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## Helicobacter pylori

### Arauro Boira, R

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GLOBAL WATER PATHOGEN PROJECT

**PART THREE. SPECIFIC EXCRETED PATHOGENS: ENVIRONMENTAL AND  
EPIDEMIOLOGY ASPECTS**

# HELICOBACTER PYLORI

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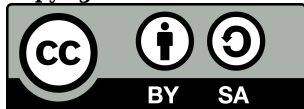
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## Summary

*Helicobacter pylori* is a human-associated bacterium, colonizing the stomach. It is estimated that *H. pylori* infection affects more than half of the adult population worldwide and it is a causative agent of chronic gastritis, having a major role in promoting the development of peptic ulcer disease and non-cardia adenocarcinoma of the stomach. Wide variations in the prevalence and incidence between regions and population groups exist. Approximately, one-third of adults in North Europe and North America carry *H. pylori*, whereas in south and east Europe, South America, Africa and Asia, the prevalence of *H. pylori* is often higher than 50%. Although the way the infection is transmitted is still unclear, interpersonal transmission appears to be the main route. Epidemiological evidence also suggests the possible role of waterborne transmission. *H. pylori* infection usually starts in early childhood gastritis and remains as a chronic, persistent infection for decades. Interestingly, only a minority of infected individuals actually develop gastric diseases, which can include: chronic gastritis, peptic ulcer, mucosa-associated lymphoid tissue (MALT) lymphoma, non-Hodgkin's lymphoma and gastric adenocarcinoma in adults. *H. pylori* is a Gram-negative helical or spiral-shaped microaerophilic bacterium with multiple sheathed flagella at each pole of the bacterial cell. It belongs to the family *Helicobacteraceae* and classe epsilonproteobacteria. The cells are 2 to 4 µm long and 0.2 to 1.0 µm wide. *H. pylori* is catalase-positive and has a potent urease enzyme, which is of particular importance in cytoplasmic pH homeostasis. This ability enables its survival in the strongly acidic gastric mucosal environment and colonization of the stomach. Glucose appears to be the only carbohydrate utilized by this obligate microaerophilic bacterium. Amino acids are potential sources of carbon, nitrogen, and energy.

There are two possible routes of transmission of *H. pylori*: oral-oral and fecal-oral. The oral-oral route is well-established and is the most likely route of infection in childhood. The fecal-oral route is much more controversial because *H. pylori* has rarely been cultivated from feces, although the presence of the bacterial antigens and DNA in the feces of infected people is common. The human stomach is considered to be the main reservoir of *H. pylori*. There are no clear trends in population susceptibility, with the exception that most of the infections are acquired during childhood. The incidence is mostly affected by living conditions of the community, such as low economic status, poor sanitation, and crowded household conditions.

Epidemiological data consistently indicate that living standards associated with improved hygiene are associated with distinct reduction in the infection prevalence. *H. pylori* is a slow growing microaerophilic microorganism, requiring a complex growth medium supplemented by blood or derivatives. Isolation of *H. pylori* by culture from water has rarely been achieved, due to its growth requirements and that in water or in biofilms the bacteria are mostly in the viable but non-culturable (VBNC) state. However, molecular techniques have successfully been applied to detect *H. pylori* in the environment. The bacterium contaminates water principally through human excreta. *H. pylori* can be identified more readily in human feces with antigen tests and PCR than with culture. The presence of *H. pylori* in feces reinforces the possibility of the fecal-oral transmission route. As well, the presence of *H. pylori* in sewage water has been confirmed by some studies. In general, *H. pylori* has been detected worldwide in water, including surface water, well water, drinking water and fecally-polluted seawater through the application of molecular techniques. Nevertheless, most works have found no correlation between the presence of *H. pylori* and fecal indicators. Protective microniches for *H. pylori* in aquatic systems have been proposed, including biofilms and protozoa. The survival of different strains has been studied under controlled conditions where culturable cells of *H. pylori* are intentionally introduced to the water. Under such conditions, bacteria lose cultivability after 1 to 14 days, but VBNC cells persist much longer. Data indicates and VBNC cells may infect and resuscitate in mice, but it is unknown if this also occurs in people. Thus, adequate sanitary conditions may be important for reducing the transmission of *H. pylori*. Nevertheless, there are few data available on the fate of *H. pylori* during sanitation management and research is needed to inform effective management practices.

