JOURNAL OF PURE AND APPLIED MICROBIOLOGY, September 2013. Vol. **7**(3), p. 2315-2324

Presence of *Helicobacter pylori* in Mumbai Water

Rupa Mulchandani[1,2], Hans-Olof Nilsson[2], Torkel Wadstrom[2] and B.R. Joshi[1]

[1]Department of Microbiology, Smt. Chandibai Himathmal Mansukhani College, Affiliated to the University of Mumbai, P.B. No. 17, Opp. Railway Station, Ulhasnagar - 421 003, Thane, Maharashtra, India.
[2]Department of Infectious Diseases and Medical Microbiology, Lund University, Solvegatan-23, 223 62, Lund, Sweden.

(Received: 11 November 2012; accepted: 15 January 2013)

***Helicobacter pylori* is found commonly in the gut of the human beings and animals. This bacterium is found usually when patients suffer from upper GI infections. *H. pylori* has been found in the patients in antral biopsies in Mumbai, India. We have found *H. pylori* in antral biopsies and faeces in another study we have done. The mode of transmission of *H. pylori* is yet not confirmed although two routes are proposed : faecal-oral and oral-oral. Many studies have found that *H. pylori* survives in water in viable or viable but non-culturable forms. We found *H. pylori* in Mumbai water samples collected from different places by culture and PCR. Thus, we propose water as the possible vehicle of transmission of *H. pylori* infection.**

Key words: *Helicobacter pylori*, environmental water, culture, PCR.

Helicobacter pylori has been a cause of gastric inflammation and has been associated with duodenal ulcers, gastric adenocarcinoma and mucosa associated lymphoid tissue lymphoma[1] since many years, viz., 28-29 years. However, the mode of transmission of this bacterium has been controversial : faecal-oral or oral-oral[2]. There have been many studies based on these two modes of transmission of *H. pylori*. Faecal-oral route of transmission has been more considered in the research studies as *H. pylori* has been found in faeces, water and other vehicles like insects[3]. There have been studies on environmental water samples for the detection of *H. pylori* and other microorganisms in faeces, food, water. *H. pylori* has been detected in the drinking water in Peru by polymerase chain reaction (PCR)[4]. *H. pylori* has been detected in spiked faeces and environmental water samples by IMS-PCR techniques[5]. *Helicobacter* spp. DNA has also been detected in Swedish water samples that is used for the daily basis[6]. *H. pylori* has also survived in milk and tapwater[7]. It has been proposed to be detected in well water[8]. It has also been found in water in Mexico city[9]. Salmonella and some other bacteria have been detected in environmental water, sewage and food samples by PCR and gene probes[10]. *H. pylori* has been found to survive and remain viable upto 28 days in mineral water[11]. It has been found to survive in environmental water like river and can transform from coccoid to spiral forms at favourable conditions of nutrient medium provided[12]. *H. pylori* has been found to persist in heterotrophic drinking-water biofilms[13] which makes it very important to check for this bacterium.

Bacteria can be transmitted from sewage to water when water is not treated properly or not stored properly. *H. pylori* may remain in viable but non-culturable forms in water and can be transmitted to humans and animals and can thus cause infection. We could culture *H. pylori* from

* To whom all correspondence should be addressed.
Tel.: +91-2512232162; Mob.: +91-9323643554;
E-mail: mulchandaniroopa803@gmail.com

water and also detect by PCR. This shows that *H. pylori* is present in treated water and environmental water resources and can thus be transmitted through water. Thus, water acts as a vehicle for the transmission of *H. pylori*.

MATERIALS AND METHODS

Collection of Water Samples

A total of 63 water samples were collected from different administrative wards of Mumbai city and suburbs (Table 1 and 2). Initially, only 30 water samples were processed to standardize the culture method (Table 1). 100 ml of each water sample from a particular area was collected in 100ml sterile bottles and processed for culture in India. Later 33 water samples 0.5–1 litre from different administrative wards were collected and filtered. These filter membranes were preserved at -20°C for culture and PCR to be analysed at Lund University, Sweden.

