Detection of *Helicobacter pylori* by FISH and PCR techniques in drinking water treatment plants (DWTP) from Colombia

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INTRODUCTION

Helicobacter pylori is a Gram-negative microaerobic curved rod bacterium. This bacterium causes gastric diseases and peptic ulcers and plays an important role in gastric cancer and lymphoma[1,2]. It has been estimated that more than half of the human population is currently infected with *H. pylori.* In Colombia the prevalence of infection is the 70-80%[3]. *H. pylori* exists in a bacillary (rod or spiral) form in the natural habitat within the human stomach, but detrimental environmental circumstances have been observed to cause a switch from bacillary to both VBNC bacillary and coccoid forms. Given the viability variations of bacteria, it is a challenge to demonstrate its presence in water and, even more, its viability and infecting capacity[4,5]. Several methodological strategies, such as cultivation in selective media, immunofluorescence, fluorescence *in situ* hybridization detection (FISH), polymerase chain reaction (PCR) and quantitative PCR (qPCR)[6,7,8] have been used to demonstrate its presence and viability in water.

Despite the prevalence of *H. pylori* infection in humans is high in Colombia, no previous studies have been reported about the presence of this pathogen in drinking water treatment plants (DWTP) from this country, and its survival during the water treatment process. Therefore, the aim of this work was to determine the occurrence of *H. pylori* in influent and effluent from a three DWTP, in Bogotá, Colombia, FISH and PCR of the *vac*A gene.

MATERIAL AND METHODS

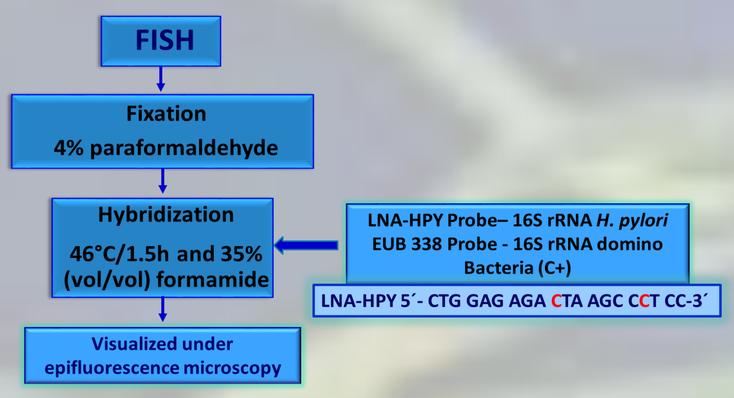

Figure 1. FISH protocol (Moreno *et al*., 2003)

Fifty-four samples, coming from the influent and effluent, were collected in three DWTP from Bogotá (Colombia), during july 2015 to august 2016. Portions of 500 mL of the influent samples were collected and centrifuged at 4,000 rpm for 20 min. Pellets of influent were resuspended in PBS buffer. For the effluent water samples, we used the "Moore hyssop" method (PAHO/WHO, 2010). Briefly, we kept the hyssop in contact with water for 72 hours. The hyssop was then transferred to Brucella Broth for 30 min, shacked at 500 rpm for elution and finally incubated at 37°C in microaerobic conditions (5% O_2, 11% CO_2, 85% N_2 and high humidity) for 24 hours. Then, both, influent and effluent samples were concentrated using Immunomagnetic Separation (IMS) and subsequently analyzed by FISH (Figure 1) and *H. pylori vac*A gene PCR (conditions see table N° 1).

Primers
VacF: 5´-GGC ACA CTG GAT TTG TGG CA- 3´
vacR: 5´-CGC TCG CTT GAT TGG ACA GA- 3´

Conditions	Cycles		T (°C)	Time
Denaturation	1		95	1 min
Amplification	33	Denaturation	94	1 min
		Annealing	57	1 min
		Extension	72	1 min
Final extension	1		72	1 min

Table 1. PCR conditions for amplified *vac*A gene of *H. pylori*

RESULTS AND DISCUSION

By FISH analysis, 14 out of 27 (51,8%) of DWTP influent and 10 out of 27 (37%) effluent samples were positive for the presence of *H. pylori* (Figure 2). *H. pylori vac*A gene was detected in 10 out of 27 (37%) influent samples and 11 out of 27 (40,7%) effluent samples (Figure 3). FISH technique yielded more positive results than PCR for *H. pylori* detection in the influent samples of the DWTP (Figure 3), similar results were obtained by Moreno *et al*. (2003). However, for effluent samples analysis, PCR technique has shown to be more sensible than FISH method.

