Transmission of *Helicobacter pylori* and the role of water and biofilms

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ABSTRACT

Documented evidence relating to the survival of *Helicobacter pylori* outside the gastric niche is extremely limited. To date the primary transmission routes of *H. pylori* have yet to be confirmed and when this is achieved preventive infection control measures can be implemented to reduce and ultimately prevent human infection from this pathogen. There is mounting evidence which suggests that the prevalence of *H. pylori* infection has a strong correlation with access to clean water, suggesting a transmission route to the host. However, there are no established culture methods for the detection of viable *H. pylori* in the environment, in particular drinking water supplies, preventing the development of true epidemiological and risk assessments. The aim of this review is to highlight the available data to date that suggests drinking water and possible survival in biofilms as a probable transmission mode for *H. pylori*.

Key words | biofilm, drinking water, *Helicobacter pylori*, viable but non-culturable (VBNC)

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INTRODUCTION

Helicobacter pylori are micro-aerophilic spiral-shaped bacteria that efficiently colonize the human gastric mucosa (Moreno *et al.* 2007). They were first identified in autopsied rabbits in 1893 (Rothenbacher & Brenner 2003), described in humans in 1906 (Rothenbacher & Brenner 2003), and successfully cultured in 1983 (Warren & Marshall 1983). Initially, research-based studies classified *H. pylori*, based on its '*Campylobacter* like' morphology and biochemistry, as *Campylobacter pyloridis* (Warren & Marshall 1983). Later the name of the bacterium was changed to *Campylobacter pylori* and then finally classified as *Helicobacter pylori* some years later (Owen 1995).

Helicobacter pylori is Latin for 'spiral rod of the lower part of the stomach'. It is known to cause inflammation in the form of chronic active gastritis, specifically in certain humans. In addition, *H. pylori* has been linked to a diverse spectrum of gastrointestinal disorders which include peptic ulcer disease, gastric adenocarcinoma and gastric mucosa-associated lymphoid tissue (MALT) lymphoma (Farinha & Gascoyne 2005). MALT lymphoma is a relatively rare condition caused by *H. pylori* and only very small percentages (1–2%) of infected individuals develop a malignant disease.

Clinical disease typically occurs decades after initial acquisition of infection. However, gastritis may progress over time from an initially superficial nonatrophic form to a more severe atrophic gastritis, with intestinal metaplasia, leading to duodenal ulceration, gastric adenocarcinoma and gastric MALT lymphoma (Farinha & Gascoyne 2005). Because of the vast array of conditions and public health concerns that surround *H. pylori*, the World Health Organization International Agency for Research on Cancer classified *H. pylori* as a class I carcinogen in humans (Sherman 2004). While only a small percentage of individuals carrying *H. pylori* ever develop clinical sequelae, asymptomatic carriage is common (Peterson & Krogfelt 2003) and if left untreated *H. pylori* infection is lifelong (Czinn 2005).

Although it is estimated that approximately 50% of the world's population is colonized with *H. pylori*, prevalence varies widely by age as well as country, ethnic background and socio-economic conditions (Czinn 2005).

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The developing world carries the greatest burden, with over 70% of children infected by age 15 (Czinn 2005). In contrast the developed world has, since the 1950s, experienced a decrease in the prevalence of *H. pylori* with each successive generation; hence at present approximately 20% to 30% of individuals harbour *H. pylori* (Rothenbacher & Brenner 2003). This reduction has been attributed to improved socio-economic status and personal hygiene but may equally have transpired because of improvements in drinking water quality.

The aim of this review is to critically evaluate published data and determine whether water and biofilms may constitute possible transmission and environmental niches for *H. pylori*. Consequently, by establishing water and biofilms as a transmission route for *H. pylori* the development of appropriate control measures to reduce the incidence and prevalence of *H. pylori* even further is of great significance, particularly in developing countries.

TRANSMISSION ROUTES OF *H. PYLORI* AND ACQUISITION

Although the natural niche for *H. pylori* is the human stomach, for widespread infection to occur the organism may need to survive in the external environment (Brown 2000). To date the precise mechanism/s involved in the transmission of *H. pylori* is/are unknown, but clearly any approach that introduces the organism into the stomach of a susceptible person may lead to that individual acquiring an infection. Many transmission routes for *H. pylori* have been proposed and have included gastric-oral (Raymond *et al.* 2008), oral-oral (Mégraud 1995), faecal-oral (Raymond *et al.* 2008), zoonotic (Fox 1995) and water/food-borne (Hulten *et al.* 1996; Herrera 2004). Clearly these proposed transmission routes indicate that *H. pylori* infection occurs through multiple acquisition pathways (Goodman & Correa 1995; Velazquez & Feirtag 1999). Despite these proposals, contamination of food by human faecal material has been found to be one of the major risk factors for the acquisition of *H. pylori* (Hopkins *et al.* 1993).

