



Isolation and identification of *Helicobacter pylori* by PCR from drinking water supplies in Erbil city

Zainab M. Alzubaidy

Diyala University/ collage science

zalzubaidii@yahoo.com

Abstract

Out of the 200 samples of drinking water, which were collected from different source in Erbil city, from the period November 2014 until April 2015 . In this study, 36 isolates of *Helicobacter pylori* were isolated from samples as well as from biofilms, this study directed for the isolation of *Helicobacter pylori* by culturing in different media and identified by biochemical tests and PCR technique, in order to evaluate the potential role of drinking water in transmission of *H. pylori*. Water samples cultured on Brain heart infusion agar with supplement, Colombia agar base with supplement and Brucella agar; the results showed that The total number and percentage of the isolates of *H. pylori* from all tested water samples were 36 (18%), and these distributed as follow: 12 (24%), 11(22%), 8(16%) and 5(10%) from biofilms, reservoir, groundwater and tap water respectively. All *H. pylori* isolates were identified by using cultural , morphological , biochemical characteristics, from all(36) of isolates:30(83%) gave positive reaction for urease indicates that the isolates had the ability to produce the urease as well as 24(67%) of the total isolates; oxidase and catalase gave positive reaction while 17(61%) of the total isolates are motile. 35(97%) of the total samples confirmed by using primers for identification of *H. pylori* based on 16SrRNA this was the first report on using 16SrRNA amplification and confirmation of *H. pylori* isolates from water samples in Iraq. The ureA genotype was expected to be present in nearly all 34(94%) *Helicobacter* positive isolates. The PCR assay employed in this work specifically targets a region of the *ureC* (*glmM*) gene which has been shown to be unique and essential for the growth of *H. pylori* and the results appeared that 28(78%) of the total isolates have this gene This study was succeeding to detect the *ureA* and C gene in most of the isolates which was already confirmed by 16SrRNA.

Key word: *H. pylori*, PCR, *Ure A* and *ure C* gene

Introduction

Helicobacter pylori colonizes the gastric mucosa of approximately half the world population, (Vyse et al. 2002). Most infected individuals are asymptomatic but in ca 10–20% of carriers chronic infection is associated with the development of gastric diseases ranging from ulcer to adenocarcinoma (Suerbaum and Michetti 2002). Infection is usually acquired in childhood and thought to be primarily spread from person to person via the faecal–oral route (Brown 2000; Go 2002). Nevertheless, the precise route and mode of transmission remain unproven and no reservoir of the organism, outside the human stomach, has been so far identified although *H. pylori*-like organisms can cause natural infections in some nonhuman primates (Suerbaum and Michetti 2002). The genus *Helicobacter* has expanded considerably over the past decade with the identification of at least 24 other species which are found in the stomach or lower bowel of various nonhuman primates, dogs, cats, rodents and birds as well as vegetables(yahaghi et al.,2014), prompted researchers to look at environmental sources as



vectors to humans. Previous studies suggested that *H. pylori* is present in groundwater, surface water, and other drinking water (Mazari *et al.*, 2001). Implying that there is a waterborne route of transmission to humans. When hygienic conditions are poor, household contamination of treated water cannot be ruled out, *H. pylori* is easily controlled by chlorination which the principal disinfection agent of water (Johnson *et al.*, 1997). Poor hygienic practices, absence of a household bath, non-hygienic drinking water and absence of a sewage disposal facility may lead to recontamination of drinking water that in this way may serve as a vehicle of transmission (Watson *et al.*, 2004). Even in treated water, the survival of *H. pylori* is possible, at least for short periods of time. Recently, a study reports that it was possible to culture a reference strain after five minutes of inoculation in chlorinated water (0.96 mg/l), and viable cell coccoid forms was higher than spiral forms after 40 sec. of chlorine exposure amplification of the specific *H. pylori* 16S rRNA gene was possible (Moreno *et al.*, 2007). *H. pylori* is easily controlled by chlorination which the principal disinfection agent of water (Lee, 1994; Johnson *et al.*, 1997), but recontamination of treated water is a widespread problem.

