antimicrobial is low. In contrast, for Bogota levofloxacin is commonly prescribed for first and second line therapies. But, in another study also performed in Bogotá the levofloxacin resistance rate was 18.2%. They compared the resistant rate between 2009 and 2014, and it was highly significant with a 15.5% difference. The prevalence of primary resistance to levofloxacin has been increasing in Colombia, this resistance to fluoroquinolone is mostly but not exclusively due to a *gyr*A mutation N871, which had not been previously reported in other countries [44, 45], leading to the search for another other treatment options.

Risk factors

The risk of infection seems to be depending on several factors and potentially contaminated environmental sources [27]. Factors such as age and ethnicity, coupled with a low socioeconomic status (which appears in the form of overcrowding when a large group of people living in the same household are infected), a low level of education, promiscuity, lack of basic services, swimming in rivers, the consumption of contaminated water and fecally contaminated fruit and vegetables, improper food handling, lack of refrigeration, the poor quality and low quantity of food available, child malnutrition, poor hygiene practices, contaminated food before and after cooking, and the lack of access to drinking water affect the incidence and prevalence of *H. pylori* infection [3, 4, 6, 7, 10, 21, 26, 27, 29, 46, 47, 48]. It can be found on different environmental sources including flowers, fruits, insects, urban surfaces, food and in different types of contaminated water sources, as drinking water, surface water, treated and untreated wastewater, groundwater, recreational waters, freshwater streams, estuaries, marine water and biofilms contaminated by sewage [27, 49]. However, as socioeconomic level varies within subpopulations of the same country, where people from rural environments are probably more exposed to infectious sources, which is compatible with different routes of infections according to the population's culture and environment. Most likely in urban (developed) countries the vertical transmission represents the main form of transmission, and in the rural (non-developed) the main form of transmission is the horizontal form, but not to the exclusion of the vertical transmission. Horizontal transmission also includes person-to-person transmission but does not exclude ingestion of contaminated water and food [26].

The infection is usually acquired in early childhood, when immune responses are immature

and gastric acid concentrations are lower since the immune system is immature at birth and gradually matures during infancy, childhood, and early teenage years. The early infection attenuates the gastric inflammatory response, resulting in a mild pangastritis, hypochlorhydria, and a low risk for peptic ulcer disease [15, 26, 30, 31]. Prevalence rates increase in developing countries, where it is present in 50% of world's population. The percentage of infection rises according to age (between 20 and 30% of children are infected) [2, 10]. Delayed acquisition of this pathogen in adulthood could cause either more severe pangastritis (predisposing to gastric ulceration) or gastritis largely restricted to the antrum of the stomach (predisposing to duodenal ulceration) [30]. Approximately 70% of people infected with *H. pylori* do not show symptoms, and they will never develop gastric problems, however the pathogen is capable of causing gastric ulcers in 15% of individuals [2, 10]. The transmission via contaminated food, water or by constant contact between children and non-parental caregivers, when a child has been taken care by a caregiver the transmission has a greater effect that when it is acquired inside the family [26]**.** Having a relative infected with *H. pylor*i is a risk factor, especially in early childhood. Nonetheless, if the infected person is an older sibling, the risk is higher [10, 21, 26].

Food and *Helicobacter pylori*

Foodborne Diseases (FBD) pose one of the most pervasive health problems in today's world and constitute a major factor for reduced economic productivity [50, 51]. FBD are the result of a whole range of contaminated products with pathogenic microorganisms, toxins or chemical substances; the prevention of these diseases depends on careful handling of raw and finished products in the food supply chain. An optimal quality control of foodstuffs results in considerable savings in social and individual costs for consumers and owners of enterprises who produce them. Ensuring innocuous and good quality food has been a constant concern among those who are involved in the food chain [52, 53, 54]. Food spoilage and the presence of pathogenic microorganisms represent an undesirable condition, foodstuffs with a water activity (a_w) above 0,97 and a pH between 4,9 and 6,0 create better conditions for *H. pylori* survival [55, 56].