## 1.0 Epidemiology of the Disease and Pathogen

### 1.1 Global Burden of Disease and Distribution

*Helicobacter pylori* is a human-associated bacterium colonizing the human stomach. It is estimated that *H. pylori* infection affects more than half of the worldwide adult population (Table 1). *H. pylori* has a major role in promoting the development of peptic ulcer disease and non-cardia adenocarcinoma of the stomach. *H. pylori* is estimated to be responsible for 75% of all gastric cancer cases (Graham, 2014). It is therefore of major public health interest.

**Table 1. Incidence of *Helicobacter pylori* infection (tested either by serology/urease/urease breath test or fecal antibody tests) among different populations**

Area	Period of Study	Patient Age Years (Assay Type, Populations Tested)	Prevalence, %	Common Risk Factors, Including Water	Reference
Bhutan	2012	Dyspeptic patients; age 17 to 75 (serology)	81 to 93	Area of living, crowed Water: No risk	Dorji et al., 2014

Area	Period of Study	Patient Age Years (Assay Type, Populations Tested)	Prevalence, %	Common Risk Factors, Including Water	Reference
Brazil	2001 to 2002	0.5 to 12 (serology)	38	Low socioeconomic conditions in childhood, dyspeptic symptoms	Parente et al., 2006
Brazil	1997 to 2003	4 to 11	28.7	Crowded environment (number of siblings, nursery attendance), deficient sanitation	Dattoli et al., 2009
Canada (Arctic communities)	2008 to 2011	Households (serology, urea breath test, culture)	63	Crowded living in family. Any type of water exposure was not a significant risk factor	Hastings et al., 2014
China	2009 to 2011	0 to 18 (fecal antigen test)	6.8	Low socioeconomic status including pre-chewed food from mother, minor handwashing facilities	Ding et al., 2015
China (Shanghai)	Not indicated	18 to 80 (serology)	73.7	As part of an epidemiological study	Li et al., 2010
Finland	1994	A city with population of 16,000 (serology)	34		Salomaa-Räsänen et al., 2010
Kazakhstan	1999	10 to 60 (serology)	90	Positive serology associated with crowded conditions, poor sanitation, river water as drinking water	Nurgalieva et al., 2002
Netherlands	Not indicated	Subjects born 1935 to 1946	48		Blankenstein et al., 2013; Hollander et al., 2013
		Subjects born 1977 to 1987	16		
		6,500 pregnant women			
Netherlands	2002 to 2006	Born in the Netherlands	24		
		Born outside Netherlands	64		
Poland	2002 to 2003	0.5 to 18 (serology)	32	Higher prevalence in children from rural areas, without running water and observation of hygiene rules. Adults: born in rural areas, lower education, low socioeconomic status and poor hygienic and sanitation conditions	Łaszewicz et al., 2014
		19 to 89 (serology)	84.2		
Portugal	1999 to 2003	18 to 30 (serology; cagA)	72.6	Age, socioeconomic status <sup>a</sup>	Bastos et al., 2013
	Reevaluation 2005 to 2008	> 71 (serology)	88.1		
Taiwan (Lanau island)	2008	21 to 89	72.1	Low education, lower income, water not studied	Chen et al., 2014
Turkey	Not indicated	> 18 (55 cities; a total of 4622 from 2382 households; UBT test)	82.5	Age, male gender, smoking, lower education and socioeconomic level, piped water (compared to bottled water)	Ozaydin et al., 2013
		Born in the USA	20.5	>20 y.: occupational contact with soil	
USA	1988 to 1994	Foreign-born	54	< 20 y: living in crowded conditions, well water as tap water Education, income, and/ethnicity	Krueger et al., 2015; Zajacova et al., 2009

