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Full Length Research Paper

Detection of *Helicobacter pylori* DNA in purified water for drinking

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***H. pylori* is a Gram negative bacterium microaerophilic that is associated to digestive human diseases such as gastritis, peptic ulcer, mucosa-associated lymphoid tissue lymphoma; this bacterium is considered a risk factor in the development of gastric cancer. Different vias have been proposed by transmission of *H. pylori*. *H. pylori* has been detected in well water, municipal water, treated wastewater and drinking water in developing countries. The present study shows an evidence of the occurrence of *H. pylori* through detection of DNA of this bacterium in purified water for drinking of Puebla city. In this work we apply nested PCR to the detection of *H. pylori* using primers that specifically amplify the gene 16S rRNA.**

Keywords: *Helicobacter pylori,* water*, 16S rRNA,* detection, drinking, PCR, nested.

INTRODUCTION

H. pylori is a Gram negative bacterium microaerophilic which is associated to different digestive human diseases such as gastritis, peptic ulcer, gastritis, mucosa-associated lymphoid tissue lymphoma (Brown, 2000; Dunn *et al*., 1997; Salama *et al*., 2013; Suzuki *et al*., 2012; Testerman and Beyond, 2014; Watar *et al*., 2014). *H. pylori* is considered a risk factor in the development of gastric cancer (Bahrami *et al,*, 2013; Dunn *et al*., 1997; Hagymási and Tulassay, 2014; Herrera and Parsonnet, 2009; Kim *et al*., 2011; Polk and Peek, 2010). The epidemiology of *H. pylori* infection appears to be different in developed and developing countries (Cullen *et al*., 1993; Graham *et al*., 1991; Khalifa *et al*., 2010; Mazari-Hiriart *et al*., 2001; Mitchell *et al*., 1992; Thomas *et al*., 1992). The prevalence of *H. pylori* is higher in developing countries than in the developed countries (Graham *et al*., 1991; Pounder and Ng, 1995). In developing countries, it has been demonstrated that *H. pylori* infection is acquire earlier in life. It is estimated that 70-90% of the population is infected with *H. pylori* in developing countries, while 25-50% of the population of developed countries is infected by this bacterium (Bahrami *et al*., 2013; Brown, 2000; Lee, 1994). For *H. pylori* different routes of transmission have been proposed, for example: person to person by faecal-oral, oral-tooral and oral-oral routes, gastro-oral route, contaminated foods (*H. pylori* has been detected from foods of animal origin, sea water, drinking

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water) (Allaker *et al*., 2002; Anand *et al*., 2014; Dore *et al*., 2001; Fujimura *et al*., 2002; Gomes and De Martinis, 2004; Madinier *et al*., 1997; Parsonnet *et al.,* 1999; Quaglia *et al*., 2008; Safaei *et al*., 2011; Thomas *et al*., 1992; Valdez-González *et al*., 2014). Also *H. pylori* has been detected in drinking water, well water, municipal water and treated wastewater in countries as Peru and Sweden (Adams *et al*., 2003; Benson *et al*., 2004; Bunn *et al*., 2002; Casasola-Rodríguez *et al*., 2013; Cellini *et al*., 2004; Engstrand, 2001; Glynn *et al*., 2002; 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 of the occurrence of *H. pylori* through detection of DNA of this bacterium in purified water for drinking of Puebla city. In this work we apply nested PCR to the detection of *H. pylori* using primers that specifically amplify the gene 16S rRNA.

MATERIALS AND METHODS

Collection of water samples and microorganism recovery

Water purified samples were colleted and examined randomly from different comertial establishments, traders dedicated to the purification of drinking water at Puebla city (México), over a period of 6 months from July to December 2014. The number of commercial establishments considered in this study was 20. For each comertial establishment 3 samples were collected in 1,000mL glass steril bottles containing 900 mL of purified water. Samples of purified water collected and transferred to laboratory within 2 hours. Then samples of purified water were filtered through 0.22 micrometer filter membrane (Millipore Co) to collect the microorganisms in them. The membranes contanining microorganims were stored at -20°C until analysis.

Preparation of genomic DNA

Bacterial DNA was extracted according to modified methodology described by Ho *et al*., (1991). Briefly, each membrane was washed with 1 mL 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 microliters of 10% sodium dodecyl sulfate and 3 microliters (2mg/100 microliters) of proteinase K. Then, 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 DNA was pelleted by microcentrifugation at 13,000 x g for 5 min, washed with -20°C 70% (V/V) ethanol, desiccated for 30 min, and dissolved in 50 microliters of molecular biology-grade water. The DNA was quantified spectrophotometry. Genomic extracted DNA was stored at -20°C.