Processing of Water samples for culture

22 water samples of 100ml each were filtered through 0.22µm cellulose acetate filter membranes (Millipore Corp, Mumbai, India) under vaccum in a horizontal laminar air-flow. The membranes were transferred to 10ml of sterile Columbia broth (Hi-Media, Mumbai, India) and the tubes were incubated in an anaerobic gas jar at 37°C under microaerophilic conditions for 48 hours. After incubation, turbidity was looked for in the broth and a loopful of it was streaked on Columbia blood agar supplemented with 7% sheep blood and Skirrow's supplement (Hi-Media, Mumbai, India). The subsequent 8 water samples of 50ml each were centrifuged at 10,000xg for 15 minutes in a refrigerated centrifuge. The sediment was plated on Columbia agar base supplemented with 7% sheep blood and Skirrow's antibiotic supplement

Enrichment of membranes for culture and PCR

All the 33 water samples (0.5–1 litre each) (Table 2) were filtered through membranes as described above. Each membrane, which was preserved at -20°C was divided into two parts with a sterile scalpel. One part was transferred to 10 ml of sterile gonococci (GC) broth supplemented with 5% horse serum[14] and the other was used for PCR assay. The GC broth tubes with membranes were incubated under microaerophilic conditions in a gas jar on a shaker at 37°C for 2-3 days. After incubation turbidity was looked for in the medium and a loopful of it was subcultured on sterile GAB-CAMP agar medium[14]; the plates were incubated at 37°C in a CO_2 incubator and were observed for 10 days.

DNA extractions from water samples

The filter membranes kept aside for PCR were cut into small pieces (approximately 2mm x 2mm each) with a sterile scalpel and transferred into a 1.5ml eppendorf tube. The pieces were digested and DNA extracted. For lysis of bacterial cell membrane, digestion buffer (250 µl of 1% Triton X-100, 10mM TNE and 12.5µl of lysozyme [10mg/ml]) was added and incubated at 37°C for 45 minutes. After incubation 25µl of proteinase-K (10mg/ml) was added and the tubes were incubated at 37°C for 48 hours. The tubes with lysate were then kept at 95°C for 5 minutes in a water bath to inactivate proteinase-K. The samples were centrifuged in a microcentrifuge at 12000xg for 5 minutes in a cold room. After centrifugation, the supernatant was transferred to a fresh sterile microcentrifuge tube. DNA was extracted by the conventional phenol:chloroform:isoamylalcohol, centrifuged at 12000xg for 15minutes. The upper phase was transferred to a new tube and further extracted with equal volume of chloroform:isoamylalcohol. The DNA was then precipitated with 0.6 vol. of isopropanol and 0.1 vol. of 3M Na-acetate at -20°C. The DNA was pelleted by microcentrifugation at 12000xg for 15 minutes, washed with 70% (vol/vol) cold ethanol, dessicated for 30 minutes at room temperature and dissolved in 100 µl of molecular biology-grade water[15,16].

PCR amplification for water samples

Two sets of primers were used : HPU and 16SrRNA. The PCR mixture consisted of 10mM Tris-HCl (pH-8.3), 50mM KCl, 2.5mM $MgCl_2$, 0.01% BSA, 0.2mM of each deoxynucleotide-tri-phosphate (Boehringer GmbH, Mannheim, Germany) and 0.5mmol of each oligonucleotide primer (Scandinavian Gene Synthesis, Koping, Sweden). Taq polymerase (1.5 U; Boehringer) was added and the reaction mixture was overlaid with 40µl of mineral oil (Sigma). PCR was performed in reaction volume of 50µl in a thermal cycler (PTC-100, MJ Research, Mass, USA). Amplification was performed for 35 cycles with the primer pair HPU and for 40 cycles with 16SrRNA. The first cycle

consisted of denaturation at 94°C for 5 minutes, primer annealing (HPU: 48°C, 30 seconds; 16SrRNA: 60°C, 1 minute), and extension at 72°C for 45 seconds for HPU and 1 minute:20 seconds for 16S rRNA; in subsequent cycles, denaturation at 94°C was done for only 1 minute. In the final cycle extension was carried out for 10 minutes. Amplified products (13µl) mixed with gel loading buffer (0.25% bromophenol blue, 30% glycerol, 40mM EDTA pH-8.0) were analysed by 2% agarose gel electrophoresis and stained with ethidium bromide (0.5mg/ml). PCR products were visualized by excitation under UV light. DNA of standard strain *Helicobacter pylori* CCUG-17874 (Culture Collection University of Gothenberg), was used as positive control. Sterile double distilled water as well as a sterile membrane treated in a similar manner were used as negative controls.

PCR for enriched water samples

100µl of enriched gonococci broth culture of each water sample was used for the PCR. The cultures were centrifuged at 12000xg for 6 minutes and the pellet was used to extract the DNA as described above. PCR assay with HPU primers was performed for all the broth cultures whereas with 16SrRNA only 5 broth cultures were analysed due to short stay in Sweden.

Analysis of Swedish water samples and spiked samples

We analysed 4 Swedish water samples from sea, river, well (Tjornarp and Furulund) for *H. pylori* detection in a similar way by HPU-PCR assay. We also spiked 4 *H. pylori* positive water samples with standard *H. pylori* strain CCUG-17874 as follows : 10^5 CFU/ml, 10^3 CFU/ml, 10^2 CFU/ml, and 10^1 CFU/ml and ran 3 negative samples as controls. These were subjected to HPU–PCR assay. The spiked samples were from Elphinstone, Ghatkopar, Bhandup and Parel railway stations respectively.