<sup>a</sup>Incidence rate was 3.6/100 person years

Wide variation exists in the prevalence and incidence among regions and populations. Approximately, one-third of adults in northern Europe and North America carry *H. pylori*, whereas in south and east Europe, South America, Africa and Asia, the prevalence of *H. pylori* is often higher than 50% (Table 1). However, the prevalence among different ethnic groups within a country can differ, for example, in the USA (Siao and Somsouk, 2014) and Canada (Jones et al., 2012). In these countries *H. pylori* infection is more prevalent among recent immigrant groups coming from countries with high endemic prevalence of *H. pylori* infection than among the general population living longer in these countries. For similar reasons (based on poorer general health), indigenous peoples, such as for First Nation groups in Canada also have high prevalence (Cheung et al., 2014). In general, the infection rate increases with age, suggesting that acquisition occurs in early childhood. Presently, in high income countries the lower prevalence of infection in the younger generations (birth cohort effect) indicates a decline in *H. pylori* prevalence and associated diseases in the coming decades. For example, in Finland, the proportion of pregnant women infected declined by nearly half from 1983 to 2001 and in the Czech Republic, from 2001 to 2011, the prevalence decreased from 30% to 10% in young adults and from 60% to 40% in older subjects. In Japan, *H. pylori* prevalence (approximately 80%) remained similar from the late 1980s to the early 1990s among older subjects, but has gradually decreased (approximately 50%) from 2007 to 2011 (Shiota et al., 2013).

The reasons for different trends are not fully understood. Low socioeconomic conditions in childhood are known to be the most important risk factors for *H. pylori* infection. This suggests that the transmission is mostly oral-oral and is associated with close contact within families and transmission of the infection from parents to children and between children. Attending daycare has also been shown increase the risk of transmission. Also, poor sanitary and hygienic conditions are reported risk factors, suggesting a potential role of water (or lack thereof) in transmission. Although the way the infection is transmitted is still unclear, interpersonal transmission appears to be the main route (Mentis et al., 2015), but there is also epidemiologic evidence for the possible role of waterborne transmission (Aziz et al., 2015; Bellack et al., 2006).

### 1.1.1 Symptomatology

*H. pylori* infection usually starts in early childhood with gastritis and remains as a chronic, persistent infection for decades. Interestingly, only a minority of infected individuals actually develop gastric diseases, which can include: chronic gastritis, peptic ulcer, mucosa-associated lymphoid tissue (MALT) lymphoma, non-Hodgkin's lymphoma and gastric adenocarcinoma in adults (Graham, 2014). It is still not clear whether early childhood colonization causes any specific symptoms. Studies have shown that the onset of colonization in adults can trigger

symptoms such as hypochlorhydria, nausea or gastric pain (Atherton and Blaser, 2009).

## 1.2 Taxonomic Classification of the Agent

*H. pylori* belongs to the family *Helicobacteraceae* and class epsilonproteobacteria. The genus *Helicobacter* contains 36 described species that have been validated at this point in time (<http://www.bacterio.net/index.html>) and is divided according to their major colonization sites as gastric or lower intestinal tract associated bacterial species.

*H. pylori* is microaerophilic, catalase-positive, and has specialized mechanisms for pH homeostasis, including a potent urease enzyme. The ability to maintain the internal pH enables its survival in the gastric mucus and to colonize the stomach. Glucose appears to be the only carbohydrate utilized by this obligate microaerophilic bacterium. Amino acids are potential sources of carbon, nitrogen, and energy (Mobley, 2001). It is a genetically-diverse gastric pathogen, carrying a range of antibiotic resistance patterns, and varies in geographic occurrence (Secka et al., 2013).

### 1.2.1 Physical description of the agent

*H. pylori* is a Gram-negative helical or spiral-shaped microaerophilic bacterium with multiple sheathed flagella at each pole of the bacterial cell. The cells are 2-4 µm long and 0.2-1.0 µm wide. In aged cultures and in water or other stressful conditions (inside and outside of gastric mucosa), viable but non-culturable (VBNC) coccoid forms of the cells dominate (Fernández-Delgado et al., 2016; Loke et al., 2016). The genome size is small, approximately 1.6 Mbp (G+C 39%). It was cultivated and described for the first time in 1982 (Marshall and Warren, 1984).

## 1.3 Transmission

### 1.3.1 Routes of transmission

Studies carried out over the last three decades indicate that there are two possible routes of transmission of *H. pylori*: oral-oral and fecal-oral. The oral-oral route is well-established and is the most likely route of infection in childhood. A large number of epidemiological studies have indicated that crowded family conditions, low socioeconomic status and poor hygienic practices, especially in childhood, are risk factors associated with acquisition of the infection. The presence of *H. pylori* in the mouth, saliva and vomit of infected persons supports the oral transmission. Moreover, several epidemiological studies have shown that the bacterium is transmitted by persons close to the children, especially mothers (Mentis et al., 2015).