Nested PCR conditions 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 microliters of each oligonucleotide primer (50 pmol/ microliter for each primer) in an Eppendorf tube, 1 microliters of extracted DNA, 2.5 microliters of 10X PCR buffer (500 mM KCl, 100 mM Tris-Cl, 15 mM $MgCl_2$, pH 8.3), 1 microliters of deoxynucleoside triphosphate mixture (final concentration, 1.25 mM each dATP, dCTP, dGTP, and dTTP). 0.3 microliters of Taq DNA polymerase and molecular biology-grade distilled water were added to make a final reaction volume of 25 microliters. 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 AGA CTA AGC 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% (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 microgram/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).

Figure 1. Aspect of the filter membrane after one water purified sample was filtered.

RESULTS

Between July and December 2014 were colleted and examined 20 water purified samples from traders dedicated to the purification of drinking water at Puebla city. 3 samples of purified water (each one 900 mL) were collected and filtered through 0.22 micrometer filter membrane to collect bacteria contained into water. The results shown that filtration method used allowed the total recovery of bacteria suspended into water samples. Colored sediments on filter membrane surfaces were obtained as shown in Figure 1. Figure 1 shows sediment obtained in major amount by filtering samples of purified water. An indirect way of demonstrating the presence of microorganisms in the obtained sediments after filtration of purified water, was carrying out the extraction and quantification of DNA accordig to Materials and Methods. So each membrane was washed with TE buffer containing sodium dodecyl sulfate and proteinase K. After incubation, DNA was extracted according to comun protocol using phenol-chloroform-isoamyl alcohol and ethanol. DNA concentrations determined from sediment samples of filtred water are shown in Table 1. As shown in Table 1, all samples of purified water were positive for the presence of sediment by filtration, which is indicative of the presence of microorganisms. These data are related to the presence of DNA in analyzed sediments. As shown in Table 1, all analysed water samples containing different concentrations of DNA from 22 to 475 ng/ microliter of DNA. DNA concentration is indicative of the presence of microorganisms in the analyzed water and it should be in proportion to the amount of microorganisms in it. DNA purity of each the samples of

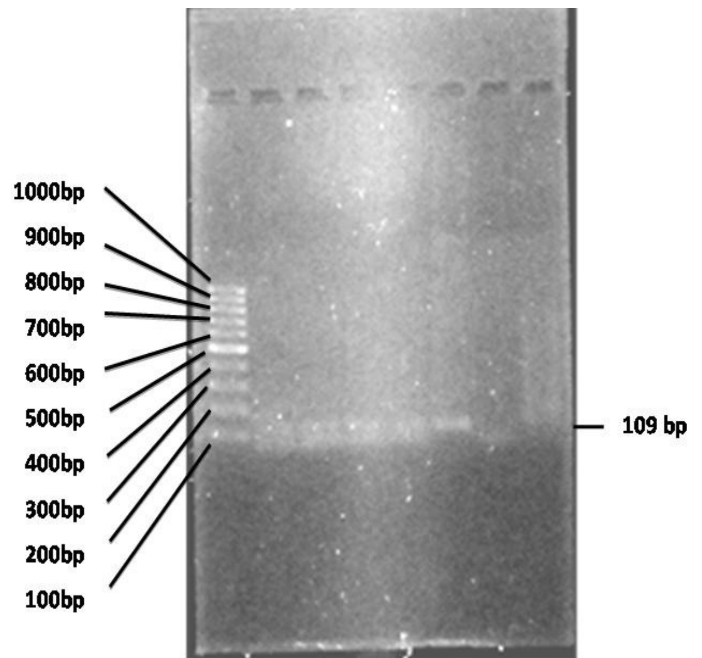

Figure 2. Some *H. pylori* PCR products isolated from water purified samples. PCR products were analysed by gel electrophoresis and ethidium bromide staining. Lane 1: MBI Fermentas 100 bp DNA Ladder used as a size marker; lane 2: sample 1; lane 3: sample 5; lane 4: sample 10; lane 5: sample 17; lane 6: DNA amplified from *H. pylori* (NCTC 26695); lane 7: sample 18; line 8: sample 20.

analyzed water showed average values of 1.5 (by rate OD260nm / 280nm) (data not shown). Subsequently, it is proceeded to the detection of *H. pylori* using primers Hp1, Hp2 and Hp3 that specifically amplify the *16S rRNA*

Table 1. Concentrations of DNA extracted from filtred water.