The infections outbreaks caused by contaminated food with this pathogen are associated with inadequate cooling techniques, cooking a long time in advance, undercooking, and improperly reheated food. Likewise, other aspects such as hygiene for food handlers, cleaning and sanitizing procedures for kitchen items and ingredients, and inappropriate establishment locations play a role in the infection process [57, 58, 59]. Other findings including presence of insects (cockroaches and house flies) or the location and ventilation of food outlets become determining factors that generate a favorable scenario for contamination and dissemination of *H. pylori* through food [4, 58, 59, 60].The increase in prevalence of infection has been linked to the raise in the consumption of food from street vendors, which confirms that products cooked in unsanitary conditions constitute a risk factor very important in the transmission of the bacterium, suggesting that food may act as a vehicle rather than a reservoir [38, 50, 61].

Under laboratory conditions, houseflies (*Musca domestica*) can carry viable *H. pylori* on their external surfaces and in their alimentary tracts. Moreover, *H. pylori* DNA has been found in wild flies, providing evidence that flies acquire *H. pylori* from the environment and therefore function as reservoirs and vectors. Flies transmit the disease through their hairs or through regurgitation and vomit since they act as mechanical vectors. When feces containing viable forms of *H. pylori* are released into the environment by someone with an acute *H. pylori* infection and/or gastroenteritis, that may directly contaminate food, water sources, other people or attract flies, initiating the cycle again. Flies become colonized and disperse, they colonized by feeding on feces and, subsequently, they contaminate food and mucous membranes [4]**.**

The food products that are mainly analyzed are milk, meat and vegetables, which suggest that these foods play an important role in the environmental transmission of this pathogen. Among these, milk and milk products are the most studied, there is an indirect evidence of *H. pylori* transmission trough milk, similar to obtained for water, but less extensive, and because the infection is mainly acquired during childhood and milk is mostly consumed during this period [26, 38]. A research aimed at assessing the

potential of raw milk from ruminants as *H. pylori* reservoirs was carried out. Its objectives were to analyze the diversity of genotypes of CagA and VacA as virulence factors and subsequently find a link between the genotypes present in human and animal strains, taking into account the likelihood of viability of the zoonotic transmission. For this assay, a wide variety of animal milk was sampled showing that s1a/m2 is the most frequent genotype in VacA across all species including human excluding buffalo. The s1b allele in sheep presented a higher statistic association with human strains, which allowed to confirm that these animals act as *H. pylori* reservoirs and transmit the bacteria to humans through their milk [25]**.** Previous publications revealed the isolation of *H. pylori* from gastric tissue of several animals such as sheep, pig and cow, taking part in the human food chain, leading to presume them as plausible reservoirs and sources of the infection, besides the human [26, 38, 62].

Quaglia *et al.*, [63] carried out survival trials in pasteurized and ultrahigh temperature (UHT) milks artificially contaminated and aerobically stored at 4°C. The obtained results revealed that the microorganisms used in the study showed a progressive reduction with an average survival of 9 days in pasteurized milk and 12 days in UHT milk, with an approximate average of initial inoculum of 10^5 and 10^6 CFU/ml, respectively [63]. The prevalence of *H. pylori* infection is higher in people who drink milk or products with these milks under unhygienic conditions compared to those who drink this product in optimal hygienic conditions, thus demonstrating the pathogen does not survive pasteurization [63, 64]. The detection of *H. pylori* DNA is higher in raw milk when compared with pasteurized milk. The microorganism can survive for short periods in milk, which is unsettling because milk is a food product with a short shelf-life [26]**.**

