

## Presence of *Helicobacter pylori* in drinking water of Karachi, Pakistan

Adnan Khan<sup>1</sup>, Amber Farooqui<sup>2,1</sup>, Shahana Urooj Kazmi<sup>1</sup>

<sup>1</sup>*Immunology and Infectious Diseases Research Laboratory, Department of Microbiology, University of Karachi, Karachi, Pakistan*

<sup>2</sup>*Division of Immunology, International Institute of Infection and Immunity, Shantou University Medical College, Guangdong, China*

### Abstract

**Background:** Prevalence of *Helicobacter pylori* (*H. pylori*) infection is an increasing problem in developing countries. Several environmental factors such as overcrowding, poverty, contaminated drinking water and food are considered to contribute toward transmission of infection; however, little is known about their definitive roles. The aim of this study is to investigate the presence of *H. pylori* in drinking water samples of Karachi, Pakistan.

**Methodology:** Samples of drinking water were collected from 18 different towns located in the metropolitan area of Karachi. Samples were concentrated by membrane filtration method and subjected to PCR for the detection of *H. pylori*.

**Results:** Two out of 50 (4%) samples collected from two different densely populated town areas were found to be positive for *H. pylori*.

**Conclusion:** The study provides evidence for the presence of *H. pylori* in municipal drinking water of Karachi.

**Key words:** *Helicobacter pylori*; drinking water; PCR; Karachi; Pakistan

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### Introduction

It is widely accepted that *Helicobacter pylori* (*H. pylori*) is a stomach colonizer of half of the world's population. The organism is often associated with a number of gastric diseases including gastric ulcer, peptic ulcer, gastritis and gastric adenocarcinoma. Despite widespread presence, little is known about the transmission of *H. pylori*. Among well-known modes, iatrogenic transmission of the organism due to invasive endoscopy is proven [1], while a previous study also presents positive association of *H. pylori* colonization with direct person-to-person contact [2]. Moreover, a high incidence rate among hospital-associated individuals and clustering of infection within families provide a valid basis for person-to-person transmission of infection. A number of human body secretions including vomitus, saliva, gastric juice and feces have been predicted as possible modes of transmission [3]. Several reports showed the prevalence of infection among infants younger than two years of age that correlate the transmission of infection through infected mothers to infants [4,5]. Studies conducted on possible zoonotic involvement show the ability of *H. pylori* to colonize in stray cats

and sheep for long periods; however, strong evidence about their roles in human transmission are lacking [6,7].

Another important factor of infection transmission is contaminated water. Due to fecal contamination, water might be an important and neglected source of *Helicobacter* transmission. Evidence of high rates of infection in those nations that rely on untreated water support this hypothesis. In 1991, a retrospective study in Peru showed the prevalence of infection in those individuals who rely on municipal water. The observation was further confirmed by a thorough investigation in which municipal water was tested positive for *H. pylori* in those areas with high prevalence of *Helicobacter* associated gastritis [8,9]. *H. pylori* have also been observed in ground water, drinking water storage reservoirs, and sewage samples, providing more suspicion toward the involvement of water in transmission of infection; however, no direct evidence was provided [10-12].

*H. pylori* is present in a coccoid and uncultivable form in water, which has different antigenicity than the cultivable form; therefore, PCR assay is

considered the most appropriate detection method. It is believed that *H. pylori* are not often detected in water because of the high amount of polymerase inhibitors present in water. However, pre-PCR treatment of water samples might increase the sensitivity of the test thus making it useful in identifying the mechanism of transmission. Although inconsistencies are present in research data, the presence of *H. pylori* in water samples from different geographical areas of the world indicates its likely involvement in the transmission of the organism.

Karachi is the largest city of Pakistan and among the largest metropolitan areas in the world with an estimated population of 16 million. We have already observed the high rate of *Helicobacter* infections among the residents of the city (unpublished data). Therefore, it is worth investigating the factors that are likely associated with the spread of infection in local communities. In this study, we investigate the presence of *H. pylori* in drinking water supplied to different regions of Karachi, Pakistan.