Southern Blot Hybridization

Southern blot hydridization was performed to confirm that *H. pylori* DNA was amplified in the PCR analysis. PCR fragments were transferred onto a nitrocellulose membrane (Schleicher and Schull, Dassel, Germany) by the alkaline blotting procedure[15]. The membrane was heated at 80°C for 2 hours and prehybridized in 5xSSC (20xSSC; 0.3M sodium citrate), 2% blocking reagent no. 6 (Boehringer Mannheim, GmbH, Biochemica, Germany), 0.1% N-lauryl-sarcosine and 0.02% sodium-dodecyl-sulfate (SDS) at 65°C for 4 hours. The amplified urease A (HPU) fragment of *H. pylori* CCUG–17874 was used as a probe and prepared by the hexanucleotide priming technique with the Digoxygenin-dUTP labeling kit according to the manufacturers instructions (Boehringer Mannheim). After hybridization, performed at 65°C for 12 hours, and washing (twice with 2xSSC, 0.1% SDS and twice with 0.1xSSC, 0.1% SDS), detection of hybridized probes was performed with the DIG Nucleic acid detection kit. Detection of the probe was performed according to the manufacturers instructions (Boehringer Mannheim). Southern blot hybridization was performed only for water samples from Mumbai City.

RESULTS

Culture

i) From enriched broth (Columbia broth) cultures, eight out of 22 were positive for *H. pylori* by filteration method. Eight out of eight water samples were found to be culture-positive for *H. pylori* by centrifugation method. *Helicobacter pylori*-like colonies were observed on Columbia blood agar plates (Fig. 1). Cultures were confirmed by positive catalase, oxidase and urease tests and showed Gram negative curved rods resembling the standard strain of *H. pylori* (Fig. 2). These cultures were from Andheri east and west, Elphinstone, Parel, Ulhasnagar, Bhandup and Thane. These cultures were *H. pylori*, but unfortunately they were lost on repeated subcultures.

ii) Enriched broth (gonococci broth) cultures of 33 water samples showed overgrowth on the GAB-CAMP agar plates, so it was difficult to identify *H. pylori* colony on the plates.

Polymerase chain reaction (PCR)

Amplification with HPU primers

Water samples

The HPU-PCR assay showed the presence of *H. pylori* DNA in 7 of 33 water samples, revealing a 411 base-pair sequence of urease A gene. These water samples were from Parel, Elphinstone, Bhandup and Ghatkopar. All these samples were from municipal tap water supply but collected from

Table 1. Source for water collection for culture

Area	Source	Number of samples
Andheri (east)	Municipal tap	3
Andheri (west)	Municipal tap	2
Andheri (east)	Community Well	1
Borivali	Municipal tap	2
Bhandup	Municipal tap	2
Elphistone Road	Municipal tap	3
Ghatkopar	Municipal tap	3
Malad	Municipal tap	1
Parel	Municipal tap	4
Thane	Lake Water	4
Thane	Pond Water	1
Ulhasnagar	Municipal tap	2
Ulhasnagar	Well	2

different sources/reservoirs. Three water samples were from railway stations where unhygienic conditions prevail (Table 2), 3 were from households stored in plastic/metal containers, and 1 sample was direct municipal tap water collected from a hospital. Water samples from Elphinstone, Bhandup and Ghatkopar railway stations gave strong positive bands, and remaining five showed weak positive bands (Fig. 3), (Table 3).

Enriched Broth Cultures

Six enriched samples were positive with strong bands. These samples were taken from Elphinstone, Bhandup, Ghatkopar, Chandanwadi, Thane and Andheri. The first three samples were from railway stations, two from well and lake respectively and last one from municipal tap water stored in a tank (Table 3).

Table 2. Source for water collection for culture and PCR

Area	Source	Number of samples
Andheri	Railway Station, household	4
Apollo Bhandar	Household, slum, tea stall	6
Borivali	Railway Station, household	3
Bhandup	Railway Station, household well	3
Chandanwadi	Household Well	3
Ghatkopar	Railway Station, household well	3
Elphinstone	Railway Station, household slam	3
King's Circle	Railway Station, household	2
Parel	Slum, canton	3
Thane	Lake	1
Ulhasnagar	Household, well	2

Table 3. PCR-assay of water sample for *H. pylori*

Source	Water samples		Enriched broth	
	HPU – Assay	16SRNA – Assay	HPU – Assay	16SRNA – Assay
1) Railway Station				
Elphinstone	+	-	+	-
Bhandup	+	-	+	+
Ghatkopar	+	-	+	-
2) Household				
Chandanwadi	+	+	+	NT
Andheri (east)	-	-	+	NT
Elphinstone	+	-	-	+
Ghatkopar	+	-	-	-
Parel +		- -	-	
3) Lake				
Thane	-	-	+	NT

NT : Not tested

Amplification with 16SrRNA primers

Water Samples

The 16S ribosomal RNA-PCR assay detected only one positive water sample out of 33 water samples, which was taken from a house in Chandanwadi. The amplified product of this sample showed a 109 base-pair band. There was no correlation between two PCR-assays with any of the water samples (Fig. 4), (Table 3).

