Epidemiology, Diagnosis and Risk Factors of Helicobacter pylori Infection

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Abstract

Helicobacter pylori is a human-specific pathogen which leads to gastric pathologies including gastric cancer. It is a highly unique bacterium considered as a carcinogenic agent. *H. pylori* remains a major human health problem, responsible for ~90% of the gastric cancer cases. Approximately four billion individuals have been detected for *H. pylori* infection worldwide in 2015. At the turn of the 21st century, the prevalence of *H. pylori* has been declining in highly industrialized countries of the Western world, whereas prevalence has plateaued at a high level in developing and newly industrialized countries. However, the infection status remains high in immigrants coming from countries with high prevalence of *H. pylori* infection. *H. pylori* can be diagnosed by invasive and non-invasive methods. Urea breath test and stool antigens detection are among the most commonly used non-invasive ones. Although the way *H. pylori* is transmitted remains still unclear, the level of contamination is strongly dependent on the familial and environmental context, with a drastic impact of living conditions with poor hygiene and sanitation. However, familial socioeconomic status is the main risk factor for *H. pylori* infection among children. In addition, food and water source have a high impact on the prevalence of *H. pylori* infection worldwide. This chapter highlights the latest knowledge in the epidemiology of *H. pylori* infection, its diagnosis and critical risk factors responsible for its high prevalence in some populations and geographic areas.

1. Introduction

Helicobacter pylori is a spiral-shaped and flagellated Gram-negative bacterium which colonizes specifically the human stomach. *H. pylori* infects about 50% of the population worldwide, making it the most widespread infection in humans, especially in developing countries where its prevalence is estimated to be around 80% (Torres et al, 2000). *H. pylori* infection leads to chronic gastritis, which can evolve either into peptic ulcer diseases or into the development of pre-neoplastic lesions (intestinal metaplasia, dysplasia) and adenocarcinoma (Correa 1992). It is until now, the only bacterium recognized as a type 1 carcinogenic agent (IARC, 1994). Persistent *H. pylori* colonization and the associated chronic inflammation are critical parameters for the development of gastric malignancies. Further knowledge on the epidemiology of the infection, its pathways of transmission and risk factors could lead to public-health measures for the prevention and control of this infection. The purpose of this chapter is to give an overview of the recent epidemiological data on the prevalence of *H. pylori* infection among the world population and the available diagnostic tools for its detection. The infection rate is known to be linked to socioeconomic factors. However environmental factors including source of water and sanitary conditions as well as factors related to food can also lead to the dissemination of *H. pylori* infection. An update of these different risk factors will also be developed in this review.

2. Epidemiology of *H. pylori* infection

A recent review with reports from 62 countries estimates that more than half the world's population is still infected with *H. pylori*. This means that, based on regional prevalence estimates, there were approximately 4.4 billion individuals with *H. pylori* infection worldwide in 2015 with a wide variation in the prevalence of *H. pylori* between regions and countries. Pre-

valence is highest in Africa (79.1%), Latin America and the Caribbean (63.4%), and Asia (54.7%). In contrast, *H. pylori* prevalence is lowest in Northern America (37.1%) and Oceania (24.4%). At the turn of the 21st century, the prevalence of *H. pylori* has been declining in highly industrialized countries of the Western world, whereas prevalence has plateaued at a high level in developing and newly industrialized countries. The widening differential gap in prevalence has important implication on the future worldwide prevalence of diseases associated with *H. pylori*, including peptic ulcer and gastric cancer. These differences in *H. pylori* prevalence likely reflect the level of urbanization, sanitation, access to clean water, and varied socioeconomic status (Hooi et al. 2017). In children, a comprehensive review and meta-analysis of original pediatric studies from 2011 to 2016 performed on healthy children estimated an overall seroprevalence rate of 33% [95% confidence interval (CI) 27–38] (Zabala Torrres et al. 2017). In the same study, a review of information available from seven cohort studies concluded that infection rates in healthy children under 5 years of age were still between 20 and 40% in high-income countries and between 30 and 50% in upper-middle income countries, indicating that the country of birth plays a role in infection prevalence. Higher rates of infection (40%), as determined by cross-sectional studies, are predominantly seen in low or low-to-middle-income areas (or in countries with severe income inequality). However, huge variations in prevalence can be observed between countries with similar living conditions. This can be observed in Europe, for example, where the prevalence of *H. pylori* infection remains high in Spain and Portugal, although the levels of sanitation and of economic development have risen in recent decades and are comparable to other European countries where the prevalence of infection is significantly lower. Similar variations from country to country can also be seen in Asia, which cannot be fully explained by only looking at the level of development (Zamani et al. 2018). There are significant differences in the *H. pylori* prevalence even

within the same country. For example, different racial groups in the United States have different *H. pylori* prevalence. It was reported that the prevalence in non-Hispanic whites ranges from 18.4% to 26.2% and that in non-whites ranges from 34.5% to 61.6% (Everhart et al. 2000; Cardenas et al. 2006).

