Review

The coccoid forms of *Helicobacter pylori*: A permanence mechanism

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**ABSTRACT**

*Helicobacter pylori* is a spiral pathogenic bacterium that colonizes the stomach of more than 50% of the world population. Although there are numerous articles reported on *H. pylori* not yet been clarified the full route of transmission of the bacteria to the host, which can be fecal-oral, oral-oral and today has also emerged as a possible vehicle of transmission hydric via. Thanks to these theories it has emerged a new knowledge about *H. pylori*. It has been described its non-culturable viable stage (coccoid form of *H. pylori*), decreasing bacterial metabolism and changing its morphology as a response mechanism to environmental stress.

**Keywords:** *Helicobacter pylori*, Cocoid form, Resistance, Environmental, Permanence, Stress.

**INTRODUCTION**

*H. pylori* is a Gram negative microaerophilic helicoidal bacterium which has about 2-6 flagella that confer mobility and allows it to penetrate the gastric mucosa; it is approximately 2.4-4.0 micrometers long and 0.5-1.0 width (Brown, 2000). *H. pylori* can generate different pathologies in infected people manifesting gastritis, ulcers, MALT lymphoma of B cells and gastric cancer. Sometimes infected patients have no clinical manifestations throughout your life (asymptomatic patients) (Duš et al., 2013; Montero-Campos et al., 2011; Percival and Suleman, 2014). The first symptoms usually take a long time to manifest after infection (Brown, 2000). The transmission of *H. pylori* infection have two routes which have been confirmed by epidemiological studies on large populations of patients: the transmission of infection through the faecal-oral and oral-oral routes (Bardhan, 1997; Duš et al., 2013).

The overall prevalence of *H. pylori* infection is higher in underdeveloped economically countries appearing in major regions from Africa, Asia and Latin America than developed countries from Western European and North American.

It is well known that infection by *H. pylori* is mainly acquired during childhood. Over 50% worldwide children have been in contact with the bacteria (Montero-Campos et al., 2011; Percival and Suleman, 2014). Other studies have shown that 20% of the population from underdeveloped economically
countries showed infection by *H. pylori* at 5 years old; infection by *H. pylori* increased to 80% when the population was about 20 years old (Goodman et al., 1996). Infection by *H. pylori* is rare during infancy in developed countries and prevalence steadily increases during adulthood, so that even at age 60, just under 50% of the population is infected (Brown, 2000; Cava and Cobas, 2003; Richards et al., 2011).

Socioeconomic status is clearly seen as one of the most important factors for the development of infection; social groups with lower per capita income in the world showed a higher prevalence (Mitchell, 2001). This hypothesis has been supported by numerous studies which indicate contrasts between developed countries and underdevelopment.

**SURVIVAL OF Helicobacter pylori IN AQUATIC ENVIRONMENTS**

Although *H. pylori* is considered a pathogen commonly associated with the human stomach, it has been reported that this bacterium is able to survive in external environments of the human stomach (Brown, 2000; Goodman et al., 1996; Velázquez and Feirtag, 1999). Dental plaque has also been reported to contain *H. pylori*; however in plaque *H. pylori* only exist in a transient state (Oshowo et al., 1998; Percival and Suleman, 2014). Another authors have proposed that biofilm formation of *H. pylori* in the oral cavity should be considered as a potential reservoir for bacterium (Souto and Colombo, 2008). There is building evidence to suggest that *H. pylori* may reside in potable water systems and that waterborne bacteria can adhere to surfaces by aggregating matrix to form biofilms. There is growing evidence that external reservoirs of *H. pylori* may exist potentially aiding transmission to the host (Costerton et al., 1999; Percival and Thomas, 2009).

Several studies have shown that *H. pylori* can survive in aquatic environments such as lakes, rivers, drinking water, surface and ground water, wastewater and coastal marine environments (Fernández-Delgado et al., 2008). Furthermore, some investigations have shown for several days survival of *H. pylori* in milk and tap water (Fan et al., 1998; Hulten et al., 1996). In both cases the morphology of this bacterium has been helical shaped. However, in another study it was shown that this bacterium can survive in river water for several months and morphology observed is not helical shape corresponded to the classical form of the bacteria; *H. pylori* have adopted the coccoid morphotype (Azevedo et al., 2007; Chen, 2004; Percival and Suleman, 2014; Vincent, 1995). Both in vitro and in vivo *H. pylori* can be found in coccoid or spiral form (Cava and Cobas, 2003). Thus it was considered that after a time that *H. pylori* is subject to stressful environmental conditions, it acquires coccoid form, which has been termed viable non-culturable (Bode et al., 1993; Richards et al., 2011).