H. pylori DNA has been identified in several water sources using various gene targets. Drinking, river, sea, ground and wastewater have provided positive results by PCR analysis (Cellini *et al.*, 2004; Queralt *et al.*, 2005). However, *H. pylori* appears to be more resistant to chlorine and ozonation than *E. coli*, and same resistant to monochlorine. Traditional indicator organisms may fail to protect the consumer from exposure to *H. pylori* (Baker *et al.*, 2002; Moreno *et al.*, 2007). Regarding spring water, the results for presence of *H. pylori* DNA were also negative (Queralt *et al.*, 2005). Likewise, upstream river water samples were negative (Fujimura *et al.*, 2008), these results suggest that faecal is responsible for the contamination of water suppliers.

For the reasons above the study aimed to evaluated drinking water samples, biofilms, reservoirs and water storage tanks in Erbil city fed by different water distribution systems were tested for the presence of *Helicobacter pylori* by culture and molecular detection methods in order to provide an understanding of their potential role in transmission of *H. pylori*, the accuracy of results varies according to the sensitivities and specificities of the detection methods employed. Objectives of this research were (i) to isolate *H. pylori* from drinking water by utilizing culturing techniques, (ii) to identify *H. pylori* by classical microbiological tests and PCR (16S rRNA), (iii) to genotype detection of gene (*ureA*) and *UreC* gene in *H. pylori* isolates.

Material and Methods

Samples were collected from different geographical locations in Erbil city, properties served by three different water distribution systems: reservoir, tap water, groundwater and biofilms. Fifty sample representative of the incoming supply water was collected from each area. Samples were collected in sterile bottles, also fifty biofilm samples from the header tank, water closet cistern (where possible, an area of 50 cm² was swabbed using a sterile cotton swaps), and the showerhead, were collected using a sterile swab. Deposit samples from the domestic water supply were collected by securing a 25-μm pore size sterile nylon tap net and flushing for 2 min following tap surface decontamination with 10% hypochlorite solution. Samples were transported to the laboratory within 24 h at ambient temperature.

The water samples were filtered through a 0.25-μm pore size nylon membrane filters (Nyflo; Pall Life Sciences, Poole, UK) and precipitates were by gently removed from the membrane with a disposable cell scraper and re suspended in 25 ml sterile phosphate-buffered saline (PBS). Swabs taken from biofilm were suspended in 10 ml PBS was used in this study.



Culturing of samples

Subsamples (25 µl) of each final concentrate of all 200 water and biofilm samples were cultured on three culture media: the first, Brain Heart Infusion Agar plus 7% HRBC plus antibiotic supplement contains (Vancomycin 5 mg, Trimethoprim lactate 25 mg, Polymyxin B 0.05 mg.), the second media was, Columbia Agar plus 7% HRBC plus growth supplement contains: a. Sodium pyruvate 0.125 g, b. Sodium metabisulphate 0.125 g and c. Ferrous sulphate 0.125 g. and Brucella agar. Small smooth colonies resembling *H. pylori* were sub-cultured, and were subsequently identified by Gram-stain and tests for rapid urease, cytochrome b oxidase and catalase activity (Owen 1998). The plates were incubated under microaerophilic conditions at 37°C, using an anaerobic jar and anaerobic Gas Pak kit without catalyst to generate a microaerophilic environment. Plates were inspected first on day 3 and then after one-day interval for a total of ten days. GasPak was changed whenever the jar was opened. *H. pylori* were identified by the following criteria: 1. Colonial morphology, Motility test, Catalase, oxidase and Urease Tests.

Identification of the isolates by PCR:

Genomic DNA Extraction and PCR amplifications

The subjected (36) isolates to the Genomic DNA Extraction was according to the kit manufacturing company information procedures was done as follows; 0.1ml. of activated bacteria in the Luria Brittani broth transferred to sterilized test tube and centrifuged at 8000 rpm for (1) min. added 180 µl of Lysis Buffer (T1) and 25ml Proteinase K solution Vortex to mix, Incubated at 56°C until complete lysis was obtained (at least 1-3h). Added 200µl Buffer B3, vortex vigorously and incubate at 70°C for 10 min. Vortex was done briefly, Dissolved Wash Buffer (B5) 7 µl in 210 µl ethanol (96-100%) Added 500 µl Buffer BW, centrifuge for 1 min at 11,000xg. Discard flow-through and place the column back into the Collection Tube. *2nd wash added 600 µl Buffer B5 to the column and centrifuge for 1 min at 11,000xg, Discard flow-through and place the column back into the Collection Tube. Centrifuged the column for 1 min 11,000 x g. residual ethanol is removed during this step. Placed the NucleoSpin Tissue Column into a 1.5 µl microcentrifuge tube and added 100µl prewarmed Buffer BE (70°C). Incubate at room temperature for 1 min, Centrifuged 1 min at 11,000xg (MACHERTY NaGeL). Stored the DNA in the freezing at -20°C (Boom *et al.* 1990).

The primers sequencing which used for the identification were appeared in table 1

**Table 1 primers sequencing design**

Target gene & Primer name	Nucleotide sequence	Amplicon Size (bp)	Refrence
<i>16 SrRNA</i> <i>16 SrRNAF</i> <i>16 SrRNAR</i>	5GCAATCAGCGTCAGTAATGTTC3 5GCTAAGAGATCAGCCTATGTCC3	500 BP	Falsafi <i>et al</i> ., 2009
<i>ureA</i> <i>ureAF</i> <i>ureAR</i>	5 GCC AAT GGT AAA GCCTTA GTT3 5CTC CTT AAT TGTTTT TAC 3	411Bp	Notarnicola, <i>et al.</i> ,1996
<i>Hp /UreC</i> <i>HpF</i> <i>HpR</i>	GAATAAGCTTTTAGGGGTGTTAGGGG AAGCTTACTTTCTAACACTAACGCGC	294Bp	Ahmad <i>et al.</i> , 2013

Detection of *16srRNA* gene and *Urea* gene and *Hp/ Urec* gene

A sterile amplification tube was used; the following orders were mixed at final volume 20 μ l for each primer (Bioneer kit), As shown the PCR reaction mixture (master mix 20 μ l, forward primer 1 μ l, reverse primer 1 μ l, nuclease free water 16 μ l and template DNA 2 μ l. The master mix composed from the following dNTP, 250 μ M each: (dATP, dCTP, dGTP, dTTP; Taq DNA Polymerase, 1U/ml; Tris-HCl (pH 9.0), 10 μ M; KCl, 30mM; MgCl₂, 1.5mM. DNA amplification was carried out for 35 cycles in 20ml total reaction mixture as follows: Temperature cycling by using *16S rRna*, denaturation at 94c° for 1 min., annealing at 55c° for 30 s extension at 72c° for 2 min., final extension at 72c° for 7min, hold at 4c°.

Temperature cycling of *UreaA* gene : Denaturation at 94c° for 1 min. annealing at 45c° for 1min, extension at 72c° for 2 min, final extension at 72c° for 7min, hold at 4c°

Temperature cycling of *HP ureC* gene : Denaturation at 94c° for 1 min. Annealing at 55c° for 1min. extension at 72c° for 2 min final extension at 72c° for 7min, hold at 4c°. Aliquots of each PCR product were separated by electrophoresis in a 1% (w/v) agarose gel in TBE buffer (90 mm Tris-HCl, 90 mm boric acid, 2.0 mm EDTA) and stained in ethidium bromide at 0.5 μ g ml⁻¹. 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 (Al-Sulami *et al.*, 2012).

Results and Discussion

In this study 200 samples from different source of drinking water and biofilms (smooth layer) in Erbil governorate were collected, the samples examined for the presences of *H. pylori* by using special selective media for this bacteria as appeared in table 1, the collected samples included 50 samples from each one. The total number and percentage of a doubted isolates of *H. pylori* from all tested water samples were 36 (18%), and these distributed as follow: 12 (24%), 11(22%), 8(16%) and 5(10%) from biofilms (smooth Layer), reservoir,