H. pylori has been cultured from the faeces of infected individuals (Thomas *et al.* 1992; Kelly *et al.* 1994) and specific DNA sequences have been amplified from raw sewage (Forrest *et al.* 1998) providing possible evidence for the faecal-oral route of transmission. However, the faecal-oral route of transmission and its interpretation has been questioned, as the concentration of *H. pylori* cells present in faecal material is considered to be low, particularly when compared with other faecal pathogens of public health significance (Vincent 1995). Furthermore, the prevalence of *H. pylori* IgG antibodies in sewage workers, compared with a control group, matched for age and socio-economic status, has demonstrated no increased risk of infection when exposure to human faecal material has occurred (Friis *et al.* 1996). Despite controversy in this area there is growing evidence that suggests a faecal-oral transmission route for *H. pylori* (Mladenova *et al.* 2006). Consequently, if *H. pylori* is excreted within faeces they may well go on to colonize surfaces present in water sources. Such surfaces subsequently then become transmittable sources of *H. pylori* (Xia & Talley 1997).

The age at which humans are exposed to *H. pylori* may influence its route of transmission. For example, in an Argentinean study, the key risk factor for acquisition of *H. pylori*, specifically in childhood, was the nature of the water source (Olmos *et al.* 2000). In developing countries, many children are infected with *H. pylori* by the age of ten, and relapses are known to occur (Dooley *et al.* 1989; Gurel *et al.* 1999). In Peruvian children *H. pylori* prevalence was found to strongly correlate with socio-economic status: children were found to be three times more likely to be infected with *H. pylori* when they drank from an external water source than those exposed to water from an internal water source (Klein *et al.* 1991). However, no difference was found when those children from high- and low-income families with an internal water source were compared (Klein *et al.* 1991). Children born into high-income families supplied with municipal water are considered 12 times more likely to become colonized with *H. pylori* than those supplied from community wells (Frenck & Clemens 2003). This suggests that municipal water is a possible risk factor in the transmission and acquisition of *H. pylori*. It is plausible to suggest that breaks in municipal pipes allow for infiltration of contaminated surface water (Frenck & Clemens 2003). A group of 3,289 residents in Italy were screened for the prevalence of *H. pylori* IgG antibodies (Dominici *et al.* 1999). The conclusion drawn from this

study was that there was a common source of exposure to *H. pylori* in the environment (Dominici *et al.* 1999).

Some research findings have shown that hands and fingernails contaminated with *H. pylori* may transfer bacteria into a water source (Dowsett *et al.* 1999; Frenck & Clemens 2003). Evidence of *H. pylori* presence in dental plaque has led to the suggestion by a number of researchers that the oral cavity may be a potential reservoir for this bacteria in the adult population (Luman *et al.* 1996; Cave 1997; Kamat *et al.* 1998; Oshowo *et al.* 1998*a*,*b*). Souton & Colombo (2008) found that the prevalence of *H. pylori* can be as high as 33.3% in subgingival biofilms and 20% in saliva. Despite this it still remains unclear whether the oral cavity acts as a permanent reservoir for *H. pylori* specifically as within this oral ecosystem *H. pylori* is considered to be transient.

In addition to humans, domestic cats and Old World macaques have been found to be colonized with *H. pylori* but it is doubtful whether these animals provide an important reservoir for human infection (Osata *et al.* 1997; Baker & Hegarty 2001). Flies have also been considered as having a potential role in the vectorial spread of *H. pylori* from human faeces to food (Grubel *et al.* 1998).