Fig. 1. Black and White Photograph of one of the culture of *H. pylori* isolated from a Mumbai water sample from Andheri (east) area.

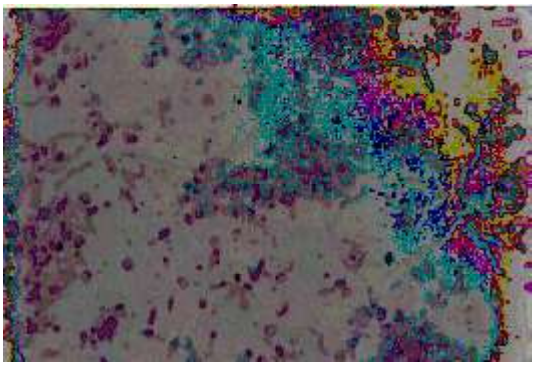

Fig. 2. Gram's Staining of Standard culture of *Helicobacter pylori* CCUG -17874. Gram negative curved rods are visible in clusters.

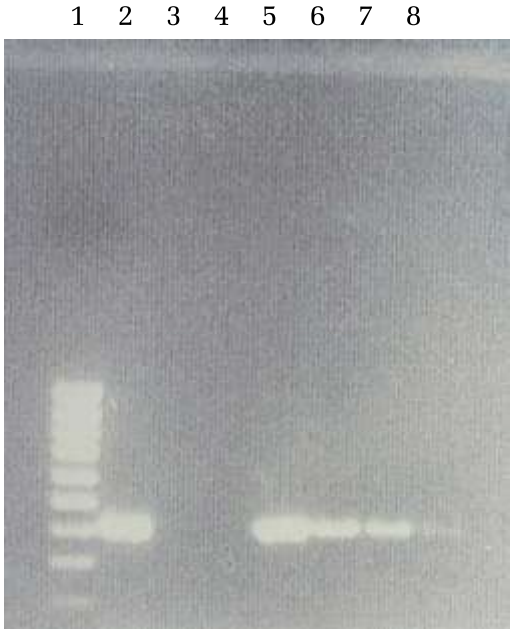

Fig. 3. Agarose gel electrophoresis of spiked water samples from Mumbai area showing HPU-PCR assay bands of 411bp DNA after amplification. Lane I shows Molecular weight marker of 100bp step ladder, Lane II positive control *H. pylori* CCUG-17874, Lane III shows negative control as dd/w, Lane IV-X shows positive and negative results for water samples for *H. pylori*.

Enriched Broth Cultures

Only five of 33 enriched broth cultures were tested by 16S rRNA-PCR assay. Only one sample which was from Bhandup railway station showed a weak positive band for *H. pylori* (Table 3).

Comparison of two PCR-assays

In three water samples collected from railway stations, the enriched broth cultures as well as direct PCR of the water samples was positive with HPU primers. The 16S ribosomal RNA-PCR assay of enriched water samples showed presence of one positive band for *H. pylori* from five samples tested. The same sample was positive for *H. pylori* PCR assay of enriched broth culture as well as direct PCR of water samples for HPU primers. This water sample was from Bhandup railway station.

Four of 24 household tap water samples were positive with HPU primers and one with 16S rRNA gene sequence; no correlation was found here. One of 24 enriched broth of household water samples was positive with HPU primers; neither of the two enriched broth cultures tested were positive. Well and lake water samples were not positive with HPU primers as well as 16SrRNA primers. Enriched broths of one well and one lake water sample were positive with HPU primers (Table 3).

Analysis of spiked filter membranes showed the positive bands of *H. pylori* in the decreasing order from 10^8 to 10 CFU/ml (Figure 5).

Southern blot hybridization

Four water samples were spiked with *H. pylori* standard strain CCUG-17874. Two PCR positive samples from railway stations and two from household taps as well as three negative tap samples by ureA-PCR were analysed with southern blot hybridization by using urease probes for *H. pylori* gene sequence A. The positive samples tested, which generated either intense or weak PCR signal after electrophoresis, all hybridized to the probe at stringent conditions. Negative samples by PCR did not hybridize in southern blot.