groundwater and tap water respectively. As shown different source of water contaminated with a suspected isolates of *H. pylori* from total samples as appeared positive and contaminated with this bacteria, this result agree with (Al-Sulami *et al.*, 2010; Al-Sulami *et al.*, 2012) in basrah Iraq studies indicated that *H. pylori* found in tap water and samples from tankers supplying, Reverse osmosis (RO), the water pipes suffer from breaks and corrosion at many sites, which cause drinking water contamination by sewage infiltration and rain leakage into the system. Sartory and Holmes, (1997) needed to establish which factors affect the ability of *H. pylori* to survive in distribution systems and be isolated from drinking water, such as the bacterial strains, density of bacteria in the distribution systems, type of water pipe materials, efficiency of disinfection process and the techniques and materials used for culture, also Degnan *et al.*, (2003) in America studies suggested that *H. pylori* was present in ground water, surface water, and other drinking water as well as Mazari *et al.*, (2001); Yingzhi *et al.*, (2002), they found positive result for isolation *H. pylori* from drinking water.

Table 1: Sources of tested Water Samples

Sample sources	No. of Samples	positive* Samples %
Reservoir	50	11 (22%)
Tap Water	50	5 (10%)
Groundwater	50	8 (16%)
Biofilm (smooth layer)	50	12 (24%)
Total	200	36 (18%)

positive samples with suspected *H. pylori

In addition to the previous mentioned, samples as a contaminated with suspected *H. pylori* the characters of isolated bacteria were determined to all positive samples after being stained with gram stain the cells shows Gram negative spiral to coccobacilli, rod shaped, and the colonies appear small to middle in size, rounded, and creamy in color, raised and smooth, the morphology characters of isolated *H. pylori* from different sources appeared in the results were comparative to that found by Giao *et al.*, (2010) found that morphology of the majority of cells was spiral shaped and did change during the duration of the experiment under any of the conditions tested, as well as Eshraghian (2014), had been demonstrated *in vitro* that *H. pylori* cells can transform from a cultivatable spiral-shaped form to a non-cultivatable coccoid form, in which the recovery of the bacterium is very difficult by routine culture methods. on the other side, Ali khan *et al.*, (2007) and Yahaghi *et al.*, (2014) Iranians studies showed that *H. pylori* growth in spiral and coccoid forms in the human gut, also Azevedo *et al.*, (2007) and Ranjbar *et al.*, (2016) isolated *H. pylori* with different shapes changes from the normal spiral-shaped bacillary form into the coccoid form when it is exposed to water or to other adverse conditions.

Identification of isolated bacteria by Biochemical characterization

The isolates examined to Urease production, which was the key for more confirmation a positive rate as shown in table3, from all (36) of the isolates 30 (83%) gave positive reaction for urease the color changing to dark pink indicates that the isolates had the ability to produce the urease; were 24 (67%) of the total isolates; oxidase and catalase



gave positive reaction while 17(61%) of the total isolates are motile. the results were comparative to that found by Virginia *et al.*, (2014) as shown Urease is one of the key enzymes in *H. pylori* pathogenesis. also urease is strongly immunogenic and chemotaxic for phagocytes (Al-Sulami *et al.*,2010), while Amieva and omar,(2008) indicated that catalase, oxidase and urease positive, three aspect of the bacterium were essential to colonization, including urease, motility and adherence, infection depends up on a combination of these bacterial. This study succeeded in isolating *Helicobacter pylori* from water samples. Few studies were done on the presences *H. pylori* in water and drinking water.

Table 3: Biochemical characters of isolated *H. pylori*

Sources	Total Samples	No. of Isolates	Urease %	Oxidase %	Catalase%	Motility%
Reservoir	50	11	10(91%)	8(73%)	8(73%)	5(45%)
Tap water	50	5	3(60%)	2(40%)	2(40%)	1(20%)
Groundwater	50	8	6(75%)	6(75%)	6(75%)	5(63%)
Biofilm	50	12	11(92%)	8(67%)	8(67%)	6(50%)
Total	200	36	30(83%)	24(67%)	24(67%)	17(61%)

Amplification of 16SrRNA and ureA Gene and ureC / HP from gene *H. pylori*

Detection of the bacterial gene of *H. pylori* general primer 16SrRNA and virulence *ureA*, *ureC* /Hp gene as shown in the figures (1 , 2 ,3) and table 4 shows the confirmation of *H. pylori* bacteria by Polymerase chain reaction (PCR) methods which used to identified *H. pylori* as its 16SrRNA gene unmistakably differentiated the *Helicobacter* genus from the closely related *Campylobacter* genus and other *Helicobacter* species (Liu *et al.*,2008),the identification of *H. pylori* which isolated from water samples by PCR has been reported 35(97%) of the total samples(36) as appeared in table 4. Studies in several countries (Mazari-Hiriart *et al.*,2001; Hegarty *et al.*,1999) demonstrated the presence of *H. pylori* in surface water.