TRANSMISSION OF *H. PYLORI* IN WATER

In developing countries, water rather than person-to-person spread plays a significant role in the transmission of *H. pylori* (Akcam *et al.* 2000). Water from streams, rivers and wells has been considered as a common source (Hulten *et al.* 1991, 1996; Klein *et al.* 1991; Mackay *et al.* 1998; Hegarty *et al.* 1999; Engstrand 2001; Mackay *et al.* 2001; Mazari-Hiriart *et al.* 2001; Lu *et al.* 2002; Imanishi *et al.* 2003; Karita *et al.* 2003; Azevedo *et al.* 2004; Gomes & De Martinis 2004; Rolle-Kampczyk *et al.* 2004). In Brazil, Zaterka & colleagues (2007) confirmed that the source of drinking water in childhood was a risk factor for *H. pylori* infection and that the prevalence of *H. pylori* infection was higher when a local river was the source of drinking water and lower when this water was filtered or boiled. In addition, Goodman & colleagues (1996) found that swimming in streams, using streams as a drinking water source, and frequent consumption of raw vegetables, cleaned in contaminated water, increased the likelihood of infection with *H. pylori*. Other research also supports an association between *H. pylori* infection and consumption of untreated well or spring water (Carballo *et al.* 1997; Benson *et al.* 2004; Reavis 2005). Further evidence concerning the importance of water as a transmission route of *H. pylori* was stressed by Fujimura & colleagues (2004) who collected and analysed a total of 24 water samples from the upper, middle and downstream reaches of four Japanese rivers for evidence of *H. pylori* by nested polymerase chain reaction (PCR). The conclusion from this study suggested that water, in the natural environment, could be a risk factor for *H. pylori* transmission (Fujimura *et al.* 2004).

PERSISTENCE, DETECTION AND CULTURABILITY OF *H. PYLORI*

Despite the numerous research findings identifying *H. pylori* in water, it is important to consider the fact that the use of PCR and other molecular methods for the detection of pathogens in environmental samples has limitations. This is principally due to the inability of PCR to differentiate between naked DNA from dead and living cells. Furthermore, the natural environment contains many microorganisms which have not yet been identified or cultured, which may interfere with molecular technologies. Consequently, to scientifically interpret data regarding the epidemiology of *H. pylori*, cultured bacteria from appropriate water sources are necessary.

The first successful isolation of *H. pylori*, by culturable methods, occurred in a municipal wastewater canal on the US–Mexico border (Lu *et al.* 2002). This canal was found to be heavily contaminated with untreated raw sewage, in an area known to have a high *H. pylori* prevalence (Lu *et al.* 2002). Aside from this study, positive culture of *H pylori* from drinking water has not been successful, despite efforts to produce a culture-specific media sensitive and selective enough to isolate and grow this organism. A simple plating medium for the detection of *H. pylori* in the environment was investigated by Degnan *et al.* (2003) and Fernández *et al.* (2007). However, the culturable methods employed were unsuccessful in the culturing of *H. pylori*.

H. pylori rapidly transforms into a viable but non-culturable state (VBNC). This state is induced by low

nutrient and hyperosmotic conditions (Mizoguchi *et al.* 1999; Zheng *et al.* 1999; Moreno *et al.* 2003). Such stressed conditions are commonly found in water and the environment. Fluorescent *in situ* hybridization (FISH) with rRNA oligonucleotide probes has been used for detection and identification of VBNC forms of bacteria (Rowan 2004). The role of the VBNC of *H. pylori*, and its associated forms, in infection and transmission remains unclear (Rowan 2004). However, what is significant is that within the VBNC state *H. pylori* cells are still alive (Moreno *et al.* 2003; Rolle-Kampczyk *et al.* 2004; Rowan 2004). Ultimately this may, when evidence becomes available, have important implications for the survival and therefore the potential infectivity of *H. pylori*. To date, little or no evidence exists regarding the resuscitation of VBNC cells of *H. pylori* or on the ability of the VBNC cells to cause infection.

Initial evidence of *H. pylori* presence in environmental water samples has come from PCR amplification of samples obtained from Colombia, where infection rates are over 90% (Handwerker *et al.* 1995). In addition to this a number of PCR assays have been utilized over the years for the detection of *H. pylori* in water (Engstrand *et al.* 1992; Weiss *et al.* 1994; Hulten *et al.* 1996; Sasaki *et al.* 1999; Benson *et al.* 2004; Gomes & De Martinis 2004; Shahamat *et al.* 2004). Furthermore, in the United States, actively respiring *H. pylori* from surface and well water has been detected using fluorescent antibody-tetrazolium reduction (FACTC) microscopy (Hegarty *et al.* 1999) and confirmed using species-specific PCR (Azevedo *et al.* 2006*a*). Sen *et al.* (2007) investigated the development of internal controls for PCR assays by spiking drinking water with 100 cells of *H. pylori* and demonstrated similar cycle thresholds to those of recombinant *Escherichia coli* during chlorine disinfection. In addition to PCR, FISH was validated as a quick and sensitive method for detection of *H. pylori* in environmental samples (Moreno *et al.* 2003). These findings suggest the presence of *H. pylori* in the natural environment and a possible waterborne route of transmission. Nayak & Rose (2007) demonstrated that quantitative polymerase chain reaction (qPCR) could determine *H. pylori* concentrations in water. In this study real time qPCR was shown to be a specific, sensitive and rapid method to quantify *H. pylori* in sewage. Prior to these studies a two-stage *in vitro* method for detection of *H. pylori* in spiked water and faecal samples using immunomagnetic separation followed by PCR detection (IMS/PCR) has been described (Enroth & Engstrand 1995).

