Presence of *Helicobacter pylori* into municipal water in common use

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**ABSTRACT**

*Helicobacter pylori* is a microaerophilic bacterium which causes chronic gastritis, duodenal ulcers, gastric ulcers and it plays an important role of cases of cancer gastric. Epidemiology and prevalence of *H. pylori* infection is different in developed and developing countries. It is higher in developing countries than in the developed countries. Different routes are described for the transmission of bacteria such as: fecal-oral route (bacteria have been isolated from human faeces); oral-oral route (DNA of the bacterium has been found in saliva and dental plaque) and gastro-oral route (through endoscopes and biopsy forceps). Over time it has also been considered water as a vehicle for the transmission of *H. pylori*. This bacterium has been detected in drinking water, well water, municipal water and treated wastewater in different countries. Therefore, the present study aimed to seek evidence the occurrence of *H. pylori* through detection of 16S rRNA gene of this bacterium in municipal water commonly used.

**Keywords:** *Helicobacter pylori*, Municipal water, 16S rRNA, PCR, Disease.

**INTRODUCTION**

*Helicobacter pylori* is a Gram negative bacterium microaerophilic which causes chronic gastritis in half the world's population and it is the etiological agent of 95% of duodenal ulcers, 70-80% of gastric ulcers (Brown, 2000; Hagymási and Tulassay, 2014). *H. pylori* plays an important role in 60-70% of cases of cancer gastric, which represents one of the most common cancers worldwide (Graham *et al*., 1991; Hagymási and Tulassay, 2014; Herrera and Parsonnet, 2009; Kim *et al*., 2011; Lee, 1994; Polk and Peek, 2010; Watar *et al*., 2014). Epidemiology and prevalence of *H. pylori* infection is different in developed and developing countries: it is higher in developing countries than in the developed countries (Brown, 2000; Cullen, 1993; Glynn *et al*., 2002;
Graham et al., 1991; Khalifa et al., 2010; Pounder and Ng, 1995). Epidemiological studies suggest fecal-oral route of infection as naturally, since the bacteria have been isolated from human faeces (Parsonnet et al., 1991; Pounder and Ng, 1995; Safaei et al., 2011; Thomas et al., 1992). The oral-oral route is also considered a transmission route, since DNA of the bacterium has been found in saliva and dental plaque (Allaker et al., 2002; Anand et al., 2014; Madinier et al., 1997; Valdez-González et al., 2014; Vincent, 1995). Gastro-oral route is also contemplated through endoscopes and biopsy forceps, circumstance of an increased risk of infection in endoscopy personnel, especially those who handle these instruments without gloves (Bahrami et al., 2013; Brown, 2000). It is also suggested that can be transmitted by water, since H. pylori can survive in this environment for several days. So H. pylori has been detected in drinking water, well water, municipal water and treated wastewater in different countries (Adams et al., 2003; Benson et al., 2004; Cellini et al., 2004; Engstrand, 2001; Gomes and De Martinis, 2004; Hegarty et al., 1999; Hulten et al., 1996; Hulten et al., 1998; Klein et al., 1991; Lu et al., 2002). The present study aimed to seek evidence the occurrence of H. pylori through detection of 16S rRNA gene of this bacterium in water commonly used at the municipality of Puebla.

MATERIALS AND METHODS

Collection of water samples

Water samples in common use in the municipality of Puebla were collected from the 4 distribution systems of water at Puebla city (México): San Rafael system (9 water samples), Loreto system (9 water samples), San Baltazar system (9 water samples) and La Constancia system (5 water samples), over a period of 12 months from August 2013 to December 2014. So number of water samples considered in this study was 32. For this, keys to the standpipes were cleaned before sampling and it was careful not to splash the sampling. Water was allowed to flow for two minutes prior to sample collection. Water samples were taken from midstream (Mazari-Hiriart et al., 2001). 3 samples of water in common use (900 mL) were collected from each monitored site. Each water sample was collected directly from the water supply coming from the municipal water supply network in bottles of 1000 mL wide-mouthed sterile polypropylene, being transported to laboratory within 2 hours and stored under refrigeration (4°C) until analysis. Then samples of water were filtered through 0.22 µm filter membrane (Millipore Co) to collect the microorganisms in them. The membranes containing microorganisms were stored at -20°C until analysis.