Identification of the isolates confirmed by using primers specifically designed for the identification of *H. pylori* based on 16SrRNA gene, and *ureA* gene, based primers gave bands on agarose gel corresponding to a 500 base pair and 411bp respectively, when compared to the molecular ladder, this was the first report on using 16SrRNA amplification and confirmation of *H. pylori* isolates from water samples in Iraq. The ureaA genotype was expected to be present in nearly all 34(94%) *Helicobacter pylori* positive isolates. However, our study was succeeding to detect the *ureA* gene in most of the isolates which was already confirmed by 16SrRNA. This result agrees with Percival and Thomas, (2009) who used *ureA* gene. On the other hand, the detection of *ureC* gene also was done for *H. pylori* isolates and the results appeared that 28(78%) have this gene as well as appeared as band 294bp on agarose gel electrophoresis as shown in figure 3 .

Studies results of Ahmad *et al.*, (2013) they extracted the genomic DNA and amplification for *ureC* (g/mM) HP. Extracted genomic DNA was amplified for the *ureC* (*glmM*) gene and detected with the specific primers, the gene product was 294 bp. Vale and Vito(2010); Bahrami *et al.*,(2013) they found in their studies, *ureC* gene of *H. pylori* was detected in tap water, dental units' water, refrigerated water with filtration ,and public cooler water samples. *H. pylori* DNA has been identified in several water sources using diverse gene targets. The PCR assay employed in this work specifically targets a region of the *ureC* (*glmM*) gene which



has been shown to be unique and essential for the growth of *H. pylori* (Safaei *et al.*, 2011; Bahrami *et al.*, 2013).

Table 4: Detection of *H. pylori* genes by PCR

Type of Sources	No. of Isolate	PCR +ve <i>16srRna</i>	PCR +ve <i>UreaA</i>	PCR+ve <i>ureC</i> HP
Reservoir	11	11(100%)	11(100%)	10(91%)
Tap Water	5	4(80%)	4(100%)	3(60%)
Groundwater	8	8(100%)	8(100%)	6(75%)
Biofilm (smooth layer)	12	12(100%)	11(92%)	10(83%)
Total	36	35(97%)	34(94%)	28(78%)

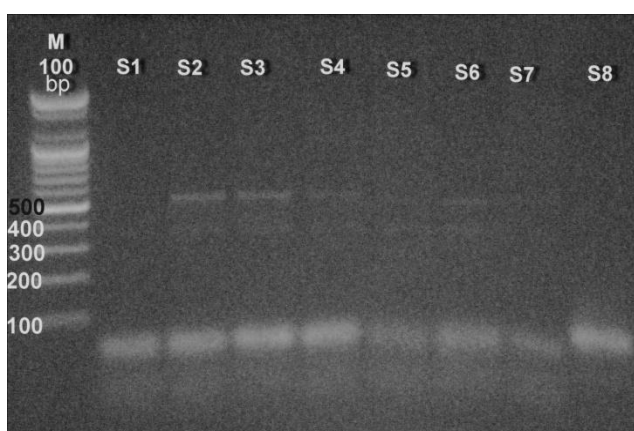


Figure 1: Gel electrophoresis for amplification of 16s rRNA gene (product size 500 bp). Electrophoresis was performed on 1% agarose gel. Lane M is (100 bp) ladder. S1 S2 S3 S4 S5 S6 S7 are negative control and S8 shows positive control. Based on this figure the 8th ladder was positive and completed other samples