The fecal-oral route is much more controversial because *H. pylori* has rarely been cultivated from feces, although the presence of its bacterial antigens and DNA in the feces of infected people is common (Ford and Axon, 2010). Thomas

*et al.* isolated *H. pylori* for the first time in 1992 from the feces of 10 Gambian people living in a high prevalence area (Thomas *et al.*, 2004). A few other studies have reported isolation of the bacterium from feces (Kelly *et al.* 1994; Parsonnet *et al.* 1999). These works together suggest the feasibility of a fecal-oral transmission route for the pathogen. Growth in aquatic environments is also possible within free-living amoebae and in cooperation with biofilm-associated mycobacteria (Gião *et al.*, 2011; Moreno-Mesonero *et al.*, 2016; Santiago *et al.*, 2015).

### 1.3.2 Reservoirs: human, animal, and environmental

The human stomach is considered to be the main reservoir of *H. pylori*. Although it is unclear if environmental strains are important in human infection, mounting evidence exists for environmental sources (Moreno-Mesonero *et al.*, 2016). The genome of *Helicobacter pylori* has evolved over the millennia along with the migration out of Africa with its human host approximately 60,000 years ago. Human migrations and permanent settlement in different continents, has resulted in coevolution of different genetic populations with distinct geographical distributions (Correa and Piazzuelo, 2012). Evidence suggests that the association of *H. pylori* with the *Homo* genus originated approximately 100,000 years ago in Africa (Correa and Piazzuelo, 2012; Montano *et al.*, 2015).

There are no confirmed data on zoonotic transmission of *Helicobacter pylori* from animals to humans.

### 1.3.3 Incubation period

*H. pylori* can cause a chronic infection. The infection is acquired during childhood, but symptoms generally do not appear until adulthood and vary from person to person (Graham, 2014; Mentis *et al.*, 2015).

### 1.3.4 Population susceptibility

With the exception that most of the infections are acquired during childhood, there are no clear trends in population susceptibility. The incidence is mostly affected by living conditions, such as low economic status, poor sanitation, and crowded household conditions. A few studies have demonstrated that transmission can occur between adults, especially within couples or persons living together (Kivi *et al.*, 2003; Perez-Perez *et al.*, 1991).

Variable prevalence within social groups living in the same area may exist. In Brazil, different infection rates have been observed among children of low versus high socioeconomic status. In Colombia, people living in the Andes Mountains have a much higher gastric cancer incidence compared to the low coastal region. In both areas, the incidence of *H. pylori*, the origin of the gastric cancer, is the same. Notably, differences in cancer occurrence rate have been related to the ancestral origin of *H. pylori* strains in both populations. The prevalence of the highly-virulent European strains is more dominant among people

inhabiting the mountain area, while the African strains are more frequently detected in coastal areas, likely explaining the higher incidence of the gastric cancer (de Sablet *et al.*, 2011).

## 1.4 Population and Individual Control Measures

### 1.4.1 Vaccines

No vaccines are currently available, but there has been some effort towards development (Blanchard *et al.*, 2004).

### 1.4.2 Hygiene measures

All epidemiological data show that the living standards associated with improved hygiene (toilets, running water, safe drinking water, possibility for hand washing within families) are associated with distinct reduction in the infection prevalence (Gomes and De Martinis, 2004). In most of the high standard of living countries, the prevalence is nowadays low and a decreasing trend has been noted (Graham, 2014; Mentis *et al.*, 2015).

## 2.0 Environmental Occurrence and Persistence

### 2.1 Detection Methods

The gold standard for *H. pylori* detection from clinical samples is culture. However, the applicability of this method to *H. pylori* detection from environmental samples is problematic due to the predominance of VBNC states (including coccoidal cells) in the environment. Most attempts to isolate *H. pylori* from the aquatic environment have failed. Nonetheless, other methods based on molecular techniques have demonstrated that it does exist as potentially active cells in the aquatic environment (Table 2, 3, and 4).

Isolates have been obtained from clinical samples, which present the advantage of enabling bacterial strain characterization. *H. pylori* is a fastidious microorganism, requiring a complex growth medium supplemented by blood or derivatives (Velázquez and Feirtag, 1999). While there is not a standard method, Brucella or Columbia agar supplemented with 5% to 15% of horse or sheep blood or fetal calf serum and antibiotics, such as Skirrow or Dent supplements, are commonly used. Specific growth media can be obtained from commercial companies.