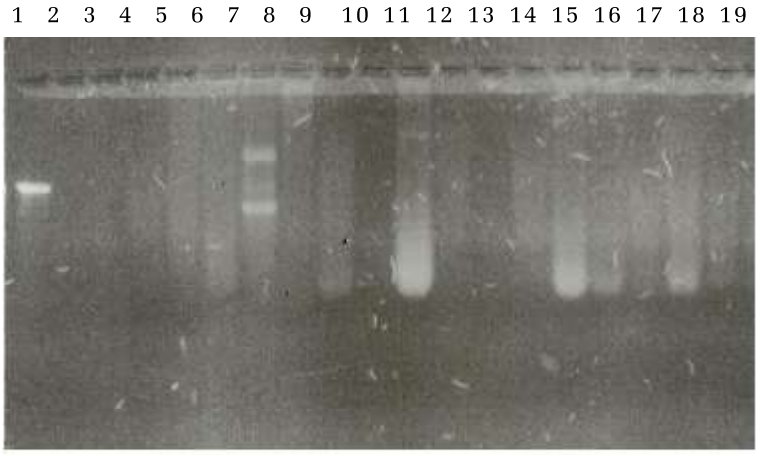

Fig. 4. PCR amplified products of water samples. Lane I shows a positive control of *H. pylori* DNA standard strain CCUG-17874. Lane II shows a negative control of sterile double distilled water. Lane III-XVIII shows PCR results after amplification of DNA for 16SrRNA primers for *H. pylori* from water samples. Lane VII shows a positive band of 109 bp product for *H. pylori* DNA for 16SrRNA gene sequence from a water sample obtained from Bhandup railway station.

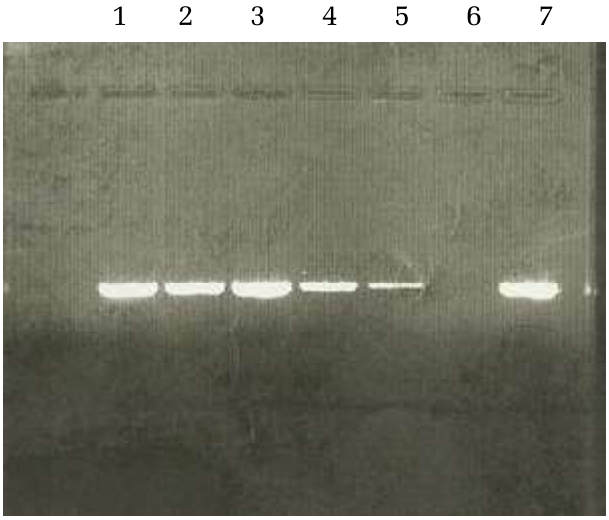

Fig. 5. Agarose gel electrophoresis of 0.22µm filter membranes spiked with CCUG-17874 *H. pylori* strain in d/w samples showing HPU-PCR bands of 411 bp DNA after amplification. Lane I - V shows 108, 106, 104, 102, 10 CFU/ml. Lane VI negative control and Lane VII positive control.

DISCUSSSION

We found 16 of 30 water samples culture positive for *H. pylori*. These cultures showed Gram negative curved rods or Gram negative rods to cocoid forms. Eight water samples from sewage also showed similar results but it was not possible to isolate *H. pylori* colony in pure form. *H. pylori* has never been isolated from an environmental source before, but recent studies, have shown that, like *Campylobacter jejuni*, it may survive in fresh water microcosms in a viable state for more than 10 days and as viable but non-culturable coccoidal bodies for up to one year[17]. The coccoid forms of *H. pylori* have been shown to remain viable but non-culturable for several months[18]. The lower positivity rate of culture in our study may be due to the presence of coccoid forms of bacteria in water which may be viable but non-culturable. It has been shown that *H. pylori* in coccoid form is more difficult to detect by PCR probably because of different antigenicity and or DNA content compared to rods. It may be that some of the coccoid bacteria are dead after storage in water, not dormant or viable but non-culturable and therefore do not contain intact DNA. Coccoid forms of *H. pylori* serotypes have been found to be revived in the laboratory[28]. It has been recommended that samples as fresh as possible should be analysed to avoid the conversion of rod-shaped *H. pylori* to the coccoid form[19].