Purified water Samples	Obtained sediment	DNA concentration (ngram/microliter)[a]
1	+	80
2	+	197
3	+	137
4	+	125
5	+	27
6	+	40
7	+	45
8	+	32
9	+	475
10	+	30
11	+	35
12	+	30
13	+	25
14	+	22
15	+	32
16	+	42
17	+	35
18	+	187
19	+	37
20	+	35

[a]Average value of DNA concentration from 3 measurements.

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). In this study, *16S rRNA* gene of *H. pylori* was detected in most samples analyzed of purified water: 18 of 20 samples of analized water were positive, which resulted novel because the DNA of *H. pylori* was found in 90% of samples tested. Some *H. pylori* PCR products isolated from water purified samples are shown in Figure 2. Lanes 2, 3, 4, 5, 7 and 8 are examples of positive PCR; lane 6 represents positive control of *H. pylori*.

DISCUSION

As mentioned above, the transmission pathways of *H. pylori* to humans is not clear at all. It has been suggested that the transmission of *H. pylori* occurs by oral-oral and fecal-oral vias, including contaminated foods and water (Allaker *et al*., 2002; Anand *et al*., 2014; Brown, 2000; Dore *et al*., 2001; Engstrand, 2001; Fujimura *et al*., 2002; Gomes and De Martinis, 2004; Madinier *et al*., 1997; Parsonnet *et al.,* 1999; Quaglia *et al*., 2008; Thomas *et al*., 1992). Water seems to be an important vehicle to dissemination of bacteria. So *H. pylori* has been detected from major water reservoirs, for example: sea water, well water, drinking water (Adams *et al*., 2003; Bahrami *et al*., 2013; Benson *et al*., 2004; Bunn *et al*., 2002; Casasola-Rodríguez *et al*., 2013; Cellini *et al*., 2004; Engstrand, 2001; Glynn *et al*., 2002; Hegarty *et al*., 1999; Hulten *et al*., 1996; Hulten *et al*., 1998; Klein *et al*., 1991; Lu *et al*., 2002). In this context, several epidemiological studies have reported that infection with *H. pylori* is associated particularly to an inadequate water sanitation (Mazari-Hiriart *et al*., 2001). Some evidences have been provided to sustain the presence of *H. pylori* in aquatic environments, such as observation of coccoid forms in water samples and survival of *H. pylori* in water, presence of DNA in water samples, growth of *H. pylori* from water samples, formation of biofilm by *H. pylori* in water reservoirs (Azevedo *et al*., 2007; Bahrami *et al*., 2013; Bunn *et al*., 2002; Cellini *et al*., 2004; Hulten *et al*., 1996; Hulten *et al*., 1998; Vale and Vítor, 2010). It has been reported that *H. pylori* is able to remain viable in water storage systems possibly held in the biofilms and coccoid forms (Azevedo *et al*., 2007; Bunn *et al*., 2002; Flores-Encarnación *et al*., 2015; Percival *et al*., 2014). It has been reported that *H. pylori* can survive in aquatic environments (lakes, surface and ground water, wastewater and coastal marine environments, rivers, drinking water (Fernández-Delgado *et al*., 2008). *H. pylori* can survive in river water for several months adopting the coccoid morphotype (Azevedo *et al*., 2007; Chen, 2004; Percival and Suleman, 2014; Vincent, 1995). 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). In this study the positivity to presence of microorganisms into analyzed samples of purified water were determinated. At first glance it was observed abundant sediment recovered from water filtered samples (Figure 1). 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 microorganisms present in the water samples. DNA concentrations were an indicative of the presence of microorganisms in the analyzed water. In the present study, only two water samples (10%) were not found to be contaminated with *H. pylori* using nested PCR (Table 1). *H. pylori* was found in 90% of water analized samples. There are several papers 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 (Benson *et al*., 2004; Bunn *et al*., 2002; Gomes *et al*., 2004; Hulten *et al*., 1996; Hulten *et al*., 1998; Klein *et al*., 1991; Lu *et al*., 2002; Mazari-Hiriart *et al*., 2001; Smith *et al*., 2004; Watson *et al*., 2004). PCR has been used by many authors as a methodology in detection of *H. pylori* due to its valuable high sensibility and the variety of primers reported in literature (Ho *et al*., 1991; Hulten *et al*., 1996; Lage *et al*., 1995; Mazari-Hiriart *et al*., 2001).