This seems to be the mode of transmission of the bacterium that has been demonstrated in studies in China, Peru, Colombia and other countries, which water could be a vehicle for the transmission of this bacterium. Hypothesis was supported by a strong association between the consumption of well water contaminated with *H. pylori* and infection of this bacterium in people who consumed water well (Baker and Hegarty, 2001). It has been reported that both the spiral and viable coccoid form of *H. pylori* are present in the oral cavity (Young et al., 2001).

**THE COCCOID FORM OF H. pylori**

One of the attributes that have *H. pylori* to survive in the environment is changing spiral morphology to the coccoid form in some stressful situations for example a exposure to oxygen, changes in pH (alkaline), increased temperature, nutrient starvation, prolonged incubation time, exposure to inhibitors and antibiotics affecting the proton pump (Andersen and Rasmussen, 2009; Azevedo et al., 2004; Chaput et al., 2006; Chen, 2004; Fan et al., 1998; Janas et al., 1995; Mizoguchi et al., 1998; Richards et al., 2011). This responsivenes to environmental stress, has been named viable non-culturale (VNC), because it maintains the ability to perform metabolic functions and non cultivable because it can not be isolated by conventional methods of culture in laboratory.

This property may be reversible if the bacteria again in favorable conditions and it can return to cultivable state or spiral form (Fernández-Delgado et al., 2008). It has also been proposed that the change in morphology to coccoid form is a temporary adjustment presenting bacteria to environmental stress to survive, and thus may play a role in the survival and how transmission through water (Andersen and Rasmussen, 2009; Bode et al., 1993; Saito et al., 2003). Both forms are commonly observed in the human stomach (Chan et al., 1994; Chaput et al., 2006). The integrity of the membrane, ATP production and mRNA transcription are indicators of the viability of the bacterium. *H. pylori* in viable non-cultivable state can maintain the integrity of the cell membrane, producing ATP and mRNA long after losing the ability to grow on a solid culture medium (Adams et al., 2003). Also it has been proposed that some viable cells retain full energy supplies (Enroth et al., 1999). Coccoid forms of *H. pylori* contain a reasonable quantity of ATP, an active respiratory chain, viable as assessed by viability staining. Proteins such as VacA, CagA, urease are detectable. Several groups have reported colonization of mice with coccoid bacteria and have subsequently isolated coccoid bacteria from their stomachs, indicating that under certain conditions coccoids may revert back to spiral bacteria (Adams et al., 2003; Cellini et al., 1998; Chaput et al., 2006; Mizoguchi et al., 1998; Nilsson et al., 1996; Velázquez and Feirtag, 1999). Some investigations have shown that *H. pylori* in river water can survive for several months (Oshowo et al., 1998; Percival and Suleman, 2014). The integrity of the membrane, ATP production and mRNA transcription are indicators of the viability of the bacterium. *H. pylori* in viable non-cultivable state can maintain the integrity of the cell membrane, producing ATP and mRNA long after losing the ability to grow on a solid culture medium (Adams et al., 2003). Also it has been proposed that some viable cells retain full energy supplies (Enroth et al., 1999). Coccoid forms of *H. pylori* contain a reasonable quantity of ATP, an active respiratory chain, viable as assessed by viability staining. Proteins such as VacA, CagA, urease are detectable. Several groups have reported colonization of mice with coccoid bacteria and have subsequently isolated spiral bacteria from their stomachs, indicating that under certain conditions coccoids may revert back to spiral bacteria (Adams et al., 2003; Cellini et al., 1998; Chaput et al., 2006; Mizoguchi et al., 1998; Nilsson et al., 1996; Velázquez and Feirtag, 1999).
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were fully degraded and they expressed the urease gene. This thesis holds that bacteria in this state can remain viable.