Culture conditions are critical for successful isolation of *H. pylori*. As it is a microaerophilic bacterium, an adequate atmosphere between 3% to 7% of O<sub>2</sub> and 5% to 10% of CO<sub>2</sub> is needed. After an incubation time between 3 days to 7 days at 37°C with humidity, small, translucent colonies should appear, about 0.5 mm to 2 mm in diameter. Bacterial identification has been based on a battery of phenotypic tests, the most important being morphology of the cells, staining gram-negative, and positive reaction for urease, catalase and oxidase tests.

*H. pylori* have been isolated from cathartic human feces (Parsonnet and Shmueli, 1999) and from probably malnourished children from Gambia (Thomas et al., 1992) but not from routine culture fecal samples, where the bacteria is frequently in a VBNC state. As a consequence, its presence is mostly detected with other methods, such as PCR, or especially with stool antigens enzyme immunoassay.

*H. pylori* has been repeatedly detected in the aquatic environment, applying other techniques such as microscopy (Hegarty et al., 1999), autoradiography, ATP, or molecular techniques. Molecular techniques that have successfully been applied to detect *H. pylori* in the environment include fluorescence *in situ* hybridization (FISH), PCR or qPCR (Moreno-Mesonero et al., 2016). Microarrays to detect its presence jointly with other pathogens from food and in sewage have been also designed (Kostić et al. 2010; Li et al., 2015).

Often a pre-treatment before bacterial detection, such as sample concentration by filtering or immunomagnetic beads, is applied to reduce inhibitors and /or competitors. Monoclonal-based immunomagnetic separation has been very useful to enable detection both by molecular methods (Flanigan and Rodgers, 2003; Hulten et al., 1996; Monteiro et al., 2001; Sasaki et al., 1999) and culture (Lu et al., 2002; Moreno and Ferrús, 2012; Watson et al., 2004).

PCR is the most widely-applied technique, with several gene fragments targeted: urease (*ureA*) or (*ureC*), adhesion (*hpsA*), cytotoxin *CagA* (*cagA*), vacuolizing (*vacA*), *glmM* (Labigne et al., 1991; Shahamat et al., 2004) and also 16S rRNA of genus and species specific (Chisholm et al., 2001; Ho et al., 1991; Logan et al., 2000). The sensitivity and specificity of these techniques has not been established, in addition they might be subject to variability: according to the characteristics of the strains, samples, type of matrix, presence of inhibitors, concentration of the bacteria, extraction method, etc. Often more complementary techniques have been applied. For example, its 16S rRNA gene has been confirmed along with the presence of *ureA* or *vacA* (Mazari-Hiriart et al., 2001; Nayak and Rose, 2007) or by sequencing (Twing et al., 2011).

More research is needed to develop techniques that allow determination of both the presence and infectivity of water-associated *H. pylori*. Such techniques should be effective for both chlorinated and unchlorinated water samples and should also take into consideration possible protective niches of *H. pylori*, such as biofilms and/or protozoa. In particular, new media better adapted to isolate the bacteria from different matrices, and new molecular techniques such as PMA qPCR that distinguish viable cells, would be very useful.

## 2.2 Data on Occurrence

Isolation of *H. pylori* by cultivation from water has rarely been achieved. Lu et al. (2002) isolated for the first time the bacteria from untreated sewage water. Since then few groups have isolated the bacteria, probably because in

water or in biofilms the bacteria are mostly in a VBNC state or, if not, they are fastidious slow-growing microorganisms (Lu et al., 2002). In the last two years *H. pylori* has been cultured from drinking water, but not in pure culture, with its presence among accompanying microbiota confirmed by PCR (El-Sharouny et al., 2015; Santiago et al., 2015). Recently Ranjbar et al. (2016) published the isolation of *H. pylori* from 8 out of 450 bottled mineral water samples in Iran, with a low-level of co-culture of non-target microbiota (Ranjbar et al., 2016).

The bacterium contaminates water principally through human excreta. However, as it is difficult to culture, most of the environmental data about its presence are qualitative, with little quantitative information. Lack of quantitative data makes it difficult to estimate the fate of the bacterium through the water cycle. Further research is needed in order to fulfil this gap.

DNA/RNA of *H. pylori* has been detected worldwide in water applying molecular techniques. The application of PCR-based methods (Engstrand, 2001; Gomes and De Martinis, 2004; Hulten et al., 1996; Nayak and Rose, 2007), FISH (Moreno et al., 2003) and some others (Enroth and Engstrand, 1995; Hegarty et al., 1999) have demonstrated the presence of *H. pylori* in different types of waters: sewage, rivers, lakes, ground water, wells and drinking water (Table 2, 3, and 4).