The presence of *H. pylori* in water purified samples indicates the poor microbiologic quality of the water used for human consumption (Mazari-Hiriart *et al*., 2001). In this study the isolation of bacteria in water analyzed samples was not performed, however previous studies by other authors reported the presence of coliforms and mesophilic aerobic bacteria in the water expended in some places at Puebla city (Cabrera-Maldonado *et al*., 2009). It indicates the disease potential of the water to be a vehicle for the storage and dissemination of pathogenic bacteria to humans (Bahrami *et al*., 2013). The use of these purified waters for drinking 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 (Brown, 2000; Dunn *et al*., 1997; Salama *et al*., 2013; Suzuki *et al*., 2012; Testerman and Beyond, 2014; Watar *et al*., 2014). Use of these purified waters represents a potential risk to human health. According to the results of this study, purified water for drinking (distributed for some comertial establishments dedicated to the purification of drinking water at Puebla) are a risk factor to health and a potential vehicle for transmission of *H. pylori* to the population consuming this water.

CONCLUSION

The presence of *H. pylori* into water purified samples suggests water contamination because to an inadequate sanitation process to purify water. The presence of amplifiable *H. pylori* DNA from purified water adds weight to the view that this bacterium may be transmitted through contaminated drinking water. The consumption of contaminated drinking 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 purified water and provide further information of the potential risk of human infection with *H. pylori* via consumption of purified water with poor microbiological quality.

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REFERENCES

Adams BL, Bates TC, Oliver JD (2003). Survival of *Helicobacter pylori* in a natural freshwater environment. Appl. Environ. Microbiol. 69:7462-7466.

Allaker RP, Young KA, Hardie JM, Domizio P, Meadows NJ (2002). Prevalence of *Helicobacter pylori* at oral and gastrointestinal sites in children: evidence for possible oral-tooral transmission. J. Med. Microbiol. 51: 312-317.

Anand PS, Kamath KP, Anil S (2014). Role of dental plaque, saliva and periodontal disease in *Helicobacter pylori* infection. World J. Gastroenterol. 20:5639-5653.

Azevedo NF, Almeida C, Cerqueira L, Dias S, Keevil CW, Vieira MJ (2007). Coccoid form of *Helicobacter pylori* as a morphological manifestationof cell adaptation to the environment. Appl. Environ. 73:3423-3427.

Bahrami AR, Rahimi E and Safaei HG (2013). Detection of *Helicobacter pylori* in city water, dental units' Water and bottled mineral water in Isfahan, Iran. The Scient. World J. 2013:1-5.

Benson JA, Fode-Vaughan KA and Collins MLP (2004). Detection of *Helicobacter pylori* in water by direct PCR. Lett. Appl. Microbiol. 39:221-225.

Bode G, Mauch F, Malfertheiner P (1993). The cocoid forms of *Helicobacter pylori*. Criteria for their viability. Epidem. Infect. 111:483-490.

Brown LM (2000). *Helicobacter pylori*: epidemiology and routes of transmission. Epidem. Rev. 22:283-297.

Bunn JEG, MacKay WG, Thomas JE, Reid DC, Weaver LT (2002). Detection of *Helicobacter pylori* DNA in drinking water biofilms: implications for transmission in early life. Let. Appl. Microbiol. 34:450-454.

Cabrera-Maldonado C, León-Tello G, Calzada-Martínez JA, Flores-Encarnación M, Bonilla y Fernández N (2009). Control sanitario del agua purificada de venta en las "llenadoras" ¿contaminada o apta para el consumo humano?. En: Tornero CMA, Bonilla y FN, Cabrera MC, Velasco H Ma de los A (Coordinadores). Química y sustentabilidad ambiental. 1ª Edición. México. Benemérita Universidad Autónoma de Puebla. pp. 1-16.

Casasola-Rodríguez B, Orta de Velásquez MT, Luqueño-Martínez VG, Monje-Ramírez I (2013). Quantification of *Helicobacter pylori* in the viable but nonculturable state by quantitative PCR in water disinfected with ozone. Water Sci. and Technol. 68:2468-2472.

Cellini L, Del Vecchio A, Di Candia M, Di Campli E, Favaro M, Donelli G (2004). Detection of free and plankton associated *Helicobacter pylori* in seawater. J. Appl. Microbiol. 97:285-292.

Chen TS (2004). Is the coccoid form of *Helicobacter pylori* viable and transmissible?. J. Chin. Med. Assoc. 67:547-548.