Saito et al., (2003) conducted a study of the induction of coccoid form of H. pylori. During the course of the experiment they observed the change in morphology; after fourth day H. pylori become coccoid forms. Subsequently, coccoid forms of H. pylori were classified into 2 groups: type A and type B. Type A showed an irregular surface and it was appreciated that they adhered to each other. Type B had a smooth surface with flagella coiled around its body and strictly membranous structure. It was observed that both types had similar internal structure and that they had lost the ability to be cultivated (Saito et al., 2003).

It has observed that viable spiral and coccoid forms of H. pylori decreased the concentrations of DNA and RNA after 31 days of incubation in liquid medium. DNA and RNA of coccoid forms of H. pylori not were fully degraded and they expressed the urease gene. This thesis holds that bacteria in this state can remain viable.

When H. pylori is contacted with water takes coccoid form. It has also been shown that In this state H. pylori respires by a oxidative metabolism and it possesses DNA and mRNA in smaller quantities (Cellini et al., 1998; Narikawa et al., 1997). Although as described above, it can disprove the theory that the viable non-cultible state is a manifestation of cell death, as had been considered by some authors.

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It is well known that the transcript levels of genes are greatly affected by environmental stimuli. For example, mRNA molecules usually have a rapid turnover (from seconds to 20 minutes), so mRNA disappears after dying cells (mRNA molecules are useful indicators of the viability of the bacteria even in batch culture) (Conway and Schoolnik, 2003).

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It has observed that viable spiral and coccoid forms of H. pylori have intact cytoplasm structures and a cell membrane that is characteristic for Gram negative bacteria, while nonviable coccoid forms have degenerative organelles and a disintegrated cell membrane (Andersen and Rasmussen, 2009). The viable coccoid form is usually smaller and more condensed than the degenerative coccoid forms. It has been shown that H. pylori can survive as culturable forms in distilled water and saline for 414 days and in artificial seawater for 47 days (West et al., 1992). Viable culturable H. pylori can persist for 410 days and viable nonculturable coccoid H. pylori can survive for up to 1 year in fresh water (Shahamat et al., 1989). One H. pylori strain stored in deep ground water or in natural seawater at 4°C survived significantly better for 7 days than the same strain stored in nutrient-rich media (Konishi et al., 2007). These studies indicate that H. pylori may survive as culturable forms for weeks in water and that they survive better in natural systems than in artificial nutrientrich systems. The nonculturable coccoid form was able to persist for several months and it is unlikely that the fractions were contaminated with spiral forms after such a long period (Andersen and Rasmussen, 2009).

It has generated much controversy since it began to write about the viable non-culturable form, because some authors consider this stage as the manifestation of cell death while other authors consider this state as a viable form or latent form (Azevedo et al., 2004; Chen, 2004; Saito et al., 2003). On the other hand, other researchers have shown that viable non-culturable form may be a reversible state from the spiral typical form to coccoid form and coccoid form to spiral form again (Bode et al., 1993). From a broader perspective, it is now accepted that what happens is that these bacteria are not able to cultivate under routine and conventional laboratory methods, however it has been shown that coccoid forms of H. pylori are able to colonize the gastric mucosa and cause gastritis in mice (Touati et al., 2003).

There is not sufficient information about the reversal of the coccoid form of H. pylori to typical spiral (resuscitation). Unlike other bacteria where it has been observed that rich media supplementation promotes resuscitation of bacteria (for example in Salmonella sp), in H. pylori was not found any factor that causes regression of the coccoid form to spiral form (Zeng et al., 2013). Sörberg et al., (1996) were the first to attempt to reverse the coccoid form of H. pylori to the helical form by supplementation with fresh medium for 4 weeks, however failed to reverse in cultures. Kurokawa et al., (1999) proposed that could reverse spiral form of H. pylori to the coccoid form through two processes: stimulation and proper nutritional supplementation (Kurokawa et al., 1999). Azevedo et al., (2007) reported that once the cells of H. pylori enter to coccoid form can not be reversed to the helical form even though the cells be placed in optimal growth conditions. Recently it has been published that there is not conclusive evidence about the reversal of coccoid form of H. pylori to helical form by manipulation of culture medium (Rudnicka et al., 2014).