The prevalence of *H. pylori* infection in both children and adults is, however, still decreasing in developed countries. One study from Iceland involved 205 children aged 7–17 years and found only 3.4% of infection (Asgeirsdottir et al. 2017). Furthermore, the prevalence was 2.6% among children when both parents were born in a low prevalence country compared with 17% among those with at least one parent born in a high prevalence area ($p = 0.026$). This confirms results obtained in Belgium some years ago (Mana et al. 2013). In Poland, the prevalence of *H. pylori* infection in 8,661 symptomatic and untreated children from 2000 to 2013 assessed by culture was 16.1%. The highest prevalence of infection was found in the year 2000 (23.1%) and the lowest in 2010 (8.9%) (Biernat et al. 2016). However, in Latvia, no evidence of a fall in prevalence in children was found during the last 10 years and the prevalence determined by stool antigen test was 15.5% (Daugule et al. 2016).

The prevalence is also decreasing in some countries in Asia and in the Middle East. Indeed, studies from Japan have shown a considerable fall in *H. pylori* prevalence in childhood. One study from a high gastric cancer incidence area found only 85 of 1765 (4.8%) students aged 13–15 years to be infected (Kusano et al. 2017) and, in another study, the prevalence in school children aged 12–15 years was 3.1% (Nakayama et al. 2017). The same fall has been observed in Iran, where former reports of *H. pylori* infection rate indicated a global prevalence of more than 85% and recent ones estimated an overall prevalence of 54%, with a prevalence of 42% in children (Moosazadeh et al. 2016). Similar trends are seen in the chinese

city Hangzhou, where the infection rates in three age groups (3–6, 7–11, and 12–17 years) were 14.8, 20.2, and 25.8%, respectively. The overall prevalence decreased from 21.6 to 17.2% between 2007 and 2014 (Shu et al. 2017). In contrast, in Vietnam, the seroprevalence in 1,094 subjects from 278 households remained stable at 51.4% in adults and 41.4% in children (Nguyen et al. 2016). Conversely, the prevalence of *H. pylori* infection remains high in newly arrived refugees attending the migrant health service in South Australia, where 922 adults and children were screened in a cross-sectional study using a monoclonal stool antigen test. *H. pylori* infection was detected in 198 of them (21.5%), almost 1.5 times that of the Australian population's estimate when both adults and children are included (Abdul Rahim et al. 2017). A systematic review involving 28 studies described the prevalence of *H. pylori* among migrants. In all but two, the prevalence was similar to or lower than in their country of origin but higher than in their country of destination. Second and later generations of migrants had a lower prevalence than the first generation (Morais et al. 2017).

As mentioned above, *H. pylori* has been identified as a Group I carcinogen by the International Agency for Research on Cancer (IARC 1994) and currently is considered as necessary, but insufficient cause of gastric adenocarcinoma (Eslick et al. 1999; Uemura et al. 2001). Approximately 89% of all gastric cancers can be attributable to *H. pylori* infection. In Africa, despite the high *H. pylori* prevalence, the reported incidence of gastric cancer was considerably lower compared with China or Japan and was postulated to be related to the predominant non-atrophic gastritis pattern in Africa; the archetypal *H. pylori Africa2* type strain largely restricted in South Africa, which lacks the *cag* (cytotoxin-associated genes) pathogenicity island; and lastly intestinal parasitic infestation modulating the immune response against *H. pylori* toward a Th2 type response, which may reduce the risk of gastric cancer (Correa and Piazuelo 2011; Kodaman et al, 2014). The now defunct phenomenon known as "African

enigma" was attributed to the inadequate sampling of the African population obtained through endoscopic data, limited access to health care, and a relatively short life expectancy in the population. More recent and robust data on the African gastric ulcer and cancer prevalence confirmed that it is not as low as reported previously (Graham et al. 2009).

Ongoing efforts to monitor *H. pylori* prevalence and its disease burden in a systematic manner is crucial, as it will minimize any skewed data, which can adversely affect the allocation of health care resources.

3. Diagnosis of *H. pylori* infection

H. pylori infection can be confirmed by invasive methods, requiring gastric biopsies obtained during an endoscopy (histology, culture, PCR: polymerase chain reaction, RAP: rapid urease test) or non-invasive methods (SAT: stool antigen test, UBT: urea breath test, and serology). The invasive tests are used in clinical practice and the non-invasive ones mostly in epidemiology and to assess the outcome of an eradication treatment. It is necessary to have at least two concordant tests to confirm or deny an infection in clinical practice (Fallone et al. 2016) (Malfertheiner et al. 2017; Jones et al. 2017). Indeed, false negative results of any diagnostic tests for *H. pylori* can occur since the sensitivity of any test never reaches 100% and the sensitivity is lower in case of antimicrobial use within the previous 4 weeks, of proton pump inhibitor within the previous 2 weeks or in case of Upper GI: gastrointestinal bleeding. False-positives are rare, but can occur and when present may be due to the occurrence of other urease containing bacteria such as *Proteus mirabilis*, *Citrobacter freundii*, *Klebsiella pneumoniae*, *Enterobacter cloacae* and *Staphylococcus aureus* (Osaki et al. 2008). Non-invasive tests are also used in test and treat strategies, that should now be restricted to adults living in high prevalence countries presenting dyspepsia without any alarm symptoms

(Fallone et al. 2016; Malfertheiner et al. 2017). However, the number of patients to treat successfully to improve dyspepsia symptoms in one is around 12 (Moayyedi et al. 2006). In the context of a prudent use of antibiotics, it may appear not justified to prescribe antibiotic which will induce resistance to the drug in case of failure, be responsible of adverse events and have a high cost (although the cost-effectiveness may vary according to the cost of care in a given country).