### 2.2.1 Human feces

*H. pylori* can be detected in human feces using different techniques. Most of the analyses include the stool antigen test with a mean sensitivity and specificity of 92% and 92.2% respectively (Gisbert and Pajares, 2004). PCR has also been used to identify the presence of *Helicobacter* in feces (Mapstone et al., 1993; Queralt et al., 2005). However, the presence of the antigen or DNA does not prove that infective organisms are present. Some researchers have successfully cultured *H. pylori* from feces (Kelly et al. 1994; Parsonnet and Shmueli, 1999; Thomas et al., 1992), but the recovery percentages are very low, because media were not appropriate to isolate this fastidious organism (Dore et al., 2005) or because many cells in stool are transformed into coccoid or VBNC cells (Kabir, 2001). The presence of *H. pylori* in feces reinforces the possibility of a fecal-oral transmission route.

### 2.2.2 Sewage

The presence of *H. pylori* in sewage water has been confirmed by some studies. In 2007, Nayak and Rose detected by qPCR this species in 87% of the analyzed samples with a concentration between  $2.0 \times 10^3$  to  $2.8 \times 10^4$  cells/L from a wastewater plant in Michigan (Nayak and Rose, 2007) (Table 2). More recently, Moreno and Ferrús (2012) cultivated the bacterium in six out of 23 positive wastewater plant samples in Spain (Moreno and Ferrús, 2012) and in a range of wastewater and receiving waters in Michigan, USA (Bai et al., 2016).



**Table 2. Occurrence of *H. pylori* in sewage and treated wastewaters**

Area	Period of Study	Matrix	Detection Method	Percent Positive (# of Samples)	Concentrations GC <sup>a</sup> /L	Reference
Mexico	1997 to 2000	Raw sewage	PCR <sup>b</sup> (16S rRNA confirmed with <i>cagA</i> genes)	20%	Qualitative	Mazari-Hiriart et al., 2001
		Treated wastewater		17%		
Mexico (Ciudad Juarez)	2002	Raw sewage	Immunomagnetic separation, culture and confirmed by PCR16S rRNA gene <sup>c</sup>	17.4% (23/132)	Qualitative	Lu et al., 2002
Spain (Catalonia)	2005	Urban raw sewage	Seminested PCR	69.2% (9/13)	Qualitative	Queralt et al., 2005
USA (Michigan)	2007	Raw sewage	qPCR <sup>d</sup>	87.0% (20/23)	2.0E+03 to 2.8E+04 GC/L	Nayak and Rose, 2007

<sup>a</sup>GC: Gene copies; <sup>b</sup>PCR: Polymerase chain reaction; <sup>c</sup>First *H. pylori* isolates from water. Two isolates were sequenced and confirmed similarity to clinical strains. Sample volume was 1 ml; <sup>d</sup>qPCR: Quantitative polymerase chain reaction.

2.2.3 Surface, ground and drinking waters Helicobacter has been detected in water aqueducts 3.6 x10<sup>4</sup> copies/L and 3.3x10<sup>3</sup> copies/L (Montero-campos et al., 2015) (Table 3).

**Table 3. Occurrence of *H. pylori* in drinking waters**

Area	Period of Study	Matrix	Detection Method	Percent Positive (# of Samples)	Concentrations GC <sup>a</sup> /L	Reference
Canadian (Arctic)	1999	Chlorinated lake water	PCR <sup>b</sup>	50.0% (3/6)	Qualitative	McKeown et al., 1999
Costa Rica & Panama	2015	Aqueducts with chlorine		qPCR <sup>c</sup>	79.5% (35/44)	
		Aqueducts without chlorine	86.4% (38/44)		3.3E+03 GC/L	Montero-campos et al., 2015
Egypt	2014	Drinking water	PCR Culture and PCR	3.8% (2/52)	Qualitative	El-Sharouny and El-Shazli, 2015
Iran	2014	Wells	PCR ( <i>UreC</i> , <i>UreA</i> )	55.9% (66/118)	Qualitative	Amirhooshang et al., 2014
Iran	2016	Bottled mineral water	Culture and PCR 16S rRNA	1.8% (8/450)	Qualitative	Ranjbar et al., 2016
Japan (Tokyo)	2001	Well water	PCR ( <i>Adhesin</i> subunits, <i>ureA</i> and 16S rRNA genes )	33.3% (2/6)	Qualitative	Horiuchi et al., 2001
		Tap water		0.0% (0/10)		