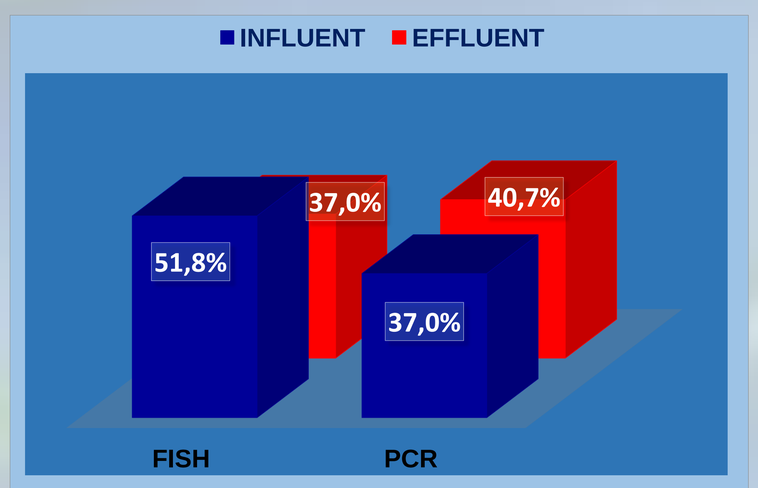

Figure 3. Detection of *H. pylori* in samples water by FISH and PCR.

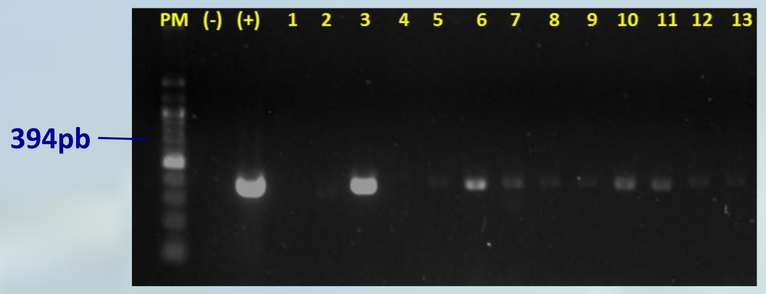

Figure 3. *H. pylori* PCR of water samples. *vac*A gene detection in water samples: Lane PM: MW bp 100; Lane (-): -control; Lane (+): +control NCTC 11637 *H. pylori*; Lanes 1-6: samples influent; Lanes 7-13: samples effluent.

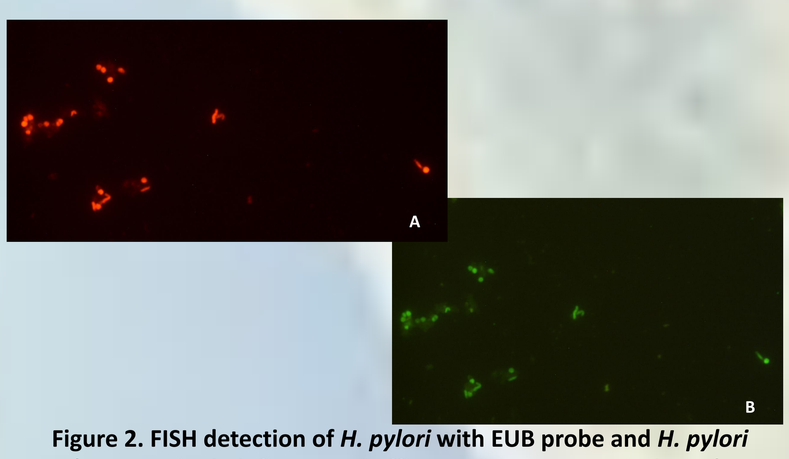

Figure 2. FISH detection of *H. pylori* with EUB probe and *H. pylori* LNA/rRNA probe in influent and effluent water. A. *H. pylori* LNA/rRNA probe. B. EUB 338 probe.

CONCLUSION

Our results demonstrate the presence of *H. pylori* in the influent and even in the final effluent of DWTPs from Colombia. Both, FISH and PCR methods, are rapid and specific techniques to identify this pathogen in drinking water samples. Moreover, by FISH analysis the different *H. pylori* morphologies were observed in the sample. According with other authors (Moreno *et al*., 2007; Santiago *et al*., 2015) this work has shown that *H. pylori* could survive to disinfection practices normally used in drinking water treatment. In addition, these findings contribute to elucidate the role of water in the persistence of *H. pylori* in environment and the risk that this poses to human health.

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