Numerous studies have shown that *H. pylori* may survive for prolonged periods in water over a range of physical variables (West *et al.* 1992). In fact *H. pylori* strains have been shown to survive for long periods under physiological saline concentrations, low temperatures and a pH range of 5.8 to 6.9. A study by Shahamat & colleagues (1993) has found that *H. pylori* were able to remain viable for periods ranging from 48 hours to between 20 and 30 days when exposed to different temperatures.

A study in Leipzig, Germany, showed a positive correlation between the drinking of *H. pylori*-contaminated well water and the acquisition of a *H. pylori* infection (Rolle-Kampczyk *et al.* 2004). *H. pylori* DNA has been amplified from drinking water samples in Japan (Sasaki *et al.* 1999), Mexico (Mazari-Hiriart *et al.* 2001) and Peru (Hulten *et al.* 1996), from water samples taken from a delivery truck in the Canadian Arctic (McKeown *et al.* 1999) and from drinking water storage pots in the Gambia (Bunn *et al.* 2002). A study by Hulten *et al.* (1998) used two PCR assays to examine municipal treated and well water samples from all 25 counties of Sweden for the presence of *Helicobacter* DNA: 37.5% of wells, 12% of municipal sources and 12% of wastewater samples were found to be positive for *Helicobacter* DNA.

The argument for a waterborne route of *H. pylori* transmission is supported by the maintenance of viability in spiked natural water (West *et al.* 1990, 1992; Shahamat *et al.* 1993; Hunter 1997; Fan *et al.* 1998; Jiang & Doyle 1998; Sato *et al.* 1999; MMWR 1999). While attempts to culture *H. pylori* from environmental water samples have been largely unsuccessful, closely related microaerophilic organisms, *Campylobacter jejuni* and *Arcobacter butzleri*, have been cultured from ground and surface waters (Arvanitidou *et al.* 1994; Stanley *et al.* 1998; Rice *et al.* 1999) and associated with waterborne outbreaks (MMWR 1999). As mentioned previously, viable *H. pylori* cells could be transmitted through faecal material (Thomas *et al.* 1992), which may well provide a route for contaminating drinking water. *H. pylori* have been shown to survive for short periods in water when present in their coccoid morphology (Mizoguchi *et al.* 1999; She *et al.* 2003). This coccoid form of *H. pylori*, because of its increased tolerance to outside

perturbations, may allow the bacteria to survive the extremes of conditions associated with drinking water and water distribution systems. In addition, it has been speculated that *H. pylori* coccoid cells may be able to tolerate the levels of disinfectant normally used in distribution systems and therefore remain viable (Azevedo *et al.* 2008). To date the survival of *H. pylori* is poorly understood in the aquatic environment; likewise we do not know how this environment affects its viability.

DISINFECTION DATA

Present data concerning the effectiveness of standard drinking water disinfection processes on *H. pylori* are limited by the number of published articles in this area. Baker & colleague (2002) found that *H. pylori* were more resistant to low levels of free chlorine than *E. coli* or *C. jejuni*. Conclusions from this research have highlighted the fact that it was possible, under conditions of inadequate disinfection, for *H. pylori* to persist in water. Based on the studies by Johnson *et al.* (1997) and Baker *et al.* (2002) it is possible that reduced chlorine residuals might not provide adequate inactivation of *H. pylori*. Consequently this would not prevent the entry and persistence of *H. pylori* in drinking water systems. This may be particularly so if the bacterium grows within a biofilm state.

A number of drinking water studies have identified *H. pylori* in water pre- and post-chlorination (Mazari-Hiriart *et al.* 2003). Moreno & colleagues (2007) have shown that *H. pylori* could survive disinfection practices that are normally used in drinking water treatment when *H. pylori* are found in the VBNC state. However, they did find that culture of *H. pylori* was lost after 5 min in water despite free chlorine levels of 0.96 mg l^{-1}.