Area	Period of Study	Matrix	Detection Method	Percent Positive (# of Samples)	Concentrations GC <sup>a</sup> /L	Reference
Mexico	1997 to 2000	Water systems		42.0% (58/138)	Qualitative	Mazari-Hiriart et al., 2001
		Wells	PCR (16S rRNA confirmed with cagA genes)	68%		
		Dams pre-treatment		100%		
		Dams post-treatment		0%		
Perú (Pueblos jóvenes)	1996	Municipal water	Immunomagnetic concentration and two consecutive PCR (Adhesin subunits and 16S rRNA genes)	50.0% (10/20)	Qualitative	Hulten et al., 1996
		Wells		25.0% (1/4)		
		Tanker truck 50 ml sample volume		0.0% (0/14)		
Spain	2015	Drinking water, biofilm	PCR	66.7% (16/24)	Qualitative	Santiago et al., 2015
			Fish	25.0% (6/24)		
			Culture	4.2% (1/24)		
United Kingdom (England)	2004	Treated water distribution systems		151: No culture	Qualitative	Watson et al., 2004
		Biofilm	PCR (16S rRNA and VacA)	42.1% (16/38)		
		Water 10 liters sampled		29.2% (14/48)		
		Water nets		16.7% (3/18)		
USA (Pennsylvania and Ohio) <sup>d</sup>	1999	Surface water	Monoclonal antibody against actively respiring bacteria	65.0% (13/20)	Qualitative	Hegarty et al., 1999
		Wells 10 to 1000 ml sample volume		59.5% (25/42)		
USA (Pennsylvania) <sup>e</sup>	2001	Untreated well water 100 to 1000 ml sample volume	Monoclonal antibody against actively respiring bacteria	59.1% (13/22)	Qualitative	Baker and Hegarty, 2009

<sup>a</sup>GC: Gene copies; <sup>b</sup>PCR: Polymerase chain reaction ; <sup>c</sup>qPCR: Quantitative polymerase chain reaction; <sup>d</sup>No correlation with total coliforms or *E. coli*; <sup>e</sup>Correlated with *E. coli*.

Other studies by Park et al (2001) detected the bacteria for the first time in biofilms of distribution systems in Scotland using nested PCR (16S rRNA); Bunn et al. (2002) found *Helicobacter* in water pots biofilms in Gambia.

Isolations were first reported from polluted canal water in 2002 (Lu et al., 2002). Since then, there have been a few reports of cultivation from water. For example, isolation from municipal drinking water in Iraq (Al-Sulami et al., 2010), in Spain from drinking water using an elaborate isolation technique (Moreno and Ferrús, 2012; Santiago et

al., 2015) and in Iran from tap water and dental unit water (Bahrami et al., 2013).

The difficulty in obtaining colonies on agar plates from environmental samples principally lies on the fact that *H. pylori* is a fastidious bacterium with a relatively slow

growth rate. Therefore, its isolation from polluted water can be easily masked by outcompeting microbiota with simpler nutritional requirements and faster growth rates. Also, as discussed above, VBNC coccoid cells rapidly form with exposure to aquatic environments.

Biofilms and protozoa are of special interest as protective microhabitats for viable *H. pylori* in the aquatic environment. The presence of *H. pylori* in biofilms has been demonstrated. In this habitat the bacteria are protected from harsh conditions and may persist longer than in water (Azevedo et al., 2006; Gião et al., 2008). As well, survival within the free-living amoeba, *Acanthamoeba*, has been demonstrated under lab conditions (Moreno-Mesonero et al., 2016; Winięcka-Krusnell et al., 2009). In any case more evidence is needed to understand the importance of these habitats.

It is difficult to relate the presence of *H. pylori* with specific water characteristics. However, its presence has generally been related with fecal pollution, accordingly the most probable sources of the bacteria in the environment (Lu et al., 2002; Moreno and Ferrús, 2012; Queralt et al., 2005). Thus, adequate sanitary conditions are likely to pose benefits for reducing transmission of *H. pylori*.