Genomic DNA

Genomic DNA was extracted according to methodology described by Ho et al., (1991). For this, each 0.22 µm filter membrane was washed with 1000 µL of TE buffer (10 mM Tris-Cl pH 7.5, 1 mM EDTA) in a 1.5-mL Eppendorf tube and it was added 30 µL of 10% sodium dodecyl sulfate and 3 µL (2 mg/100 µL) of proteinase K. Eppendorf tube was incubated at 37°C for 1 h. The mixtures were extracted with an equal volume of phenol-chloroform-isoamyl alcohol and centrifuged at 12,000 x g in a microcentrifuge for 3 min; the aqueous layer was transferred to a fresh Eppendorf tube. Two further extractions were performed with equal volumes of phenol-chloroform-isoamyl alcohol and chloroform-isoamyl alcohol. The DNA was precipitated with 0.7 volume of isopropanol at -20°C. The genomic DNA was pelleted by microcentrifugation at 13,000 x g for 5 min, washed with -20°C 70% (vol/vol) ethanol, desiccated for 30 min, and dissolved in 50 µL of molecular biology-grade water. The DNA was quantified spectrophotometry. Genomic extracted DNA was stored at -20°C.

PCR for detection of H. pylori

Amplification of the DNA template was carried out using primers Hp1, Hp2 and Hp3, previously described by Ho et al., (1991) and Mazari-Hiriart et al., (2001). Assay conditions were following. It was added 0.5 µL of each oligonucleotide primer (50 pmol/µL for each primer) in an Eppendorf tube, 1 µL of extracted DNA, 2.5 µL of 10X PCR buffer (500 mM KCl, 100 mM Tris-Cl, 15 mM MgCl2, pH 8.3), 1 µL of deoxynucleoside triphosphate mixture (final concentration, 1.25 mM each dATP, dCTP, dGTP, and dTTP). 0.3 µL of Taq DNA polymerase and molecular biology-grade distilled water were added to make a final reaction volume of 25 µL. For nested PCR, 30 cycles were used for each round of amplification. The temperature profile was as follows: 4 min at 94°C, 45 s at 94°C, 45 s at 60°C, 45 s at 72°C. The last cycle was identical, except that the 72°C extension period was increased to 7 min and the mixture was subsequently refrigerated at 4°C before analysis. Primers were used to amplify H. pylori by nested PCR with the following sequences: Hp1: 5'-CTG GAG AGC GTA GCC CCT CC-3', Hp2: 5'-ATT ACT GAC GCT GAT TGT GC-3', and Hp3: 5'-AGG ATG AAG GTT TAA GGA TT-3'.

Analysis of PCR products

Aliquots of each PCR product were separated by electrophoresis in a 1.5% (w/v) agarose gel (Ultra Pure, Invitrogen) with the MBI Fermentas (Amherst NY) 100 bp DNA Ladder Plus used as a size marker, in TAE buffer
(90 mM Tris-HCl, 90 mM acetic acid, 2 mM EDTA and stained in ethidium bromide at 0.06 µg/mL). Positive and negative controls were included in all assays to monitor specificity and laboratory contamination during the analyses. The specificity of the PCR assays has been previously reported by Ho et al. (1991). Assays on all samples were repeated in duplicate. Samples were interpreted as being positive for the presence of Helicobacter DNA if one or more of the assays produced a fragment comparable in size to that of the positive control DNA (H. pylori 26695).