3.1 Histology

Demonstration of the presence of *H. pylori* by histological analysis of gastric biopsies is facilitated by special stains such as Giemsa or immunohistochemical techniques (Lash and Genta 2016) using antibodies directed against surface antigens of the bacterium. This diagnostic method remains the most commonly used and allows the gradation of gastritis: classification of Sydney (Dixon et al. 1996) (Fig. 1), OLGA (Rugge et al. 2007) (Table 1) or OLGIM (Capelle et al. 2010) (Table 2). As the diagnostic sensitivity increases with the number of biopsies, it is advisable to take at least 2 biopsies in the antrum at the level of the large curvature, one on the small curvature and 2 at the fundus (Jones et al. 2017; Fallone et al. 2016). To properly assess atrophy, a biopsy should also be performed on the small curvature (Rugge et al. 2007).

3.2 Rapid urease test

The rapid urease test is based on the activity of the urease produced by live *H. pylori* (McNulty and Wise 1985). To perform the test, a gastric biopsy is placed in a medium containing urea and a colorimetric pH indicator. Following the ammonia production associated with

urease activity, the pH change is indicated by the colorimetric shift of the pH indicator. The advantages of this test are its simplicity, its low cost and its ease of execution.

3.3 Culture

H. pylori culture is difficult because this bacterium is fragile and required microaerophilic conditions to grow. However, its culture can be facilitated by the use of a transport medium in the endoscopy room (Bontems et al. 2001; Koletzko et al. 2006; Miendje Deyi et al. 2011; Jones et al. 2017). Culture has the advantage of being able to provide information on the susceptibility of strains to antibiotics, to adapt antimicrobial therapy and to improve the rate of eradication (Miendje Deyi et al. 2011; Chan and Mackenzie 1986; Jones et al. 2017; Fallone et al., 2016). Given the possible mixed infection with susceptible and resistant strains to a given antimicrobial agents and the distribution of the bacteria in the stomach, it is recommended to take several biopsies (at least one in the antrum and one in the fundus) (Selgrad et al. 2014; Aguilera-Correa et al. 2017; Malfertheiner et al. 2017). If the transport time to the laboratory exceeds 4 hours, biopsies should be kept frozen for a maximum of 24 hours. Beyond this, it is best to freeze at -70°C or in liquid nitrogen (Miendje Deyi et al. 2011).

3.4 Polymerase chain reaction (PCR)

Molecular biology techniques can replace culture for the diagnosis of *H. pylori* infection if a medical center does not have the technical capability and/or cannot send the frozen samples to a microbiology department with that expertise. These techniques also allow the detection of mutations causing resistance to certain antibiotics and the detection of infections with several strains with different susceptibility profiles to antibiotics (Miendje Deyi et al. 2011; Kalach et al. 2015).

3.5 Serology

Serology is a simple and very accessible method. A comparative study of 29 commercially available serological kits to detect *H. pylori* infection came to the conclusion that some of the available kits are excellent, with performance parameters such as sensitivity and specificity above 90% (Burucoa et al. 2013). However, local validation of these tests is still needed as their performance may vary depending on the antigenic composition of the circulating strains in a given population. The persistence, sometimes prolonged of antibodies against *H. pylori* does not allow to distinguish between an active and a cured infection. In addition, sensitivity is low in young children although still frequently used in epidemiological studies (Westblom et al. 1992; Andersen et al. 1994; Raymond et al. 1996; Corvaglia et al. 1999; Okuda et al. 2002; Douraghi et al. 2013). However, some recent publications suggest that newer serological tests are more reliable in children (Shady et al. 2015; Kalach et al. 2017; Raj et al. 2017). Enzyme-linked immunosorbent assay (ELISA)-based methods are always preferred over rapid near-patient tests, whose performances are usually not satisfactory and with low reproducibility (Best et al. 2018) .

3.6 Urea breath test

The marked urea breath test consists in having the patient swallow carbon-labeled urea, low-radioactive ^{14}C or, particularly in children, the non-radioactive ^{13}C, and then assay this stable isotope in the CO_2 expired. If the patient is infected, the labeled urea is metabolized by the urease produced by *H. pylori* and the expired $^{13}CO_2$ increase is detected by a mass spectrometer (Graham et al. 1987; Vandenplas et al. 1992; Cadranel et al. 1998). This test, undeniably very sensitive, but expensive, has the advantage to detect the presence of the bacterium in the entire stomach. However, children younger than 6 years appear to have a higher rate of false

positive urea breath test (Leal et al. 2011). The reported performance of the urea breath test for detection of *H. pylori* infection in a recent study, in comparison with biopsy-based histologic examination in 60 children, is low with a sensitivity of 76.2% and a specificity of 69.2% in this age group (Honar et al. 2016). Many pitfalls were underlined to explain these poor results: patient compliance, consumption of PPIs and/or antibiotics for example. Therefore, stool antigen tests with a monoclonal antibody are regarded as more convenient among young people (Honar et al. 2016; Osaki et al. 2008). It is recommended for eradication control at least 4 weeks after cessation of eradication treatment (Osaki et al. 2008; Malfertheiner et al. 2017; Jones et al. 2017).