## Methodology

### *Sample collection and processing*

As shown in figure 1, the metropolitan city of Karachi is divided into 18 administrative sectors that are known as towns. A total of 50 drinking water samples were collected from the taps of public drinking water supplies located in 18 towns. Sample collection spots were identified on the basis of frequency of use, the properly installed tap and water supply system, and average sanitary conditions around the collection area. Sampling was avoided from the places that were located in the close proximity of public toilets. The number of samples from each town varied according to the socioeconomic status of the people and population size, which helped us to identify high-risk populations. Table 1 shows the geographical distribution of areas from where samples were collected. Samples were collected as follows: first the surface of tap was cleaned with water; then water was flushed away from the taps for five minutes and one liter of the midstream sample was collected directly from the tap into clean plastic bottles. The bottles were capped and transported to the lab at room temperature, where the water was filtered through a filter assembly using a 0.45µm membrane. Two to four membranes were used for each sample depending on the quality of water. All membranes from each sample were immersed in 5 ml of sterile Milli-Q water (Millipore, Billerica, USA) for 30

minutes and centrifuged for 10 minutes at 9000 rpm. Supernatant and membranes were discarded while sediment was resuspended in 200 µl sterile Milli-Q water and stored at -80°C.

### *DNA extraction*

DNA extraction was performed using QIAamp Mini DNA Mini Kit (Qiagen, Valencia, USA) as previously described for *H. pylori* detection from water samples [13]. A total of 200 µl of each sample was added to the tubes containing 20 µl of protease followed by the addition of 4µl of RNase A stock solution and 200 µl of buffer to get RNA-free genomic DNA. After 10 minutes of incubation at 56°C, the mixture was carefully applied to the QIAamp Mini Spin Column (Qiagen, Valencia, USA) for subsequent cleaning and rinsing steps. DNA elution was achieved in 100µl of sterile Milli-Q water and purified DNA was stored at -20°C.