The survival of *H. pylori* in fresh, semi processed, ready-to-eat and raw food was assessed at 4°C under aerobic conditions. Some of the products used for sampling included tofu, tofu storage water, plain yogurt, pasteurized skim milk, lettuce leaves and prepacked boneless, skinless chicken thighs. These products were stored for two days and then were cultured on blood agar for five days and then diluted with Tryptic soy broth. They are inoculated with $6x10^4$ CFU/g of bacteria for solid food and $6x10^3$ CFU/ml for liquids. Subsequently, Samples were analyzed at the start of the experiment and after day 1, 2, 3, 4, 5, 7, 10 and 14 of storage at 4°C. The pathogen was recovered from pasteurized milk and tofu water samples up to 5 days. As far as lettuce leaves and raw chicken were concerned, the pathogen survived up to 2 days after inoculation, the reason for this rapid loss of detectability may be to the lack of protection against oxygen and desiccation of lettuce surfaces and that the chicken didn't have a protecting liquid phase as the remaining products [65]. Furthermore, these foods contain high levels of their natural bacterial microbiota which hampers their colonization given that *H. pylori* is at a disadvantage in the competition for nutrients and is less resistant to metabolites synthesized by commensals. Neither the yogurt nor the tofu showed evidence of microorganism survival by the methods used. It has been postulated that this pathogen is not able to survive in acetate buffers and at a pH of 3.5 to 5.0; moreover, it is inhibited by organic acids like the ones derived from lactic acid bacteria normally found in yogurt. The bacterium has been recovered from ready-to-eat foods making them a potential source of transmission. Although heat treatments may kill the bacteria (milk pasteurization and preparation of tofu). the possibility of a secondary contamination remains as oral-oral and fecal-oral routes are very likely, this bacterium can survive in the external environment for considerable periods**.** Even when foodstuffs are kept under refrigerated conditions they may act as a vehicle of transmission of *H. pylori* If they are not handled correctly somewhere in the food chain [65].

It is unlikely that *H. pylori* grows on food, but it may survive in a viable but non-culturable state (VBNC), therefore the pathogen's ability to survive in VBNC state in association with spinach (*Spinacia oleracea* L.) was examined, but no growth in culture media was observed. Nevertheless, mRNA transcripts were detected 6 days after the cells were introduced to the spinach. Exposure to white light induced the VBNC state in *H. pylori*, suggesting that sunlight may be a factor contributing to the lack of bacterial growth [66].

Some studies state that VBNC *H. pylori* can be found inside the vacuole yeasts, which could serve as a niche for this bacterium outside the human stomach. vacA and ureAB genes has been amplified from the ubiquitous yeast *Candida albicans* total DNA. Foodborne yeasts, such as *Candida* spp., often found in nature, foods (raw milk and vegetables), water and various human organs such as the oral cavity and the gastrointestinal and genitourinary tracts of humans, since yeasts can resist stressful conditions, they can act as a protector/reservoir vehicle in natural environments. They play an important role in the bacterial reinoculation of the same host or the transmission to a new host [26, 38, 49].

In Siavochi *et al*., [49] study, *H. pylory* DNA was isolated from 29 samples, including oral swaps of villagers, flowers, fruits, honey, honeybees, and different urban sources. They select an isolated village as a natural place far from human activities for the yeast isolation from different sources. Different genera of osmotolerant yeasts from flowers, fruits, honey, and honeybees contained *H. pylori* in their vacuole. It was mainly found in fruits and flowers (83,3%) and, honey and honeybees (83.3%), rather that the oral swaps (14,3%). Insects such as honeybees and flies, facilitate the transference and easy access of these yeasts to nectars, which are the main reservoirs of these yeasts, playing an important role in their protection and dispersal [49].

The study carried out by Momtaz *et al*., [67] revealed similar results and conclusions due to a high DNA sequence homology between the isolation of the pathogen from human beings and sheeps; researchers suggest that these animals may act as reservoirs and they share in to a certain degree the ancestral host for the bacteria with humans. Some studies have addressed the high prevalence of *Helicobacter pylori* among shepherds and stated that sheep may play a vital role in *H. pylori* transmission in these people and their family members and the infection may originate from these animal [38], *i*n other study, the prevalence of the bacteria was almost 100%, 42 shepherds and 28 members of their families where determined by 13C–urea breath test, it was revealed that the prevalence is significantly less, 65.1%, in controls without contact with sheep. Therefore, this evidence supports the theory of zoonotic transmission of *H. pylori* [26, 38].