Our HPU-PCR assay detected *H. pylori* DNA in 7 of 33 (21%) water samples and 6 of 33 (18%) enriched broth cultures (Table 3). 16S rRNA PCR assay detected only 1 of 33 (3%) water samples and 1 of 5 (20%) enriched broth cultures positive for *H. pylori* DNA. The majority of positive water samples came from municipal tap water supplied to railway stations and home which was stored in tanks or containers. 4 of 24 municipal water samples, 1 of 2 well water and 1 of 1 lake water were positive by PCR assay. Westblom et al[20], detected *H. pylori* DNA in 3 of 20 sewage water samples from Lima, Peru, by ureA-PCR assay. They concentrated 40 ml of sample and subjected the concentrate to PCR. Our attempts to culture *H. pylori* from sewage were in vain as other bacteria overgrew along with it. Hulten et al[4], detected *H. pylori* DNA in 24/48 (50%) water samples by *H. pylori* specific adhesine subunit PCR. In another study, the same group detected *H. pylori* by hybridization. They detected *H. pylori* DNA in 9 of 24 private wells, 3 of 25 municipal tap water and 3 of 25 waste water samples[6]. The percentage of positive municipal water samples was lower in Sweden compared with Peru and also in Mumbai, India. In both the studies they concentrated the water samples first by filtration and centrifugation and subjected the concentrate to immunomagnetic bead separation procedure where the bacteria were bound to the anti-*H. pylori* antibodies. We followed filtration prior to PCR but our results are in marked contrast with the results found in west. It could be due to complex inhibitors in water samples. Municipal water supply to the city of Mumbai comes from rain water collected in lakes and rivers; this is pumped to the purification plant where it is purified by sedimentation, flocculation and chlorination. The water is not membrane-filtered. Chlorination suppresses the growth of bacteria but its effect decreases with time; this permits bacterial contamination in supply lines and collection points.

Sample preparation prior to PCR plays an important role in detecting a particular gene sequence of a pathogen. Environmental water samples have to be concentrated to recover cells by centrifugation or filtration. We used membrane filtration and subjected the membranes to PCR. Many methods have been described for removal or inactivation of PCR inhibitors. We used phenol:chloroform extraction, which is the most commonly used. Immunomagnetic bead separation prior to PCR has been shown to efficiently separate out bacteria of particular interest from heterogeneous samples; yet, the coccoidal form of *H. pylori* is difficult to detect[5]. There have been substantial difficulties in removing the complex inhibitors of PCR which lowers the number of bacteria being detected.

We found the HPU primers to be more sensitive in detecting the organism as compared to 16SrRNA primers. Amplification of a target gene sequence by PCR coupled with gene probes can achieve the sensitivity and specificity required for monitoring bacterial pathogens in environmental water samples[21]. The ureA gene sequence which we used has been shown to be sensitive and specific for *H. pylori*, from antral biopsy samples[22] and from sewage water[20]. HPU primers have been

used successfully to detect the organism from dental plaques, antral biopsy tissue and faecal samples. The ure A primers are sensitive and specific to detect 10 *H. pylori* cells/reaction and have been shown to have no cross–reaction with other urease positive bacteria like *Campylobacter sordellii*, *Bacillus ureolyticus*, *Proteus mirabilis*, *M. morganii*, *P. rettgeri*, *K. pneumonia*, *U. urealyticum*, *U diverus*[20]. Other *Helicobacter* spp. *H. felis*, *H. hepaticus*, *H. muridarum*, *H. canis* and *H. acinonyx* are found to be non-reactive with these primers and are thus specific for *H. pylori*[16]. Bacteria other than *H. pylori* have been found in gastric biopsies by temperature gradient gel electrophoresis and 16SrDNA analysis[23]. Other *Helicobacter* species might be present in GI tract along with *H. pylori* in humans as they have been detected in animals[24]. Thus, HPU coding for Urease A gene sequence are more reliable as compared with other primers.

16SrRNA primers have also been widely used to identify *H. pylori* from various clinical specimens and environmental samples[4,6,25,26]. These primers have been found to be sensitive to detect as little as 0.01 pg of *H. pylori* DNA and is specific to *H. pylori* as the DNA from the closest relatives of *H. pylori* was not amplified[22]. But recently, these primers have been shown to amplify human genome[27]. Hulten et al[6], used *H. pylori* adhesine subunit and 16SrRNA RT-PCR assays. Their results showed adhesine PCR to be 10 times more sensitive than 16SrRNA RT-PCR. In our assays also, we observed HPU-PCR to be more sensitive then 16SrRNA-PCR. However, we did not use the reverse transcriptase enzyme procedure which could have increased the sensitivity of our PCR assay. *H. pylori* may be present in lower numbers in the water samples or may be in coccoid form, limiting its detection by 16SrRNA primers. Thus, for PCR amplification of *H. pylori* two targets are promising, the urease and 16SrRNA genes, because partial or whole sequence information is available for both.