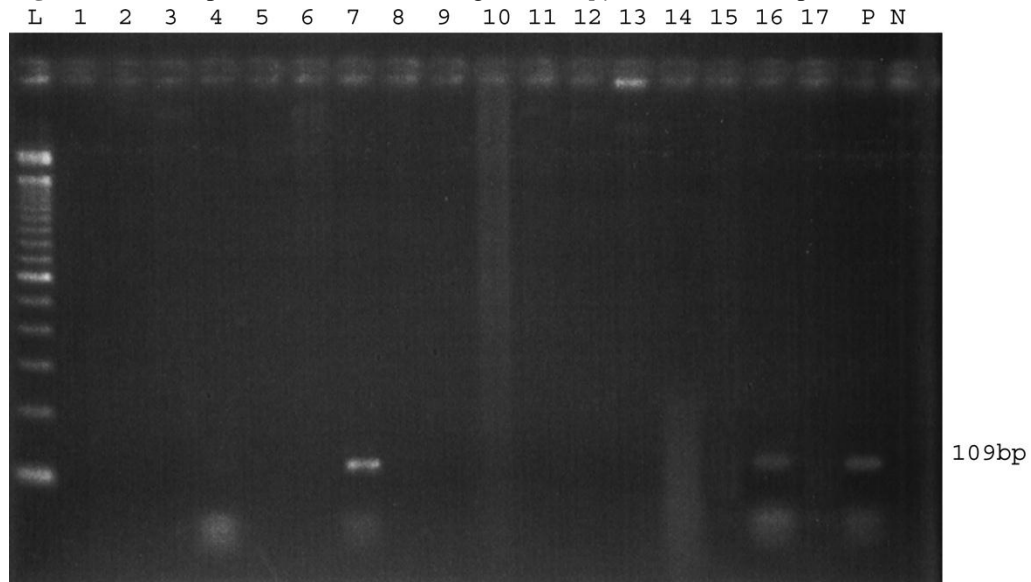
### *Detection of H. pylori by PCR*

Samples were subjected to PCR for the presence of *H. pylori* using primer pair HP1: 5'-CTGGAGAGACTAAGCCCTCC-3'' and HP2: 5'-ATTACTGACGCTGATTGTGC-3' targeting the *16srRNA* gene of *H. pylori*. Primers were used at a final concentration of 0.4 µM in a reaction mixture containing 1.5 mM MgCl<sub>2</sub>, 1 U of Taq polymerase, 2.5µl PCR buffer, 200µM each dNTPs and 2 µl of genomic DNA as the template. Amplification conditions were as follows: initial denaturation at 95.0°C for 5 minutes; 35 cycles for 30 seconds each of 95.0°C; annealing temperature of 60°C; and an elongation temperature of 72°C with a final extension of 72°C for 5 minutes. PCR products were resolved by electrophoresis on 1.5% gels with positive and negative controls and an amplicon of 109 bp was considered positive [14].

### *Results and discussion*

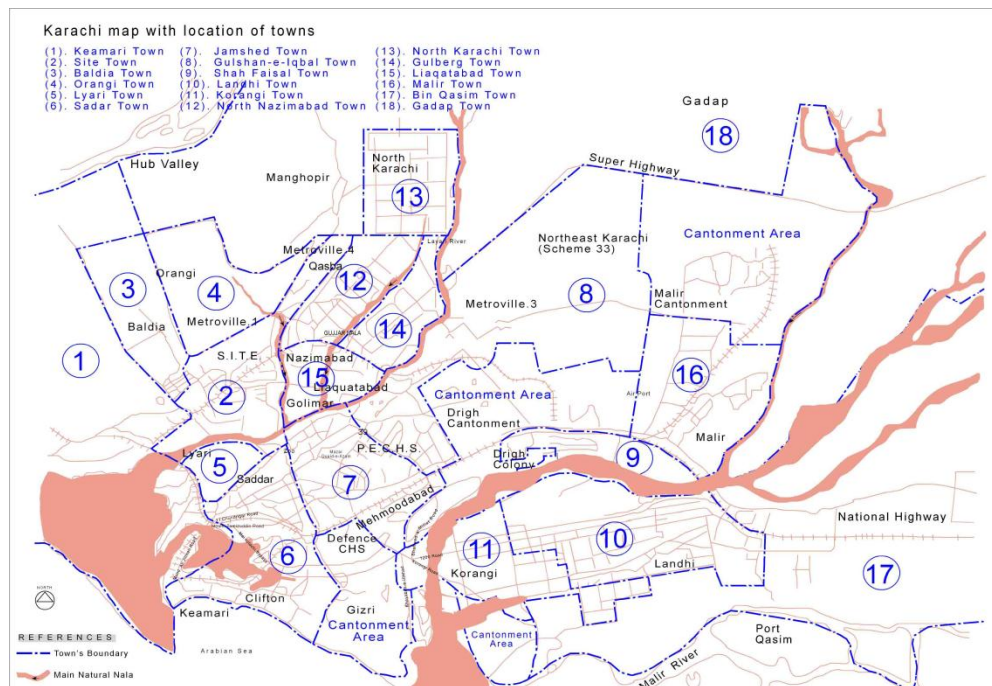
Out of 50 samples collected from 18 different towns of Karachi, two (4%) were successfully amplified (Figure 1). Positive samples belonged to two different densely populated town areas, specifically Landhi and Malir. Landhi Town is comprised of 13 union councils with a population of 564,505 people living in low socioeconomic conditions, whereas Malir Town is inhabited by 398,289 people who belong to an average socioeconomic level (Figure 2).

**Figure 1.** PCR amplification of 16S rRNA gene of *H. pylori* in water samples



The product were electrophoresed on 1.5% agarose gel and stained with ethidium bromide. Lane N: negative control; Lane P: positive control; Lanes 1-17: water samples; Lane L: molecular size marker (100 bp ladder)

**Figure 2.** Map of Karachi presenting locations of sample collection



Malir and Landhi towns from where *H. pylori* samples were recollcted are illustrated on number 16 and 10 respectively. The figure is excerpted from the official website of the OrangiPilot Project Research and Training Institute (opp-rti).