Ahmed *et al*., [68] performed a comparative analysis of the transmission of *H. pylori* in India. The study involved 1000 people who consumed food prepared under hygienic and unsanitary conditions. Those who usually eat products from street vendors presented a bacterial prevalence of 70,8% whereas those who consume food prepared in hygienic conditions show a lower prevalence (60%). On that basis, it was concluded that good hygiene, thorough cooking, and proper techniques for handling and preserving food minimize the chances of pathogen transmission [68].

To obtain evidence of these transmission routes, a study sought to detect *H. pylor*i in raw and ready-to-eat food. To this end, eight raw chickens were collected from a local grocery store and eighteen ready-to-eat raw tuna samples were also obtained from a restaurant in the Chicago area. The pathogen was identified in 36% of the samples of raw chicken and in 44% of the raw tuna using the Multiplex PCR technique. This fact demonstrates that food can be a vehicle in the transmission of this bacterium [69]. The bacterial prevalence is higher in people who consume meat under unhygienic conditions versus those who consume this product in good conditions, this was evaluated in a bovine meat test. It has been proven that the viability of *H. pylori* rapidly disappears in ground beef even if refrigerated or frozen [70].

In a recent study the aim was trying to identify the factors (lifestyles, dietary factors, and hygiene conditions) that are related to the to the prevalence of *H. pylori* infection, it was carried out an observational cross-sectional study with a community sample of 166 adults from the municipalities of Viseu and Sátão in Portugal. Data were collected thought a questionnaire with question regarding sociodemographic aspects and lifestyles. The infection was identified using 13C-urea breath test. For the prevalence of *H. pylori* infection among their dietary factors, lifestyles, and hygiene conditions, it was found a significant association for *H. pylori* infection with the lower frequency in handwashing before going to the bathroom and the consumption of well water. The association between food and *H. pylori* infection is related not only to the type of diet (healthy, unhealthy), but also especially to the consumption of contaminated food, in which

this contamination will be higher when consuming contaminated raw vegetables and fruits [62].

The detection of bacteria in food and water samples by culture media has been demonstrated to be time-consuming, difficult, and in most cases, unsatisfactory, which means that the real acquisition through environmental reservoirs, such as untreated drinking water and rivers, may be undervalued. Taking these facts into account, molecular biology (FISH and qPCR) tests are therefore very useful to detect and identify the organism from complex environments [27, 71, 72].

Water, Biofilms and *Helicobacter* spp.

The transmission of *H. pylori* through water is becoming increasingly important. The World Health Organization [73] refers to this microorganism as a water contaminant and invite people to develop complementary studies on water contamination by applying control measures as "prevention of contamination from human wastes and proper disinfection" [73]. The prevention of nosocomial and enteric diseases raised a new challenge for the control of water supplies and the surveillance and treatment of plumbing systems especially in health-care facilities. *H. pylori* it is being discussed as a possible waterborne organism, investigations have showed that the acquisition of this pathogen and the quality of the drinking water are closely related, especially when there is a consumption of untreated well water. *H. pylori* can enter the viable but non-culturable state (VBNC) in unfavorable conditions in which the organism could be metabolically active and keeps most virulence genes giving them the ability to survive for several months, thus rarely can be cultured from water samples and biofilms. As a consequence of the VBNC state, which makes it difficult to cultivated and also it gives the organism the ability to survive in water distribution systems for longer periods [27, 74].