Cullen DJ, Collins BJ, Christiansen KJ, Epis J, Warren JR, Surveyor I, Cullen KJ (1993). When is *Helicobacter pylori* infection acquired?. Gut. 34:1681-1682.

Dore MP, Sepulveda AR and El-Zimaty H (2001). Isolation of *Helicobacter pylori* from milk sheep-implications for transmission to humans. Am. J. Gastroenterol. 96:1396-1401.

Dunn BE, Cohen H, Blaser MJ (1997). Helicobacter pylori. Clin. Microbiol. Rev. 10:720-741.

Engstrand L (2001). *Helicobacter* in water and waterborne routes of transmission. J. Appl. Microbiol. 90:80S–84S.

Fernández-Delgado M, Contreras M, García-Amado MA, Michelangeli FY, Suárez P (2008). Evidencias de la transmisión acuática de *Helicobacter pylori*. Interciencia. 33:412-417.

Flores-Encarnación M, Nava-Nolazco RM, Aguilar-Gutiérrez GR, González-Gutiérrez JY, Herrera-Romero AU, Cabrera-Maldonado C (2015). The coccoid forms of *Helicobacter pylori*: A permanence mechanism. Basic Res. J. Med. Clin. Sci. 4:50-54.

Fujimura S, Kawamura T, Kato S, Tateno H, Watanabe A (2002). Detection of *Helicobacter pylori* in cow's milk. Let. Appl. Microbiol. 35:504-507.

Glynn MK, Friedman CR, Gold BD, Khanna B, Hutwagner L, Lihoshi N, Revollo C, Quick R (2002). Seroincidence of *Helicobacter pylori* infection in a cohort of rural Bolivian children: acquisition and analysis of possible risk factors. Clin. Infect. Dis. 35:1059-1065.

Gomes BC, De Martinis ECP (2004). The significance of *Helicobacter pylori* in water, food and environmental samples. Food Control. 15:397-403.

Graham DY, Malaty HM, Evans DG, Evans DJ, Klein PD, Adam E (1991). Epidemiology of *Helicobacter pylori* in an asymptomatic population of the United States. Gastroenterol. 100:1495-1501.

Hagymási K, Tulassay Z (2014). *Helicobacter pylori* infection: new pathogenetic and clinical aspects. World J. Gastroenterol. 20:6386-6399.

Hegarty JP, Dowd MT, Baker KH (1999). Occurrence of *Helicobacter pylori* in surface water in the United States. J. Appl. Microbiol. 87:697-701.

Herrera V, Parsonnet J (2009). *Helicobacter pylori* and gastric adenocarcinoma. Clin. Microbiol. Infect. 15:971-976.

Ho S, Hoyle J, Lewis F, Secker D, Cross D, Mapstone NP, Taylor GR (1991). Direct polymerase chain reaction test for detection of *Helicobacter pylori* in humans and animals. J. Clin. Microbiol. 29:2543-2549.

Hulten K, Enroth H, Nyström T, Engstrand L (1998). Presence of *Helicobacter pylori* species DNA in Swedish water. J. Appl. Microbiol. 85:282-286.

Hulten K, Han SW, Enroth H, Klein PD, Opekun AR, Gilman RH, Evans DG, Engstrand L, Graham DY, El-Zaatari FA (1996). *Helicobacter pylori* in the drinking water in Peru. Gastroenterol. 110:1031-1035.

Khalifa MM, Sharaf RR, Aziz RK (2010). *Helicobacter pylori*: a poor man's gut pathogen?. Gut Pathog. 2:2-14.

Kim SS, Ruiz VE, Carroll JD, Moss SF (2011). *Helicobacter pylori* in the pathogenesis of gastric cancer and gastric lymphoma. Cancer Let. 305:228-238.

Klein PD, Graham DY, Gaillour A, Opekun AR, Smith EO (1991). Water source as a risk factor for *Helicobacter pylori* in Peruvian children. Lancet. 337:1503-1506.

Lage AP, Godfroid E, Fauconnier A, Burette A, Butzler JP, Bollen A, Glupczynski Y (1995). Diagnosis of *Helicobacter pylori* infection by PCR: comparison with other invasive techniques and detection of *cagA* gene in gastric biopsy specimens. J. Clin. Microbiol. 33:2752-2756.

Lee A (1994). The microbiology and epidemiology of *Helicobacter pylori* infection. Scand. J. Gastroenterol. 29: 2-6.