**Table 1.** List of water samples collected from different geographical locations of Karachi

S. No	Name of Town	Population <sup>§</sup> (1998)	Union Councils <sup>§</sup>	Socio-economic conditions	samples collected	Samples Positive for <i>H. pylori</i>
1	New Karachi	240,000	13	+	3	0
2	Landhi	564,505	12	+	3	1
3	Shah Faisal	335,823	07	++	2	0
4	SITE	467,560	09	+	3	0
5	North Nazimabad	500,000	10	+++	1	0
6	Saddar	616,151	11	++	2	0
7	Malir	398,289	07	++	2	1
8	Kemari	383,788	08	+	4	0
9	Jamshed Town	733,821	13	+	4	0
10	Gulshan-e-Iqbal	646,662	13	+++	1	0
11	Gulberg	453,490	08	++	1	0
12	Gadap	289,564	08	+	4	0
3	Bin Qasim	315,684	07	+	0	0
14	Orangi	723,694	13	+	4	0
15	Korangi	564,505	09	+	4	0
16	Baldia	406,165	08	+	4	0
17	Lyari	607,992	11	+	4	0
18	Liaquatabad	649,091	11	+	4	0

+ = low, ++ = average, +++ = above average <sup>§</sup> information is collected from <http://www.karachicity.gov.pk>

Approximately 25% of Karachi's total population of 16 million people live below the poverty line. Overcrowding, poverty and low nutrition status are usually considered important factors in the transmission of gastric diseases; therefore, the presence of *H. pylori* DNA in drinking water samples of densely populated area is interesting.

Although, water-borne infections and gastrointestinal outbreaks have been reported earlier [15], no data is available regarding the presence of *H. pylori* in drinking water supplies of Karachi. In this study, the samples were collected from public water supplies. These public spots provide treated water through a central distribution system of the city. The presence of *H. pylori* was observed in 4% of the treated water samples from Karachi. A recent study reports the presence of *H. pylori* in 40% of water samples collected from Lahore, a city located in a different province of Pakistan [16]. The sharp contrast in the positivity rates of these two cities is surprising and raises serious concerns about the quality of water and the water distribution system.

Increased rates of *H. pylori* infection in developing nations such as Pakistan, Vietnam, Bangladesh and India, which combat poverty, poor

sanitary conditions and overcrowding, indicate the role of environmental factors in the transmission of infection [17-20]. In 2007, Ahmed *et al.* indicated that the risk of *H. pylori* infection can be reduced by improving personal hygiene and regular use of boiled drinking water [20]. On the other hand, Janzon *et al.* reported the less likely involvement of drinking water in a South Asian region [21].

The ability of *H. pylori* to form biofilms is an area of growing concern. It has been reported earlier that chlorine disinfectant can inactivate the organism, which thus hampers the detection of *H. pylori* in treated water [22, 23]. However, the organism can form a biofilm on the surface of water supplies and can invade the heterotrophic biofilms of treated water [10]. Therefore, the detection of *H. pylori* by PCR also indicates the possible presence of viable bacteria in drinking water supplies. However, it is necessary to adapt molecular methods to detect the viability of *H. pylori* in drinking water supplies. Recently, Buck *et al.* reported the presence of a viable but uncultivable (VBNC) form of *H. pylori* in raw vegetables that indicates the possible contribution of water or soil [24].

In conclusion, this study provides evidence of the presence of *H. pylori* in drinking water samples in Karachi, Pakistan. Further investigations of treated and untreated water supplies needs to be performed.

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### Corresponding author

Adnan Khan, PhD  
 Assistant Professor  
 Department of Microbiology, University of Karachi  
 University Road, Karachi-75270, Pakistan  
 Telephone: +9221 99261389  
 Fax: +9221 99261342  
 Email: adnankh@uok.edu.pk

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