BIOFILMS

It is possible that there are environmentally adapted forms of *H. pylori* within a biofilm community. *H. pylori* readily form biofilms (Carron *et al.* 2006) and in so doing produce a novel antibacterial peptide, which may confer increased persistence in a heterogeneous biofilm environment (Putsep *et al.* 1999). *H. pylori* is also known to produce a water-insoluble biofilm when grown under high carbon:nitrogen ratio conditions (Stark *et al.* 1999). However, environmentally adapted *H. pylori* have to be isolated and cultured for an evaluation of their true environmental persistence, virulence and ability to be transmitted in water (Vincent 1995).

It is well known that waterborne bacteria can attach to surfaces by aggregating in a hydrated exopolymer known as a biofilm (Costerton *et al.* 1999; Percival *et al.* 2000). The association of bacteria, particularly pathogens, with biofilm communities within a water distribution system may offer vulnerable and susceptible bacteria protection from disinfection and protozoan predation (Sibille *et al.* 1998). In fact microorganisms in drinking water are predominantly associated with biofilms rather than in the planktonic state (Costerton *et al.* 1999; Percival *et al.* 2000, 2004). Consequently, for *H. pylori* to survive the extremes of water it is probable that it would have to reside within a biofilm. In addition to this, *H. pylori* has recently been shown to remain viable and proliferate inside *Acanthamoeba castellanii* for up to 8 weeks when evident in a co-culture (Winiecka-Krusnell *et al.* 2002). Various species of Acanthamoebae are common components of drinking water biofilms and have been shown to shield bacteria from disinfectants and enhance their proliferation when evident in biofilms (Greub & Raoult 2004).

There is evidence that biofilms in water distribution systems may harbour *H. pylori* (Mackay *et al.* 1999; Mackay *et al.* 2001; Park *et al.* 2001). In addition a study undertaken in Western Africa, utilizing 16S rDNA sequences, has shown evidence that *H. pylori* can be detected in natural biofilms (Bunn *et al.* 2002). A more recent study by Watson & colleagues (2004) showed a close link between *Helicobacter* DNA in showerhead biofilm used in domestic households. Furthermore *H. pylori* has been found to have the ability to incorporate itself into lab-grown biofilms (Mackay *et al.* 1999; Azevedo *et al.* 2003). The study by Mackay & colleagues (1999) concluded that *H. pylori* could incorporate itself, and persist in a laboratory-based, mixed-species heterotrophic biofilm, for over 8 days. However, Watson *et al.* (2004) concluded from their study that monitoring the cold water supply to a particular property did not appear to be a reliable means of predicting the *Helicobacter* status of the water distribution system in that property. Azavedo *et al.* (2006*a*,*b*) and Bragança *et al.* (2007) have also shown that *H. pylori* may

be present as biofilms on pipe work in drinking water systems. In the study by Azavedo & colleagues (2006*a*) *H. pylori* was shown to have the ability to adhere to different plumbing materials, namely copper and stainless steel. Copper surfaces were found to be especially suitable for the maintenance of the bacteria in the spiral form.

CONCLUSION

Although inconclusive, epidemiological studies strongly suggest person-to-person transmission of *H. pylori* (Raymond *et al.* 2008). However, recent experimental findings suggest that *H. pylori* transmission may involve the consumption of contaminated drinking water (Bellack *et al.* 2006). Although *H. pylori* is not classified as a food or waterborne pathogen, it is acknowledged as an important pathogen. The ability of *H. pylori* to survive in a coccoid VBNC form and its survival correlated with water should be of interest and possible concern to epidemiologists and public health microbiologists.

Outside the developing world the evidence for waterborne transmission of *H. pylori* is sparse. However, recent evidence has shown that *H. pylori* can survive in water for over 96 hours at 25°C (Azevedo *et al.* 2008). While this timescale is relatively short, it is evident in the developing world that this would be long enough for *H. pylori* transmission and possible infection of a compromised individual to occur.

H. pylori remains a significant problem in the developing world and will continue to be a concern as a result of the poor level of sanitation and hygiene that exists in these regions of the world. Subsequently the importance of *H. pylori* as a primary pathogen is unlikely to diminish in the foreseeable future in these vulnerable areas. Consequently, it remains important that we acquire a better understanding of the risk factors associated with the acquisition of *H. pylori* infection but at the same time we should not overlook the increasing potential that *H. pylori* can be transmitted via a waterborne pathway. Accordingly the 'environmental' biofilm in which *H. pylori* may reside, resuscitate, proliferate, interact socially with other sessile microorganisms and then disseminate warrants further investigation as a source of infection (Gião *et al.* 2008).

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