3.7 Stool Antigen test

Antigen testing in stool is an alternative to the respiratory test for monitoring patients after eradication treatment, epidemiology or test and treat strategies in selected populations, with the proviso that only monoclonal antibody tests with good sensitivity should be used (Makristathis et al. 1998). *H. pylori* specific antigen is tested in fresh or frozen stool samples (Guarner et al. 2010).

4. Risk factors of *H. pylori* infection

It is now well established that *H. pylori* infection is mostly acquired during childhood, mainly during the first 5 years of life and it is significantly influenced by geographical context and specific living conditions (Mendall et al. 1992). In developing countries, the prevalence of the infection is 30 to 50% in children and reaches 90% in adults. In contrast, in developed countries the prevalence of the infection in children is between 1 to 12% and reaches 30 to 50% in

adults (Suerbaum and Michetti 2002). These differences among underdeveloped and industrialized countries are mainly due to the impact of risk factors during childhood. According to a large number of studies, the main routes of *H. pylori* transmission are person to person by oral-oral or fecal-oral routes. The level of contamination is strongly dependent of familial and environmental parameters, with a more drastic impact of living environment including poor hygiene and sanitation which are promoting factors for *H. pylori,* especially in developing countries. However, either for developing or developed countries, familial socioeconomic status is the main risk factor for *H. pylori* infection among children.

4.1 Socioeconomic status and environmental conditions

4.1.a Familial context and source of transmission

The socioeconomic status is defined as occupation, family income level, parent's education level, and living conditions including crowding occupancy. Abundant evidences from many studies demonstrated that low socioeconomic status is a major risk factor in the acquisition of *H. pylori* infection (Fig. 2). This is particularly true as while a declining trend in overall prevalence over time of *H. pylori* infection among industrialized countries, poor socioeconomic conditions still remain associated with a high prevalence. There is no difference in this respect between the developed and the developing countries. For example, in the United States, the prevalence of *H. pylori* infection was approximately twice as high among black and hispanic populations compared to age-matches whites (Malaty et al. 1992). More recently, the prevalence of *H. pylori* infection has been determined in a cohort of Portuguese adolescents (EpiTeen) at the age of 13 and the incidence after a 3 years follow-up was analyzed. An inverse association was found between the prevalence of the infection and the parent's educa-

tion level. The adolescents studying in private school were less likely to be infected (Bastos et al. 2013). A recent nationally representative cross-sectional study involving adults ≥18 years old in Turkey, where the overall prevalence of *H. pylori* infection is about 82.5%, also confirmed an inverse association of education level and *H. pylori* infection. In addition, individuals who had access to social security were at lower risk of *H. pylori* infection (Ozaydin et al. 2013). While education was the only significant factor for women, residential region, housing tenure were among risk factors for men.

Although the link between several risk factors and a high prevalence of *H. pylori* infection is well established, the way of its transmission is still unclear. However, interpersonal and intrafamilial transmissions either oral-oral or fecal-oral appear to be the main route, as supported by a recent study which confirmed that bed sharing, and two affected parents were positively associated with the presence of *H. pylori* infection (Hasosah et al. 2015). Already in the 90's, confined-living conditions as crowded sleeping accommodation was strongly associated with the presence of infection among children (Mendall et al. 1992). In line with this, Bastos et al. (2013) showed that more siblings and living in a house with higher crowding index were also positive risk factors. In urban and rural Beninese populations, 406 healthy individuals including 240 and 206 subjects, respectively, were selected from 96 households. The risk of *H. pylori* infection in children was 13-fold higher when both parents were *H. pylori* positive (95% OR=13.6, 95% Confidence Interval 3.63-51.22), compared with when only one parent was positive (95% OR=5.3, 95% Confidence Interval 1.52-18.45) (Aguemon et al. 2005). In this study, while the spread of infection is facilitated by living conditions with high promiscuity and a high transmission from infected parents to children, a high number of siblings has been reported as another risk factor. A multilocus sequence typing (MLST) DNA analysis using the stools of parents belonging to three families showed an intrafamilial trans-

mission in all selected families with a mother-to-child transmission in at least two families (Osaki et al. 2013). In addition, a grand-mother to child transmission has also been suggested by Urita et al. (2013), who tested 838 children from a small town in Japan. Furthermore, a transmission from sibling to sibling has also been proposed by a macroarray study on selected *H. pylori* coding sequences (CDS) on three families including one child with persistence of gastric symptoms after antibiotics treatment (Raymond et al. 2008). The analysis on 684 children from rural Andean area screened for *H. pylori* with the ^{13}C-urea breath test indicates that the infection is mostly transmitted among siblings which are close in age, and most frequently from the older to youngers ones (Goodman and Correa 2000). This was also confirmed in population with low overall prevalence of *H. pylori* as reported by Sykora et al. (2009) who developed a cross-sectional population-based study on 1,545 asymptomatic Czech children (aged 0-15 years) where the prevalence was 7.1%. A positive association with *H. pylori* infection was found with the number of children in a household and the lack of formal education of fathers. Also related to the social status of the family, the access to good hygiene living condition limits the prevalence of the infection, indicating that important risks factors are associated to poverty. There is no difference in this respect between the developed and the developing countries. In addition to intrafamilial transmission, spread of *H. pylori* infection can be promoted by community living conditions. Indeed, the institutionalization of children between 1 and 6 years old was significantly associated with *H. pylori* infection as reported in asymptomatic Czech children (Sykora et al. 2009). Among children living in urban area, a higher risk of *H. pylori* seropositivity was significantly found associated for those who attended day care centers or nurseries (Dore et al. 2002).