RESULTS

Between August 2013 to December 2014 were examined 32 samples of water in common use in the municipality of Puebla from 4 distribution systems of water: San Rafael, Loreto, San Baltazar and La Constancia systems. 3 samples of water in common use (each 900 mL) were collected and filtered through 0.22 µm filter membrane to collect bacteria. The results shown that filtration method used allowed the total recovery of bacterial flora contained in water samples (Figure 1). Figure 1 shows sediment obtained after filtration of water. As can be seen the pellet was abundant and it presented in most water samples were analyzed. Then it was carrying out the extraction and quantification of DNA from each obtained sediments. Each membranes was washed with TE buffer containing sodium dodecyl sulfate and proteinase K. After incubation, DNA was extracted according to protocol using phenol-chloroform-isoamyl alcohol and ethanol. DNA average concentrations were obtained from sediment samples were: San Rafael system (22.42 µg/µL), indicating a DNA maximum concentration of 105 µg/µL (at Santa Rosa colony); Loreto system (18.96 µg/µL); San Baltazar system (409.72 µg/µL), indicating DNA maximum concentration of 982.5 µg/µL (at Coatepec colony), and La Constancia system (576.1 µg/µL), with a DNA maximum concentration of 990.6 µg/µL (at Xonacatepec colony). From DNA in analyzed sediments it can be concluded that samples of water in common use were positive for the presence of microorganisms (for example: bacteria) and that different concentrations of DNA should be in proportion to the amount of microorganisms in water. Thus it became clear that at least two colonies (among which were monitored) of Puebla city showed the highest microbial load and these were: Coatepec and Xonacatepec, showing DNA values around 1000 µg/µL. DNA purity of each the samples of analyzed water showed average values of 1.69 (by rate OD260nm / 280nm OD) (data not shown). Subsequently, it is proceeded to the detection of H. pylori using primers Hp1, Hp2 and Hp3, which specifically amplify the 16S rRNA gene. The amplification conditions for 16S rRNA gene detected by nested PCR were described in Materials and Methods. PCR analysis amplified an approximately 109 base pair DNA fragment (Figure 2 below). In this study, 16S rRNA gene of H. pylori was detected in samples of water in common use in the municipality of Puebla from 4 distribution systems: 14 of 32 samples of analyzed water were positive for H. pylori according to the methodology of nested PCR. This resulted novel because the DNA of H. pylori was found DNA purity of each the samples of analyzed water showed average values of 1.69 (by rate OD260nm / 280nm OD) (data not shown). Subsequently, it is proceeded to the detection of H. pylori using primers Hp1, Hp2 and Hp3, which specifically amplify the 16S rRNA gene. The amplification conditions for 16S rRNA gene detected by nested PCR were described in Materials and Methods. PCR analysis amplified an approximately 109 base pair DNA fragment (Figure 2 below). In this study, 16S rRNA gene of H. pylori was detected in samples of water in common use in the municipality of Puebla from 4 distribution systems: 14 of 32 samples of analyzed water were positive for H. pylori according to the methodology of nested PCR. This resulted novel because the DNA of H. pylori was found in 44% of samples tested. Some H. pylori PCR products isolated from water samples (San Baltazar system) are shown in Lanes 2 and 3 of Figure 2 below.

DISCUSSION

The transmission of H. pylori occurs by fecal-oral and oral-oral routes (Anand et al., 2014; Brown, 2000; graham
Figure 2. Some *H. pylori* PCR products isolated from water samples. PCR products were analyzed by gel electrophoresis and ethidium bromide staining. Lane 1, MBI Fermentans 100 pb DNA Ladder used as a size marker; Lane 2, water sample from Mayorazgo colony; Lane 3, water sample from Coatepec colony; Lane 4, water sample from Guadalupe colony; Lane 5, DNA amplified from *H. pylori* (NCTC 26695); Lane 6, negative control.
samples of common use in the municipality of Puebla. Likewise it was possible to carry out the extraction of genomic DNA from collected sediments. These two tests were an evidence of the presence of bacteria in the analyzed water samples. DNA was found from water samples and it was indicative of the presence of bacteria. In the present study, 14 water samples of 32 (44%) were found to be contaminated with *H. pylori* using nested PCR. There are reports which demonstrates the presence of *H. pylori* in drinking water from diverse cities using PCR because the culture from this sources of water results difficult (Adams et al., 2003; Bahrami et al., 2013; Benson et al., 2004; Bunn et al., 2002; Engström, 2001; Gomes et al., 2004; Hegarty et al., 1999; Hultén et al., 1996; Hultén et al., 1998; Klein et al., 1991; Lu et al., 2002; Mazari-Hiria et al., 2001; Watson et al., 2004). The presence of *H. pylori* in water samples indicates the poor microbiologic quality of the water used for human consumption being water a vehicle for the storage and dissemination of pathogenic bacteria to humans (Bahrami et al., 2013; Mazari-Hiria et al., 2001). The consumption of these waters increase the possibility of acquiring infectious gastrointestinal diseases and in the case of *H. pylori*, gastritis, peptic ulcer, development of gastric cancer, could be acquired especially in immune compromised persons (Allaker et al., 2002; Bahrami et al., 2013; Brown, 2000; Bunn et al., 2002; Gomes and De Martinis, 2004; Glynn et al., 2002; Graham et al., 1991; Kim et al., 2011; Klein et al., 1991; Mazari-Hiria et al., 2001; Testerman and Beyond, 2014; Watar et al., 2014).

**CONCLUSION**

The presence of *H. pylori* into water samples in common use in the municipality of Puebla suggests contamination because to an inadequate sanitation process of water. The presence of amplifiable *H. pylori* DNA from purified water adds weight to the view that this bacterium may be transmitted through contaminated water. So consumption of contaminated water would be a potential risk of *H. pylori* infection for the consumer. Further studies will be necessary to determine the presence of *H. pylori* in water in common use and provide further information of the potential risk of human infection with *H. pylori* via consumption of water with poor microbiological quality.

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