Furthermore, it has been described in Vibrio vulnificus that autoinducers (quorum-sensing) are required for the resuscitation of bacterium (Ayrapetyan et al., 2014; Li et al., 2014; Zeng et al., 2013). It has been reported that autoinducers found in Gram negative and positive bacteria are thermostable, can be removed from the supernatants of the culture medium and added to the bacterial cells in viable non-culturable form, inducing resuscitation (Zeng et al., 2013). Age of the bacterial cells in viable non-culturable form also influences resuscitation, since it has been observed that cells that reached more quickly this form lose their ability to resuscitation. Age and residence time are determinants of the phenomena associated with the conversion between different stages (Pinto et al., 2015). An equally important factor is the interaction of cells in viable non-culturable form of H. pylori with the host cells to perform resuscitation, also stressors and increased nutrients (provided by the host) appear to promote resuscitation (Li et al., 2014, Pinto et al., 2011). She et al., (2003)
observed helical forms of *H. pylori* in the gastric mucosa tissue, after a mice was inoculated with coccoid forms of bacterium, so that they concluded that the reversion *in vivo* is possible. In recent years has increased significantly the knowledge of resuscitation in different Gram positive and Gram negative bacteria, however this phenomenon remains largely unknown in *H. pylori*. There is still much controversy between about reversal coccoid form to helical form.

**Role of gene cdrA in cell morphology**

Takeuchi et al., (2012) found that gene *cdrA* had a high sequence similarity with a protein of family FtsK/SpoIIIE, important protein for cell division in *E. coli*. Putative gene product-CDRA is a 367 amino acid polypeptide that exhibited a high homology to HP0066 ATP-binding protein of *H. pylori* 26695 (conserved hypothetical protein), and it showed some similarity to the protein of family FtsK/SpoIIIE that encodes a ATP-binding protein probably involved in cell division (Takeuchi et al., 1998). The gene *cdrA* not only have an inhibitory role for cell division, it is also involved in elongation and cell death via cell wall synthesis. Previously it thought that gene *cdrA* had an important role in the loss of the ability to culture of *H. pylori*, however another studies have indicated an increase in the transcription of gene when *H. pylori* lost the ability to be grown. A reverse event is presented when stressed cells of oxygen showed a decrease in the transcription of this gene, in comparison to healthy cells. Although gene *cdrA* not plays a role in the loss of the ability of *H. pylori* during culture in a state of stress, it is believed may be important if cell takes a morphological conversion by a process of physical or chemical stress (Richards et al., 2011).

**Detection of *H. pylori* by molecular methods**

Saito et al., (2003) indicated that the cultivation of bacteria by conventional methods is the best way to assess the viability of *H. pylori*. But *H. pylori* is demanding to be grown under *in vitro*, due to all the requirements necessary to maintain viability. However, when *H. pylori* has a coccoid form major difficulties for cultivation (Sen et al., 2007). The cells in coccoid forms will not grow in the traditional bacteriological methods and conditions (Andersen and Rasmussen, 2009). So by detection of *H. pylori* from environmental samples, the first choice should be the molecular methods (Bunn et al., 2002; Sen et al., 2007). In the last 20 years has increased the number of studies aimed at the detection of *H. pylori* in water samples, which have supported the theory of aquatic transmission of this bacterium. It has been required of methods more and more sensitive and specific than conventional culture media methods, so that most researchers have opted for the molecular detection methods for nucleic acids, among them reaction polymerase chain (PCR), immunofluorescence and fluorescence in situ hybridization (FISH).

**CONCLUSION**

*H. pylori* is a bacterium that has had a significant impact in the clinic for the past 20 years, even though more than 50% of the general population could be infected with this bacterium, some people are asymptomatic carriers. However, when clinical manifestations are most evident in patients, they may trigger gastric cancer, so it must make detections in early stages of the disease to eradicate bacterium. Although still not been fully elucidated transmission routes, in recent years it has been proposed transmission of *H. pylori* through water by the viable non-culturable state of the bacteria, which keeps the feasibility with a slow metabolism to survive in adverse conditions as it is environmental stress, although it may be a form of latent infection. Furthermore, the gene *cdrA* plays an important role in the change of morphology of *H. pylori*, which previously was thought to be implicated in the loss of the ability to be cultivable. Despite the many published works need to know more about this bacteria primarily on their pathways and the role of coccoid forms in the infection through water.

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**REFERENCES**