4.1.a-1 Breast-feeding

Whether or not children were breast-fed did not have a statistically significant effect on *H. pylori* seropositivity among children living in adjacent urban and rural areas as reported by Dore et al. (2002) in Northern Sardinia, Italy. However, the prospective population-based study from the Czech Republic among asymptomatic children reported a higher prevalence of *H. pylori* infection among children who had never been breast-fed (Sykora et al., 2009). In contrast, no correlation among children from low socioeconomic backgrounds in Lagos, Nigeria was observed either with exclusive breast-feeding or its duration, and *H. pylori* infection (Senbanjo et al. 2014). According to the history of breast-feeding (ever *vs.* never), a recent systematic review (Carreira et al. 2015), did not find a significant association between breast-feeding and *H. pylori* infection in either high- or middle-income countries, excepted children having been breast-fed for 4-6 months which showed a lower risk of *H. pylori* infection only in the middle-outcome countries. It was then proposed that breast-feeding may protect children against infection by acting as natural antibiotics. Accordingly, children whose mothers had breath milk with higher levels of anti-*H. pylori* IgA had a lower risk to be infected compared with those whose mothers had lower levels (Thomas et al. 1993).

4.1.a-2 Microbiota

Gut microbiota composition can be affected by many factors among which diet, environmental compounds, lifestyle habits, infection and disease (Rodriguez et al. 2015). A recent study reported different profile of gastric microbiota composition when comparing between gastric cancer and chronic gastritis patients (Ferreira et al. 2018). In this study, the gastric carcinoma dysbiosis is characterized by reduced microbial diversity with a reduced *Helicobacter* abundance. A reduced microbial diversity is recognised as associated with disease states, as

also reported for inflammatory diseases and cancer (Gevers et al. 2014 ; Ahn et al. 2013). The presence of *H. pylori* not only influence the composition of gastric microbiota but also indirectly it modifyes the intestinal microbiota, as demonstrated by Kienesberger et al (2016) in the mouse model. As also reported by Arnold et al. (2011) the presence of *H. pylori* infection induces a differential immune response, based on mouse age, that could affect the susceptibility of its host to disease and infection. Family microbiota is shared between parents and children and may play an important role in the composition of infant microbiota. Whether intestinal microbiota can affect *H. pylori* intrafamilial infection has been investigated (Osaki et al. 2018). The microbiota composition of 18 fecal specimens from five *H. pylori*-infected children and their family members were analysed in five families. The microbiota from *H. pylori*-positive children and adults showed a lower diversity than that of *H. pylori*-negative children and parents. This study indicates that the similarity in microbiota composition and its poor diversity can be a risk factor for *H. pylori* intrafamilial transmission.

4.1.b Environmental context

The environment may serve as a reservoir for *H. pylori* infection and directly associated risk factors may play an important role, even more important than familial socioeconomic status especially in developing countries.

4.1.b-1 Rural vs. urban living conditions

The difference between urban and rural living conditions is also among factors influencing the prevalence of *H. pylori* acquisition in children, in different countries. A cross-sectional study conducted among Italian children residing in different environments (urban *vs.* rural) in

the north of Sardinia showed a prevalence of *H. pylori* infection of 26% among children aged 14-16 compared to 20% for those of 5-7 years-old. The prevalence was higher among children living in rural area (36%) compared to among children residing in the urban area (13%). This difference was age-independent (Dore et al. 2002). Especially in the rural areas, contact with dogs is a promoting factor. While no association was seen between the prevalence of the infection and the occupation of the head of the house in rural area, the seroprevalence of *H. pylori* was directly associated with the socioeconomic status of parents in urban area. The same data are also reported on population with different living conditions compared to European countries, as in a study on dyspeptic patients in Andkhoy (Afghanistan) where the positive prevalence for *H. pylori* has been associated with epigastric pain and rural occupancy with a positive correlation with illiteracy (Hamrah et al. 2017). In contrast, in the Beninese population, no significant differences were observed on the prevalence of the infection between rural and urban areas (Aguemon et al. 2005).

4.1.b-2 Water and access to sanitary and hygiene

According to many epidemiologic studies, water could be an important source of *H. pylori* contamination (Ozaydin et al. 2013). Particularly in developing countries waterborne infection is the main infection route of *H. pylori* due to poor sanitary distribution of water among the population. As example in Peru, the analysis of drinking water samples from different locations, based on PCR assays on specific *H. pylori* genes, showed that the majority of contaminated water came from municipal water (Hulten et al. 1996). In contrast, only three of 25 municipal water samples analyzed in Sweden showed the presence of *Helicobacter spp*. DNA, while a large number of well water samples were positive in PCR assays (Hulten et al. 1996). *H. pylori* DNA was also detected in well water in Japan, whose consumers were detected positive for *H. pylori* infection (Horiuchi et al. 2001). Accordingly, a study conducted

in Germany indicated a positive association for *H. pylori* infection and consumption of well water (Strebel et al. 2010), as also reported in Portugal (Amaral et al. 2017). In addition, a river water-associated *H. pylori* contamination has also been suggested in Japan (Fujimura et al. 2008).