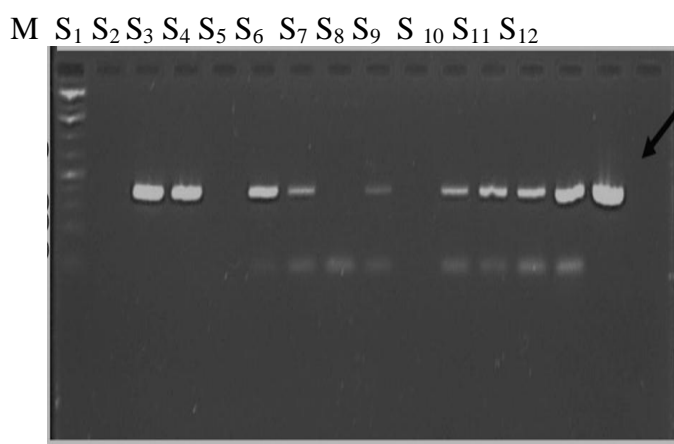


Figure 2: Gel electrophoresis for amplification of *ureA* Gene for *H. pylori* (product size 411 bp). Electrophoresis was performed on 1% agarose gel. Lane M is (100 bp) ladder, S1, S4, S7 is negative and S2, S3, S5, S6, S8, S9, S10, S11, S12 is positive.



Fig 3: Gel electrophoresis for amplification of *ureC* (*glmM*) gene for *H.pylori* product size (294 bp) electrophoresis was performed on Lane M in (100 bp) shows –ve negative control, S1 S5 are negative and S2 S3 are positive.

References

- Ahmad Reza Bahrami, Ebrahim Rahimi, and Hajieh Ghasemian Safaei(2013) Detection of *Helicobacter pylori* in City Water, Dental Units' Water, and Bottled Mineral Water in Isfahan, Iran The ScientificWorld Journal Volume 2013, Article ID 280510, 5 pages
- Ali Khani , N Sadeghiford , S. Farajina .M. Hajia, M.M,Aslani and A.R.Zamani, Evaluation of selective and Non selective media for isolation of *Helicobacter .pylori* from gastric biopsy specimens , 10(22);4156- 4159,2007.
- Al-Sulami,1 A.M. R. Al-Tae 2 and M.G. Juma'a(2010) Isolation and identification of *Helicobacter pylori* from drinking water in Basra governorate, Iraq EMHJ • Vol. 16 No. 9
- Al-Sulami,A. A., Al-Edani, T. A. and A. A. Al-Abdula(2012) Culture Method and PCR for the Detection of *Helicobacter pylori* in Drinking Water in Basrah Governorate Iraq Gastroenterology Research and Practice Volume 2012, Article ID 245167,
- Amieva M.R.,El –Omar E.M.Host – bacterial infections in *Helicobacter .pylori* infection. Rev in Basic and clin astroent 2008 ;134;306-323.
- Axon, A. T. R. (1996). The transmission of *Helicobacter pylori*: which theory fits
- Azevedo, N., Guimares, N., Figueiredo, C., Kevil, C. and Vieira, M.J. (2007) A New Model for the Transmission of *Helicobacter pylori*: Role of Environmental Reservoirs as Gene Pools to Increase Strain Diversity. *Critical Reviews in Microbiology*, **33**, 157-169
- Baker, K.H., Hegarty, J.P., Redmond, B., Reed, N.A. and Herson, D.S. (2002) Effect of oxidizing disinfectants (chlorine, monochloramine, and ozone) on *Helicobacter pylori*. *Applied and Environmental Microbiology* 68, 981–984.
- Bahrami AR, Rahimi E and Ghasemian Safaei H (2013) Detection of *Helicobacter pylori* in city water, dental units' water, and bottled mineral water in Isfahan, Iran. *ScientificWorldJournal* 2013; Article ID 280510, 5 pp.
- Boom, R., Sol, C.J., Salimans, M.M., Jansen, C.L., Wertheim-van Dillen, P.M. and van der Noordaa, N.J. (1990) Rapid and simple method for purification of nucleic acids. *Journal of Clinical Microbiology* 28, 495–503.