#### 2.2.4 Seawaters

*H. pylori* has been detected in a few studies from seawater (Carbone et al., 2005; Cellini et al., 1994; Holman et al., 2014). Nonetheless, viable *H. pylori* cells appear to persist in seawater to an even lesser degree than they do for freshwater (West et al., 1990).

**Table 4. Occurrence of *H. pylori* in surface waters**

Area	Period of Study	Matrix	Detection Method	Percent Positive (# of Samples)	Comments	Reference
Italy (Adriatic sea)	2004	Seawater	Nested PCR <sup>a</sup>	58.3% (7/12)	Free and plankton-associated bacteria	Cellini et al., 2004
Japan	2004	River water	Nested PCR	33.3 and 29.2% (8/24 and 7/24)	2 genes were detected ureA and cagA	Fujimura et al., 2004
Japan (Tokyo)	2001	Seawater	PCR (Adhesin subunits, ureA and 16S rRNA genes )	0.0% (0/10)		Horiuchi et al., 2001
		River water		0.0% (0/10)		
Spain (Catalonia)	2005	River water	Seminested PCR	10.5% (2/19)		Queralt et al., 2005
		Spring		0.0% (0/19)		

<sup>a</sup>PCR:Polymerase chain reaction.

### 2.3 Persistence

The persistence and survival of *H. pylori* in the aquatic environment has been studied applying various techniques, but there are numerous knowledge gaps. The bacteria lose their cultivability within 1 to 14 days, with reductions between 5 up to 7 log<sub>10</sub> in all the studies, but survive as VBNC cells for much longer. The survival of different strains has been essentially studied in controlled lab studies such as inoculating into autoclaved distilled water (Azevedo et al., 2008), bottled mineral water (Queralt and Araujo, 2007), chlorinated water (Moreno et al., 2007) or river water (Adams et al., 2003).

The physiological transformation to non-culturable state is usually associated with a morphological change from curved rods to coccoid forms (Adams et al., 2003; Azevedo

et al., 2007; Queralt and Araujo, 2007). Typically, coccoids are considered to be dormant forms that are more persistent in the environment than rods (Azevedo et al., 2007; Mizoguchi and Fujioka, 1999). However, VBNC cells have been resuscitated in mice, indicating that coccoid forms can caused gastritis and stimulate immune response (Cellini et al., 2004; Loke et al., 2016; She et al., 2003).

Environmental conditions can trigger morphological changes, but, only temperature has been directly associated with survival. Specifically, the bacterium survives longer at cold temperatures. Water above 20°C induces a quick decrease of cultivability (Adams et al., 2003; Nayak and Rose, 2007). Other factors such as white light rapidly induced the VBNC state (Buck and Oliver, 2010). When the persistence has been analyzed with molecular techniques, such as PCR or qPCR (Nayak and

Rose, 2007; Queralt and Araujo, 2007), the DNA can remain detectable for much longer. However, its presence in the media is not always indicative of infective cells, which is the ultimate concern.

### **3.0 Reductions by Sanitation Management**

#### **3.1 Excreta and Wastewater Treatment**

No available information could be found regarding waterless sanitation, water-based sanitation, coupled engineered and environmental-based systems, wastewater treatment facilities, sewage sludge treatment or by disinfection as a tertiary treatment.

There is no known surrogate for *H. pylori* reported in the literature, probably because it is difficult to find bacteria with similar characteristics. When *H. pylori* is in its active and free spiral form, its behavior may not be very different from that observed for other Gram-negative

bacteria, particularly *Campylobacter*. However, their sensitivity in the face of sanitation treatments may change when under stress conditions, transforming to VBNC coccoid forms or when it is within biofilms or protozoa (Moreno-Mesonero et al., 2016; Percival and Suleman, 2014).

#### **3.2 Disinfection**

Research available on disinfectants has shown rapid inactivation of culturable cells, but, the comparison of the 2  $\log_{10}$  reduction of one strain of *H. pylori* and one of *E. coli* showed that *H. pylori* is more resistant to chlorine and ozone but not monochloramine than *E. coli*, with a  $CT_{99}$  of 0.299 mg/liter.min versus 0.119 mg/liter.min for chlorine and a  $CT_{99}$  0.24 mg/liter.min versus 0.09 mg/liter.min for ozone (Baker et al., 2002). A more recent work would indicate survival following normal UV wastewater treatment (Bai et al., 2016). But further research is needed in this area.

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