The metabolism of *H. pylori* includes the formation of biofilms, an assemblage of dead and alive bacteria embedded in a matrix of exopolysaccharides, proteins, and extracellular DNA. Biofilms adhere to living and non-living surfaces, harboring bacteria and helping to ensure their long-term survival under unfavorable conditions. The microorganism present in these aggregates are highly resistant to antibiotics, disinfectants, and sanitizing agents as they prevent these compounds to fully penetrate the interior [27, 75, 76]. Furthermore, they have different metabolism, growth rate, nutrient availability, osmotic pressure, and bacterial population density. They communicate due to chemical signals (Quorum sensing) to coordinate differentiation and structure formation. Microbial cells reside in close proximity, which facilitates the exchange of genetic material found in plasmids [75, 77]. These aggregates are also being repeatedly reported as possible reservoirs in drinking water distribution systems owing to their survival for prolongates periods. They are accepted as natural habitats for most of microorganisms. Bacteria can form biofilms within almost every water distribution system, in less than 50 days they can form a stable biofilm with more than 106 CFU/cm2 by natural water bacteria [74].

H. pylori prolongs its survival thanks to the formation of biofilms and micro-colonies on vegetables. The biofilm forming capability of *H. pylori* depends on strains types and vegetables. *H. pylori* strains can be classified into high and low biofilm formers according to their relative biofilm units (BU). High biofilm formers survive longer on vegetables [76]. Stem scars and natural woundings like stomata can serve as a passage into internal plant tissues, allowing bacterial colonization and growth. The long-term survival of the pathogen inside the biofilm structure and the difficulty of eradicating this organism using conventional methods ease their transmission and increase health risks. Therefore, consumption of contaminated raw vegetables and salads pose an impending risk of infection and consequently, a serious threat to public health. In order to minimize such risk, good hygienic practices and food quality control are needed [76].

A study analyzed and characterized the extracellular DNA (eDNA) in *H. pylori* biofilm. The biomass assay suggests that eDNA may not be the main component of the biofilm matrix, which suggests that it could be involved in other processes such as recombination through transformation, contributing to the genetic variability of this bacterium defined. Its presence contributes to the dynamic exchange of information intended to

enhance the microorganism condition in the host and environment [78].

This pathogen can also be found in feces and wastewaters. In developing countries, the consumption of drinking water and contaminated vegetables represents a high risk of transmission. Eating raw and unwashed vegetables can also make them a vehicle for the infection, on the other hand wash them with contaminated water will also make them a vehicle [38, 62, 79]. Thorough cooking and consumption of chlorinated drinking water reduce such risk for human beings. In South America, it has been proven that the consumption of raw vegetables fertilized with human feces constitutes a considerable risk of infection with *H. pylori* [79].

A study conducted in 407 Peruvian children aged between 2 months and 12 years concluded that water was the vehicle of *H. pylori* infection. The kids who use municipal water supply had a higher prevalence of infection compared to those who used private wells [80]. These results were confirmed by another research that identified that *H. pylori* was present in the drinking water in Peru [81]. A more recent study proved again the presence of *H. pylori* from the drinking water from a single faucet in Lima's Lince district 5 days per week from June 2015 to May 2016, analyzing the changes in pH, temperature, free available chlorine, and conductivity. Forty-nine of 241 (20.3%) of drinking water samples were contaminated with *H. pylori* quantified by qPCR. They found statistically significant relationships between lower temperatures and a lower likelihood of the presence of *H. pylori* ($P < .05$), the water had a temperature range of 19.7-27.7°C, fairly high temperatures, good conditions for bacterial growth, and the pH range was 5.16-8.43, which 96 samples were below the EPA recommendation of 6.5 (too acidic) and could also harbor the bacterial growth [82].