- Brown, L.M. (2000) *Helicobacter pylori*: epidemiology and routes of transmission. *Epidemiologic Reviews* **22**, 283–297.
- Cellini, A. Del Vecchio, M. Di Candia, E. Di Campli Cellini, A. Del Vecchio, M. Di Candia, E. Di Campli, M. Favaro, and G. Donelli, “Detection of free and plankton-associated *Helicobacter pylori* in seawater,” *Journal of Applied Microbiology*, vol. 97, no. 2, pp. 285–292, 2004.
- Degnan, A.J., Sonzogni, W.C. and Standridge, J.H. (2003) Development of a plating medium for selection of *Helicobacter pylori* from water samples. *Applied and Environmental Microbiology* **69**, 2914–2918.
- Eshraghian A (2014) Epidemiology of *Helicobacter pylori* infection among the healthy population in Iran and countries of the Eastern Mediterranean Region: a systematic review of prevalence and risk factors. *World J Gastroenterol* **20**, 17618–17625
- Falsafi, R. Favaedi, F. Mahjoub, and M. Najafi (2009), “Application of stool-PCR test for diagnosis of *Helicobacter pylori* infection in children,” *World Journal of Gastroenterology*, vol. 15, no. 4, pp. 484–488
- Fujimura S, Kato S, Watanabe A (2008) Water source as a *Helicobacter pylori* transmission route: a 3-year follow-up study of Japanese children living in a unique district. *J Med Microbiol* **57**: 909.
- Gao MS, Azevedo N, Wilks SA, Vieira M, Keevil C (2010) Effect of chlorine on incorporation of *Helicobacter pylori* into drinking water biofilms. *Appl Environ Microbiol* **76**: 1669.
- Gao, N. F. Azevedo, S. A. Wilks, M. J. Vieira, and C. W. Keevil, (2008) “Persistence of *Helicobacter pylori* in heterotrophic drinking-water biofilms,” *Applied and Environmental Microbiology*, vol. 74, no. 19, pp. 5898–5904.
- Go, M.F. (2002) Review article: natural history and epidemiology of *Helicobacter pylori* infection. *Alimentary Pharmacology and Therapeutics* **16** (Suppl. 1), 3–15.
- Hegarty, J.P., Dowd, M.T. and Baker, K.H. (1999) Occurrence of *Helicobacter pylori* in surface water in the United States. *Journal of Applied Microbiology* **87**, 697–701.
- Janzon A, Sjöling A, Lothigius A, Ahmed D, Qadri F and Svennerholm AM (2009) Failure to detect *Helicobacter pylori* DNA in drinking and environmental water in Dhaka, Bangladesh, using highly sensitive realtime PCR assays. *Appl Environ Microbiol* **75**, 3039–3044.
- Johnson CH et al. Inactivation of *Helicobacter pylori* by chlorination. *Applied and Environmental Microbiology*, 1997, **63**:4969–4970. 16.
- Labigne, A., V. Cussac, and P. Courcoux. (1991). Shuttle cloning and nucleotide sequences of *Helicobacter pylori* genes responsible for urease activity. *J. Bacteriol.* **173**:1920–1931.
- Lee, (1994) “The microbiology and epidemiology of *Helicobacter pylori* infection,” *Scandinavian Journal of Gastroenterology*, vol. 29, no. S201, pp. 2–6.
- Liu H et al. (2008) Specific and sensitive detection of *H. pylori* by real time RT-PCR and in situ hybridization. *PLoS ONE*, 2008, **3**(7):e2689.doi:10.1371/J.
- Mazari-Hiriart, M., Lopez-Vidal, Y. and Calva, J.J. (2001) *Helicobacter pylori* in water systems for human use in Mexico city. *Water Science Technology* **43**, 93–98. Mexico border community: evidence for transitory infection. *Am. J. Epidemiol.*
- Moreno, P. Piqueres, J. L. Alonso, A. Jimenez, A. Gonzalez, and M. A. Ferrus, (2007) “Survival and viability of *Helicobacter pylori* after inoculation into chlorinated drinking water,” *Water Research*, vol. 41, no. 15, pp. 3490–3496.