Bottled water from Umuahia Metropolis, Nigeria has been analysed and was found safe but consisted of Streptococcus spp, Proteus spp, and Pseudomonas spp.[29]. In another study in Delhi, India by MPN method, drinking water of municipal corporation of Delhi showed MPN count to be 1600 and *E. coli* was identified in this water. Recently, in our Laboratory at S.I.C.E.S College, Ambernath, MPN analysis of Ambernath and Kalyan (suburbs of Mumbai) water also count was 1600 cfu/100ml and we found *E.coli*, *Proteus spp*., *Klebsiella spp*., *Pseudomonas spp*.[30]. The Upper Lake of Bhopal, India has been checked for water quality for Coliforms, BOD, COD, DO., etc and was concluded not fit for drinking purpose[31]. Thus, contamination may occur in the supply lines or at the user outlet site. In this study it is shown that *H. pylori* is present in municipal water supply samples and environmental water samples in Mumbai. But when seen in the light of the high prevalence of this organism and other bacteria in the local population this may suggest that the water may act as a possible mode of transmission of infection.

ACKNOWLEDGMENTS

We thank to all the Teachers, Management and Staff members of Smt. CHM College for their kind co-operation and support all throughout the study. We thank to Lady Tata Memorial Trust and its Tata branches for providing scholarships. We thank Tata Memorial Hospital for providing facility for filteration and among other people for support and help on the way. Above all my family support has been a great help. This work has been presented at Plenary session at a conference of Indian Society of Gastroenterology in 1998 at AFMC, Pune and has been awarded gold medal. Second prize was awarded to the work in 2008 at International festival of Science at DAV Organisation, Delhi when I used to work at All Saints High School & Junior College. This work has been presented as M.Sc. thesis for my degree in 1999. Collaborations with Lund University, Sweden, Dr. Philip Abraham, KEM Hospital, Dr. Shobna J Bhatia, B Y L Nair Hospital, Univeristy of Mumbai, India, have been a great support during this study. I also thank to my former guiding teacher, Late Dr. Neena Sandhu for her encouragement throughout the study.

REFERENCES

1. Thomson, M., Commentary: *Helicobacter pylori*-the story so far. *BMJ* 1999; 319, 541.
2. Hegarty, J.P., Dowd, M.T., Baker, K.H.,

Occurrence of *Helicobacter pylori* in surface water in the United States. *J App Microbiol*. 1999; **87**: 697-701.