Lu Y, Redlinger TE, Avitia R, Galindo A, Goodman K (2002). Isolation and genotyping of *Helicobacter pylori* from untreated municipal wastewater. Appl. Environ. Microbiol. 68:1436-1439.

Madinier IM, Fosse TM, Montiel RA (1997). Oral carriage of *Helicobacter pylori*: a review. J. Periodontol. 68:2-6.

Mazari-Hiriart M, López-Vidal Y, Castillo-Rojas G, Ponce de León S, Cravioto A (2001). *Helicobacter pylori* and other enteric bacteria in freshwater environments in Mexico city. Arch. Med. Res. 32: 458-467.

Mitchell HM, Li YY, Hu PJ, Liu Q, Chen M, Du GG, Wang ZJ, Lee A, Hazell SL (1992). Epidemiology of *Helicobacter pylori* in Southern China: identification of early childhood as the critical period for acquisition. J. Infect. Dis. 166:149-153.

Parsonnet J, Shmuely H, Haggerty T (1999). Fecal and oral shedding of *Helicobacter pylori* from healthy infected adults. J. Am. Med. Assoc. 23:2240-2245.

Percival SL, Suleman L (2014). Biofilms and *Helicobacter pylori*: dissemination and persistence within the environment and host. World J. Gast. Pathophysiol. 5:122-132.

Polk DB, Peek RM (2010). *Helicobacter pylori*: gastric cancer and beyond. Nat. Rev. Cancer. 10:403-414.

Pounder RE, Ng D (1995). The prevalence of *Helicobacter pylori* infection in different countries. Aliment. Pharmacol. Therap. 9:33-39.

Quaglia NC, Dambrosio A, Normanno G, Parisi A, Patrono R, Ranieri G, Rella A, Celano GV (2008). High occurrence of *Helicobacter pylori* in raw goat, sheep and cow milk inferred by glmM gene: a risk of food-borne infection?. " Intern. J. Food Microbiol. 124:43-47.

Safaei HG, Rahimi E, Zandi A, Rashidipour A (2011). *Helicobacter pylori* as a zoonotic infection: the detection of *H. pylori* antigens in the milk and faeces of cows. J. Res. Med. Sci. 16:184-187.

Salama NR, Hartung ML, Müller A (2013). Life in the human stomach: persistence strategies of the bacterial pathogen *Helicobacter pylori*. Nature Rev. Microbiol. 11:385-399.

Smith SI, Oyedeji KS, Arigbabu AO, Cantet F, Megraud F, Ojo OO, Uwaifo AO, Otegbayo JA, Ola SO, Coker AO (2004). Comparison of three PCR methods for detection of *Helicobacter pylori* DNA and detection of *cagA* gene in gastric biopsy specimens. World J. Gastroenterol. 10:1958-1960.

Suzuki R,Shiota S, Yamaoka Y (2012). Molecular epidemiology, population genetics, and pathogenic role of *Helicobacter pylori*. Infect. Gen. and Evol. 12: 203-213.

Testerman TL, Beyond MJ (2014). The stomach: An updated view of *Helicobacter pylori* pathogenesis, diagnosis, and treatment. World J. Gastroenterol. 20:12781-12808.

Thomas JE, Gibson G, Darboe M, Dale A, Weaver LT (1992). Isolation of *Helicobacter pylori* from human faeces. Lancet. 340:1194-1195.

Valdez-González JA, Mares-Moreno PC, Kowolik MJ, Vargas-Villareal J, González-Salazar F, De la Garza-Ramos MA (2014). Detection of *Helicobacter pylori* in dental plaque of Mexican children by real-time PCR. Health. 6:231-235.

Vale FF, Vítor JMB (2010). Transmission pathway of *Helicobacter pylori*: does food play a role in rural and urban areas?. Inter. J. Food Microbiol. 138:1-12.

Vincent P (1995). Transmission and acquisition of *Helicobacter pylori* infection: evidences and hypothesis. Biom. Pharmac. 49:11-18.

Watar J, Chen N, Amenta PS, Fukui H, Oshima T, Tomita T, Miwa H, Lim KJ, Das KM (2014). *Helicobacter pylori* associated chronic gastritis, clinical syndromes, precancerous lesions, and pathogenesis of gastric cancer development. World J. Gastroenterol. 20:5461-5473.

Watson CL, Owen RJ, Said B, Lai S, Lee JV, Surman-Lee S, Nichols G (2004). Detection of *Helicobacter pylori* by PCR but not culture in water and biofilm samples from drinking water distribution systems in England. J. Appl. Microbiol. 97:690-698.