- Moreno, Y., Ferrus, M.A., Alonso, J.L., Jimenez, A. and Hernandez, J. (2003) Use of fluorescent in situ hybridization to evidence the presence of *Helicobacter pylori* in water. *Water Research* 37, 2251–2256.
- Notarnicola, F. Russo, A. Cavallini (1996), “PCR identification of *Helicobacter pylori* DNA in faeces from patients with gastroduodenal pathology,” *Medical Science Research*, vol. 24, no. 11, pp. 785–787.
- Otto, G., S. H. Hazell, J. G. Fox, C. R. Howlett, J. C. Murphy, J. L. O’Rourke, and A. Lee (1994). Animal and public health implications of gastric colonization of cats by *Helicobacter*-like organisms. *J. Clin. Microbiol.* 32:1043–1049.
- Owen, R.J. (1998) *Helicobacter*-species classification and identification. *British Medical Bulletin* 54, 17–30.
- Percival SL and Thomas JG (2009) Transmission of *Helicobacter pylori* and the role of water and biofilms. *J Water Health* 7, 469–477.
- Queralt, R. Bartolomé, and R. Araujo, (2005) “Detection of *Helicobacter pylori* DNA in human faeces and water with different levels of faecal pollution in the north-east of Spain,” *Journal of Applied Microbiology*, vol. 98, no. 4, pp. 889–895.
- Ranjbar R, Khamesipour F, Jonaidi-Jafari N and Rahimi E (2016) *Helicobacter pylori* in bottled mineral water: genotyping and antimicrobial resistance properties. *BMC Microbiol* 16, 40.
- Redlinger, T.E., Avitia, R., Galindo, A. and Goodman, K. (2002) Isolation and genotyping of *Helicobacter pylori* from untreated municipal wastewater. *Applied and Environmental Microbiology* 68, 1436–1439.
- Safaei, E. Rahimi, A. Zandi, and A. Rashidipour, (2011) “*Helicobacter pylori* as a zoonotic infection: the detection of *H. pylori* antigens in the milk and faeces of cows,” *Journal of Research in Medical Sciences*, vol. 16, no. 2, pp. 184–187.
- Sartory PD, Holmes P. (1997) Chlorine sensitivity of environmental, distribution system and biofilm coliforms. *Water Science and Technology*, 35(11–12):289–292.
- Suerbaum, S. and Michetti, P. (2002) *Helicobacter pylori* infection. *New England Journal of Medicine* 347, 1175–1186.
- Tiveljung, K. Borch, J. Jonasson, S. Mårdh, F. Petersson, and H. J. Monstein, “Identification of *Helicobacter* in gastric biopsies by PCR based on 16S rDNA sequences: a matter of little significance for the prediction of *H. pylori*-associated Gastritis ?” *Journal of Medical Microbiology*, vol. 47, no. 8, pp. 695–704, 1998.
- Vale F.F., and J. M. B. Vitor, “Transmission pathway of *Helicobacter pylori*: does food play a role in rural and urban areas?” *International Journal of Food Microbiology*, vol. 138, no. 1-2, pp. 1–12, 2010.
- Vaira, D., P. Ferron, R. Negrini, L. Cavazzini, J. Holton, C. Ainley, M. Londei, M. Vergura, R. Dei, and A. Colecchia (1992). Detection of *Helicobacter pylori*-like organisms in the stomach of some food-source animals using a monoclonal antibody. *Ital. J. Gastroenterol.* 24:181–184.
- Virginia Montero Campos, Alejandro Hernández Soto, Jorge Camacho Sandoval (2014) Culture and Molecular Identification of *Helicobacter pylori* in Drinking Water from Areas of High and Low Incidence of Gastric Cancer in Costa Rica **Journal of Medical Microbiology**, 2014, 4, 261-269



- Vyse, A.J., Gay, N.J., Hesketh, L.M., Andrews, N.J., Marshall, B., Thomas, H.I., Morgan-Capner, P. and Miller, E. (2002) The burden of *Helicobacter pylori* infection in England and Wales. *Epidemiology and Infection* **128**, 411–417.
- Watson CL, Owen RJ, Said B, Lai S, Lee JV, Surman- Lee S and 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.
- Yahaghi E, Khamesipour F, Mashayekhi F, Safarpour Dehkordi F, Sakhaei MH, Masoudimanesh M and Khayyat Khameneie M (2014) *Helicobacter pylori* in vegetables and salads: genotyping and antimicrobial resistance properties. *Biomed Res Int* 2014, 757941.