3. Sasaki, K., Tajiri, Y., Sata, M., Fujii, Y., Matsubara, F., Zhao, M., Shimizu, S., Toyonaga, A., Tanikawa, K., *Helicobacter pylori* in the Natural Environment. *Scand J Infect Dis.* 1999; **31**: 275-279.
4. Hulten, K., Han, S.W., Enroth, H., Klein, P.D., Opekun, A.R., Gilman, R.H. Evans, D.G., Engstrand, L., Graham, D.Y. & other authors., *Helicobacter pylori* in the drinking water in Peru. *Gastroenterol*. 1996; **110**: 1031-5.
5. Enroth, H., Engstrand, L., Immunomagnetic separation and PCR for detection of *Helicobacter pylori* in water and stool specimens. *J Clin Microbiol.* 1995; **33**: 2162-2165.
6. Hulten, K., Enroth, H., Nystrom, T., Engstrand, L., Presence of *Helicobacter pylori* DNA in Swedish water. Thesis. University of Uppsala, Sweden, 1996.
7. Fan, X.G., Chua, A., Li, T.G., Zeng, Q.S., Survival of *Helicobacter pylori* in milk and tap water. *J Gastroenterol Hepatol*. 1998; **13**(11): 1096-8.
8. Hazell, S., *Helicobacter pylori* and Well Water. *ASM News*. 2000; **66**(2): 58.
9. Mazari-Hiriart, M., Lopez-Vidal, Y., Calva, J.J., *Helicobacter pylori* in water systems for human use in Mexico City. *Water Sci Technol*. 2001; **43**(12): 93-98.
10. Waage, A.S., Vardund, T., Lund, V., Kapperud, G., Detection of low numbers of Salmonella in environmental water, sewage and food samples by a nested polymerase chain reaction assay. *J Appl Microbiol*. 1999; **87**: 418-428.
11. Sato, F., Saito, N., Shouji, E., Rani, A., Takeda, H., Sugiyama, T., Asaka, M., The maintenance of viability and spiral morphology of *Helicobacter pylori* in mineral water. *J Med Microbiol*. 1999; **48**: 971.
12. Shahamat, M., Mai, U., Paszko-Kolva, C., Yamamoto, H., Colwell, R., Survival of *Helicobacter pylori* in the Environment. 1990.
13. Giâo, M.S., Azevedo, N.F., Wilks, S.A., Vieira, M.J., Keevil, C.W., Persistance of *Helicobacter pylori* in Heterotrophic Drinking-Water Biofilms. *App Env Microbiol*. 2008; **74**: 5898-5904.
14. Cederbrant, G., Kahlmeter, G., Lungh, A., Proposed mechanism for metronidazole resistance in *Helicobacter pylori*. *J Antimicro Chemother* 1992; **29**: 115-120.
15. Sambrook J, Fritsch EF, Maniatis T., Molecular Cloning: A Laboratory Manual, 2nd ed. Cold Spring Harbor: Cold Spring Harbor Laboratory Press, 1989.
16. Ho, S.A., Hoyle, J.A., Lewis, F.A., Secker, A.D., Cross, D., Mapstone, N.P., *et al.,* Direct polymerase chain reaction test for detection of *Helicobacter pylori* in humans and animals. *J Clin Microbiol*. 1991; **29**: 2543-2549.
17. West, A.P., Milar, M.R. Survival of *Helicobacter pylori* in water and saline., *J Clin Patho*. 1989; **43**: 609.
18. Bode, G., Mauch, F., Maltertheiner, P., The coccoid froms of *Helicobacter pylori*. Criteria for their viability. *Epidemiol. Infect.* 1993; **111**: 483-490.
19. Nilsson, H-O., Aleljung, P., Nilsson, I., Tyzkiewicz, T., Wadström, T., Immunomagnetic bead enrichment and PCR for detection of *Helicobacter pylori* in human stools. *J Microbiol Methods.* 1996; **27**: 73-79.
20. Westblom, T. U., Fritz, S. B., Phadnis, S., Midkiff, B., Leon-Barua, R., Recavarren, S., Ramirez, R., Ramos, A., PCR analysis of Peruvian sewage water: support for fecal-oral spread of *Helicobacter pylori*. *Acta Gastroenterol Belg*. Suppl. 1993; **6**: 47.
21. Atlas, R.M., Bej, A.K., Detecting bacterial pathogens in environmental water samples by using PCR and gene porbes. In: PCR protocols: A Guide to Methods and Applications. Academic Press. 399-406, 1990.
22. Clayton, C.L., Kleanthous, H., Coates, P.J., Morgan, D.D., Tabaqchali, S., Sensitive detection of *Helicobacter pylori* by using polymerase chain reaction. *J Clin Microbiol*. 1992; **30**: 192-200.
23. Monstein, H-J., Tivelung, A., Kraft, C.H., Borch, K., Jonasson, J., Profiling of bacterial flora in gastric biopsies from patients with *Helicobacter pylori*-associated gastritis and histologically normal control individuals by temperature gradient gel electrophoresis and 16SrDNA sequence analysis. *J Med Microbiol*. 2000; **49**: 817-822.
24. Fox, J.G., Handt, L., Xu, S., Shen, Z., Dewhirst, F.E., Dangler, C., Lodge, K., Motzel, S., Klein, H., Novel *Helicobacter* spp. Isolated from colonic tissue of rhesus monkeys and chronic idiopathic colitis. *J Med Mircobiol*. 2001; **50**: 421-429.
25. Nuygen, A.H., Engstrand, L., Genta, R.M., Graham, D.Y., El-Zaatari, F.A.K., Detection of *Helicobacter pylori* in dental plaque by reverse transcriptase polymerase chain reaction. *J Clin Microbiol*. 1993; **31**: 783-787.
26. Mapstone, N.P., Lynch, D.A., Lewis, F.A.,

Axon, A.T, Thompins, D.S, Dixon, M.F., Quirke, P., Identification of *Helicobacter pylori* in mouths and stomachs of patients with gastritis using PCR. *J Clin Microbiol*. 1993; **46**: 540-3.

27. Chong, S.K.F., Lou, Q., Lee, C.H., Fitgerald, J.F., The *Helicobacter pylori* 16SrRNA Gene PCR with the primer set HP1/HP2 amplifying a 109bp fragment is not specific and cannot be used to detect *H. pylori* in clinical specimens. *Gastroenterol*. 1996; A81.
28. Esmaeilli D., Mohabati A M., Sotodeh N., Reviving the Coccoid Forms of *H. pylori* Serotypes. *J Pure Appl Microbio.*, 2010; **4**(2): 541-544.
29. Uchechi N. E., and Chidinma U. E., Microbial Analysis of Bottled water samples sold commercially in Umuahia Metropolis in Abia State, Nigeria. *J Pure Appl Microbio*. 2008; **2**(1): 97-102.
30. Darakshan S., Wani S.P., Raghavan V., Bacteriological Examination of MCD Drinking Water with Reference to Coliforms. *J Pure Appl Microbio*. 2012; **6**(1): 471-473.
31. Magarde V., Iqbal S. A, Iqbal N., Pollution Study of Upper Lake of Bhopal, India. *J Pure Appl Microbio*. 2009; 3(2): 743-746.