The potential presence of live *H. pylori* infective cells in water samples is of public health concern. The analysis of 45 wastewater samples obtained from two secondary wastewater treatment plants in Valencia, Spain showed the presence of culturable *H. pylori* isolates (Moreno and Ferrus 2012). These findings support that fecal-contaminated water may act as a reservoir for *H. pylori* spread. It has been suggested that the ability of *H. pylori* to form biofilm may allow its survival in natural water sources and water distribution systems (Garcia et al. 2014). In addition to DNA detection, about less than 10 reports indicate that culturable form of *H. pylori* can be isolated from water samples as reviewed by Aziz et al. 2015. *H. pylori* cultivability in water should be limited in time with an optimum <10h at temperatures over 20°C (Adams et al. 2003 ; Azevedo et al. 2008). The acquisition of culturable phenotype in water is associated to morphological transition of the bacterium to the rod shape implicating the peptidoglycane (PG) turnover (Fernandes et al. 2017). Accordingly, *H. pylori* enters a viable but not culturable (VBNC) state within a few days after inoculation into water, associated to morphological changes from a spiral bacillus to coccoid form. While the exposition of mice to *H. pylori* viable strain SS1-supplemented drinking water led to infection in mice with significant gastric inflammation after 4 weeks (Boehnke et al. 2015), waterborne VBNC SS1 failed to colonize mice either through drinking water exposure or oral gavage (Boehnke et al. 2017).

4.2 Lifestyle habits

4.2.a Food

Several studies detected the presence of *H. pylori* DNA in dairy products and especially raw milk (Momtaz et al. 2014), that has been considered as the main source of food transmission. About 19% of raw milk and dairy product samples tested in Iran were found *H. pylori*-positive (Mousavi et al. 2014). Ovine milk and traditional cheese were the most commonly contaminated products as reported by Dore et al. (1999b) which detected the presence of *H. pylori* by PCR amplification in milk samples from sheeps, thus indicating a potential contamination of humans by milk consumption. Similar data were also reported for raw cow milk samples in Japan (Fujimura et al. 2002), as well as in Greeks (Angelidis et al. 2011) and American (Dore et al. 2001) herds. Supporting these data, a recent study reports a higher prevalence of *H. pylori* positively associated with the consumption of milk (Assaad et al. 2018). The contamination of milk may be also related to lack of hygiene measure during milk processing and especially from unpasteurized milk storage in some countries. An important finding from Mousavi et al. (2014) was the high presence of *H. pylori* antibiotic resistant strains isolated from milk and dairy products.

Meat has also been proposed as a possible source of *H. pylori* reservoir. Previous data identified the presence of *H. pylori* by 16SrRNA and *vacA* PCR analysis in gastric tissues from sheep, thus taking part in the human food chain. Sheeps have been proposed as a source of *H. pylori* transmission among shepherds and their family members (Dore et al., 1999a) (Papiez et al. 2003). In line with this, the prevalence of *H. pylori* infection among shepherds, who reside in Northern Sardinia and among members of their family is one of the highest in the world (98%). It may be associated with direct contact with sheeps and sheepdogs (Dore et

al. 1999a) (Dore et al. 1999b), as also reported in Colombian Andes population (Goodman et al. 1996).

Raw vegetables may be also a source of *H. pylori* food transmission to humans. Several studies reported the detection of *H. pylori* by culture and PCR in vegetable and salad samples (Goodman et al. 1996; Yahaghi et al. 2014). Contaminated water used through washing may be likely the source of the presence of *H. pylori* in raw vegetables. Thus, the lack or deficient waste water treatment may also promote *H. pylori* food contamination and it is likely to play an important role in *H. pylori* transmission to humans. However, given the link between *H. pylori* infection and low socioeconomic status, the infection could occur predominantly among individuals with deficient nutritional status. In the Andean population in Columbia, *H. pylori* infection occurred more frequently among children of short stature for their age, with a low consumption of fruits and vegetables associated with low nutritional indicators (Goodman et al. 1997).

4.2.b. Alcohol drinking and smoking habits

Studies on the association of smoking and alcohol consumption with *H. pylori* infection showed conflicting results. While Zhu et al. (2014) reported that the use of alcohol and tobacco had no impact on the prevalence of *H. pylori* infection, significant relationships between smoking habits (current *vs*. never) with *H. pylori* seropositivity have been reported in Japanese adults (Kikuchi et al. 1998). In Northern Ireland, smoking has been reported positively associated with the presence of *H. pylori* infection, however, no relationship with alcohol consumption has been found to be significant (Murray et al. 1997). In contrast, the study by Ogihara et al. (2000) which analyzed the impact of drinking and smoking on *H. pylori* serology in 8837 subjects working in textile companies in Japan, found a negative association bet-

ween current cigarette and alcohol consumption with *H. pylori* seropositivity. The increased gastric acidity associated to smoking may be a cause of the negative association between the presence of the infection and tobacco consumption. Furthermore, more recently non-smokers and regular alcohol consumers were suggested as under less risk of *H. pylori* infection than others (Ozaydin et al. 2013).