Fujimura *et al.*, [83] suggest that environmental water sources in relation to the human biosphere, such as river water could be a risk factor for *H. pylori* transmission. *Helicobacter pylori* DNA was detected in the water from the middle and downstream and not in the upper reaches of four rivers in japan. This assay tried to attest the prevalence in children that were nearby the rivers, hence, they found that *H. pylori* prevalence in the children examined was 9.8% for those living near the middle part of the river and 23.8% nearby downstream, both of which were higher than the value in an area distant from the river. They concluded that the presence of this microorganism may be relate to human biosphere. There was no detectable *H. pylori* DNA in the upper part of the river were there were no human activities [83]. In another study carry out in Venezuela, the findings showed that *H. pylori* infection frequency is significantly higher during rainy season (96%) than during dry season. The difference in prevalence of infection among asymptomatic patients lies in socioeconomic inequality rather than in gastric cancer risk. It has been stated that water has an intermediary role in the fecal-oral route, acting as a reservoir and vehicle that hosts the bacteria for a long period of time before being accidentally transmitted by bathroom surfaces, water wastes, contaminated drinking water or via contaminated food [84].

The viable but non-culturable state (VBNC) lose culturability in laboratory conditions and cannot be detected by the traditional methods, therefore, various molecular methods and antibody treatments have been used and adapted to identify *H. pylori* in wells, rivers, drinking water, biofilms found in water storage tanks, and water piping [3,27, 85], *Helicobacter pylori*-specific DNA can be attested using nested PCR [83], likewise, direct viable count method (DVC) combined with fluorescent in situ hybridization (FISH), enable the confirmation of the presence of VBNC *H. pylori* cells, as it was confirmed in 35% of the analyzed samples for the study carried by Piqueres *et al.* [86].

A study conducted in Bogotá, Colombia determinate the presence of cultivable and therefore viable *H. pylori* in influent and effluent water from 3 drinking water treatment plants (DWTP), where specific detection of *H. pylori* was achieved by pre-enrichment culture, qPCR and FISH techniques. This study demonstrate that viable *H. pylori* cells were present in both, influent and effluent water samples obtained from drinking water treatment plants in Bogotá and provide further evidence that contaminated water may act as a transmission vehicle. Besides, FISH and qPCR methods are rapid and specific techniques that allow to identify *H. pylori*

from complex environmental samples such as influent water [27].

Holman *et al*., [87] assessed fecal contamination in coastal marine environments across Georgia, Trinidad, and nine locations inside Puerto Rico. For the collection process, Membrane Filtration (EPA method 1604 and method 1600) was used. The pathogen was identified in four of the 31 places sampled. This study confirms that the widespread presence of *H. pylori* in tropical and subtropical coastal waters constitute a potential risk in public health. Likewise, the possible role of environmental transmission in marine mammals was assessed by seeking *Helicobacter* spp. in water samples from their aquatic environment and in fish otoliths regurgitated by dolphins. Water samples from six pools were collected; two inhabited by dolphins and four inhabited by seals. Samples were analyzed by culture and PCR. DNA presence in the aquatic environment and otoliths suggests that environmental contamination is one potential cause for *Helicobacter* spp. transmission. Since neither the source water for the pools nor the fish that were used for food were contaminated. Water can also be contaminated with fecal particles from these animals, and then continue the transmission route with other aquatic animals or it can get passed to the food chain through fish [87].

Conclusion

The presence of *Helicobacter pylori* is higher in non-developed countries and on rural areas, mainly because this population have a higher exposure to the risk factors that were already mentioned, but mostly due to lack of access to better water sources and poor hygiene practices, which all became consequence of a low socioeconomic background. *H. pylori* can be transmitted through water and contaminated food, posing a major problem in public health. The methods utilized for isolating this pathogen consist of clinical sampling and therefore do not provide optimal performance in the analysis of samples other than gastric epithelium. Future research should be focused on improving *H. pylori* culture conditions of water and food samples and/or propose molecular methods as a regular control water analysis and continuing the studies on different reservoirs involved in the transmission of this pathogen. It is important the maintenance and continuous improvement of drinking water quality which play a major role in the prevention in enteric infections. Microbiological quality control in food (routine control) should be implemented in order to expand the research into pathogens such as *H. pylori.*

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The present paper was prepared and reviewed by the authors. No conflicts of interest questioning the validity of this review are declared.

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