4.3 Occupational hazards

The occupational risk for acquiring *H. pylori* infection has been addressed in several studies among healthcare workers during contact with patients. The prevalence of *H. pylori* infection has been evaluated by immunoassay on stool samples from 249 subjects employed in a university teaching hospital according to three categories of hospital workers including personal from gastrointestinal endoscopy unit, personal and staff from other hospital units either with direct or no contact with patients. The results indicated that hospital work involving direct contact with patients constitutes a major risk factor for *H. pylori* contamination compared with hospital work without direct contact with patients (Mastromarino et al. 2005). Accordingly, nursing staff was also demonstrated as higher risk of *H. pylori* infection compared to administrative and technical staff (Triantafillidis et al. 2002). In addition, during the 5 years of following, a higher seroconversion for subjects initially *H. pylori* negative which became positive, was observed for the nursing staff category. It is interesting to notice, that also in that study, the level of education was inversely associated with the prevalence of the infection.

5 Conclusions

Even though recent research suggests that the prevalence of *H. pylori* infection trends to decrease in most of the countries, it remains high in most of developing countries as also illustrated with immigrant populations coming from countries with a high prevalence. The main risk factors remain associated with socioeconomic, familial living conditions and environmental factors. These data highlight the importance of the identification of population-specific risk factors as *H. pylori* reservoir that will allow to develop efficient preventive strategies to limit the prevalence of *H. pylori* infection among the most vulnerable populations.

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		Corpus			
	Atrophy score	No atrophy (score 0)	Mild atrophy (score 1)	Moderate atrophy (score 2)	Severe atrophy (score 3)
Antrum	No atrophy (score 0) (*including incisura angularis*)	Stage 0	Stage I	Stage II	Stage II
	Mild atrophy (score 1) (*including incisura angularis*)	Stage I	Stage I	Stage II	Stage III
	Moderate atrophy (score 2) (*including incisura angularis*)	Stage II	Stage II	Stage III	Stage IV
	Severe atrophy (score 3) (*including incisura angularis*)	Stage III	Stage III	Stage IV	Stage IV

Table 1: Gastritis staging according to OLGA classification.

The Operative Link on Gastritis Assessment (OLGA) system corresponds to an histological staging for gastric inflammation, considering gastric atrophy as the histological lesion representative of disease progression. From Rugge et al. (2007) with permission.

		Corpus			
	IM score	**Not fat: no IM (score 0)**	**Mild IM (score 1)**	**Moderate IM (score 2)**	**Severe IM (score 3)**
Antrum (including incisura angularis)	No IM (score 0)	Stage 0	Stage I	Stage II	Stage II
	Mild IM (score 1)	Stage I	Stage I	Stage II	Stage III
	Moderate IM (score 2)	Stage II	Stage II	Stage III	Stage IV
	Severe IM (score 3)	Stage III	Stage III	Stage IV	Stage IV

Table 2: Gastritis staging according to OLGIM classification

The Operative Link on Gastric Intestinal Metaplasia (OLGIM) system corresponds to an histological staging for gastric inflammation, considering intestinal metaplasia (IM) as the histological lesion representative of disease progression. From Capelle et al. (2010) with permission.

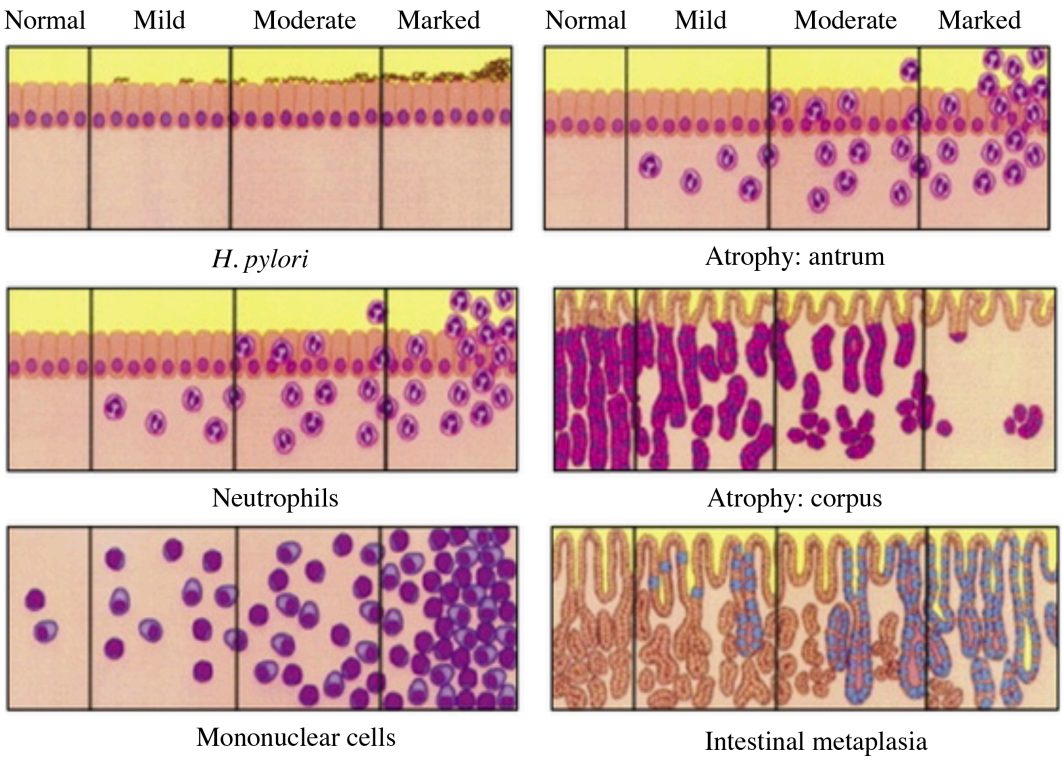
Legends of figures

Figure 1: **The updated Sydney classification for gastric lesions.**

Each feature is assigned with a semi-quantitative and descriptive evaluation to 0: absence (Normal), 1 for mild, 2 for moderate and 3 for marked corresponding modification including infiltration of neutrophils and mononuclear cells, intensity of atrophy and frequence and severity of intestinal metaplasia. Taken from Dixon et al. (1996) with permission.

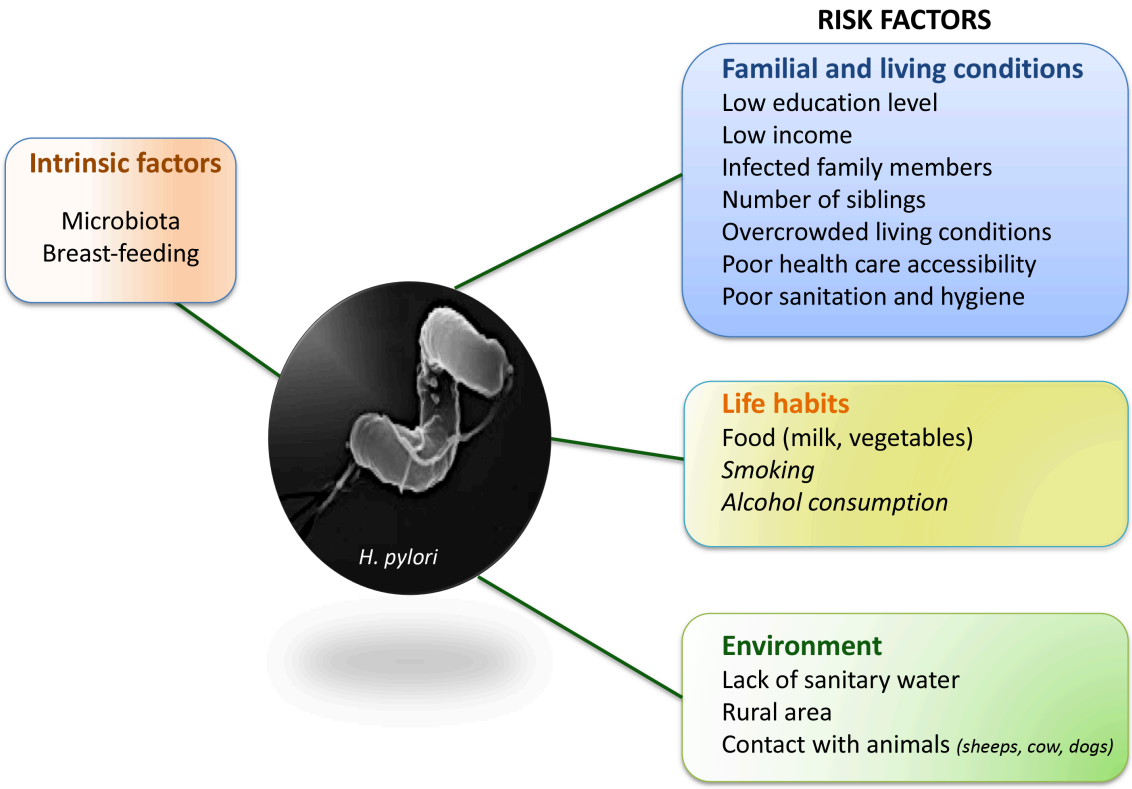
Figure 2 : Risk factors associated to *H. pylori* infection.

Major risk factors associated to the transmission and spread of *H. pylori* are of multiple origins including familial and living conditions, life style habits and environmental context. Microbiota and breast-feeding correspond to individual factors suggested to modulate the transmission/acquisition of *H. pylori* infection.


Normal
Mild
Moderate
Marked
Normal
Mild
Moderate
Marked
H. pylori
Atrophy: antrum
Neutrophils
Atrophy: corpus
Mononuclear cells
Intestinal metaplasia

1

2 **Fig.1**


RISK FACTORS
Familial and living conditions
Low education level
Low income
Infected family members
Number of siblings
Overcrowded living conditions
Poor health care accessibility
Poor sanitation and hygiene
Intrinsic factors
Microbiota
Breast-feeding
H. pylori
Life habits
Food (milk, vegetables)
Smoking
Alcohol consumption
Environment
Lack of sanitary water
Rural area
Contact with animals (sheeps, cow, dogs)

1

2 **Fig. 2**