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Finding the existence of of *Helicobacter pylori* in springs of Chaharmahal and Bakhtiari Province, Iran by polymerase chain reaction

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Original article

ABSTRACT

Background and aims: *Helicobacter pylori* is the primary cause of various diseases. This study was conducted to determine the existence of *H. pylori* in springs and surface water in Chaharmahal and Bakhtiari province.

Methods: In this study, 29 water samples were collected from three different locations of Zayanderud River, 24 springs, and two subterranean from June 2014 to August 2014 in Chaharmahal and Bakhtiari province. Samples were filtered and DNAs were extracted. Then, the existence of *H. pylori* DNA was tested by polymerase chain reaction (PCR) for 16S rRNA, glm and Cag genes.

Results: In the present study, the total infection rate of samples by *H. pylori* was 24.13%. *H. pylori* and DNA was detected in 100% of samples of Zayanderud River, 12.5% in springs of Dehcheshmeh in Farsan and Baram in Lordegan, and 50% in aqueducts.

Conclusion: These findings show the existence of *H. pylori* in springs and surface water in Chaharmahal and Bakhtiari province and additional evidence for waterborne transmission of *H. pylori* in some environments.

Keywords: *Helicobacter pylori*, Polymerase Chain Reaction, Water.

INTRODUCTION

Helicobacter pylori infection is an serious problem in developing countries,[1] which is etiologically associated with gastric cancer and peptic ulcer diseases.[2] The high infection rate of *H. pylori* is observed in overcrowded and lower socioeconomic groups in developing countries.[3] In Iran, the overall prevalence of *H. pylori* infection according to a review article is between 30.6% and 82%.[4]

Several environmental factors such as overcrowding, poverty, and drinking water contamination are considered as transmission routs of *H. pylori* infection.[1]

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However, findings of some studies have demonstrated that drinking water and distribution systems contamination are the major sources of *H. pylori* infection in human.[5,6]

Studies have reported various rates of drinking water's infection with *H. pylori*. In study of Khan et al in Karachi, Pakistan in which PCR method was used for detecting *H. pylori*, 4% of the collected samples were positive.[1] In addition, the *H. pylori* infection rate of water by the same method in Isfahan, central Iran was 7% in comparison to 56% in Kermanshah, west of Iran.[7,8]

It is necessary to mention that the drinking water of some provinces like Isfahan and Chaharmahal and Bakhtiari are supplied by the Zayanderud River as surface water. Regarding the importance of water as a possible source of transmission of *H. pylori*, this study was conducted to determine the existence of *H. pylori* in drinking water samples in Chaharmahal and Bakhtiari province, southwest of Iran.

METHODS

The province of Chaharmahal and Bakhtiari is located in middle part of the central Zagros Mountain Chain in Iran. The highlands of this province are the hotbed of the largest rivers of Iran such as Zayanderud and Karron. The drinking water of this province is produced by Zayanderud which is a surface water and mineral water spring.

In this study, a total of 29 samples including 24 springs, two subterranean canals, and three samples from different geographic regions of Zayanderud were examined in three months period from June to August 2014 in Chaharmahal and Bakhtiari province.

Twenty-four samples of spring water in seven districts of Chaharmahal and Bakhtiari province including nine springs in Shahrekord (Zaneh, Goldareh, Darbid, Below Dehak, Sarghayeh, Mayak, Beroy, Gerdabe Ben, Badi), two springs in Boroujen (Siasard, Mother and Daughter), three springs in Farsan (Dehcheshmeh or Pireghar, Tange Darkesh Varkesh, ghaleh),), three springs in Ardal (Moula, Sarab, Sarkhoon), one spring in Lordegan (Baram), three springs in Kiar (Kat, Vaghtosaat, Shalamzar), and three springs in Kohrang (Dimeh, Kohrang, sardab) were gathered and analyzed. Moreover, two samples from Pez and Laghdombeh subterranean in Saman and three samples from different geographic regions of Zayanderud (Zamankhan Bridge, Savadjan, Baba- Pirahmad) were examined.

Springs were selected in terms of their availability and accessibility to people. Springs in impossible routs or unusable cases were excluded from the study. In sterile flasks, 10 liter per sample was collected from each selected resource. Samples were kept at 4°C until the beginning of the analysis.

Samples were filtered through 0.45 µm Millipore filter membrane. Each filter was fragmented under sterile conditions and immersed in PBS solution to resolve the deposits accumulated on the filters.

DNA extraction was accomplished using commercially available DNA extraction kit (DNPTM KIT, Cina gen, Iran) according to manufacturer's protocol. Briefly, samples were incubated for three hours at 55°C after adding 100 µl (1mg/ml) proteinase K. Then, samples were treated with 150 µl pre-warmed lysis buffer and vortexed vigorously for 1 minutes. Three hundred µl ice-cold precipitation solutions were added to each tube and tubes were kept at -20°C for 24 hours. Samples were centrifuged for 10 minutes at 12000 rpm. Supernatant was removed and 600 µl wash buffer was added to each tube. Tubes were inverted 10 times and samples were centrifuged under previous conditions.

Supernatant was removed and tubes were incubated at 65°C for 5 minutes with open doors. At the final step of extraction pellet were resolved in 30 µl distilled water and quality and quantity of DNA extraction samples were analyzed by spectroscopy (UNICO 2100, USA).

PCR Amplification was performed using the following set of primers: Forward 5-CTGGAGAGACTAAGCCCTCC-3 and reverse 5-AGGATCAAGGTTTAAGGATT-3 for 16S rRNA producing a 446bp product, Forward 5-AAGCTTTTAGGGGTGTTAGGGGTTT-3 and reverse 5-AAGCTTACTTTCTAACACTAACGC-3 for glmM gene producing a 294bp product and forward 5-ATGACTAACGAAACTATTGATC and reverse 5-CAGGATTTTTGATCGCTTTATT-3 for cag producing 232bp product.[9-11]

PCR was carried out in a total volume of 25 µl mixture containing: 100ng DNA, 1.0 U Taq DNA polymerase, 3.5 µM of each primer, 200 µM of each dNTP, 3 mM MgCl2, and 1 x Taq polymerase buffer. Furthermore, PCR was accomplished under the following conditions: 95°C for 5 min following by 35 cycles of 95°C for 30 s, 58°C for 30 s and 72°C for 30 s and a final extension step of 72°C for 5 minutes.

Product analysis

Products were analyzed on 1% agarose gel containing 1 µg/ml ethidium bromide and visualized with UV light.

Data were analyzed using SPSS 16.0 statistical software (SPSS Inc. Chicago, IL, USA), Chi-square test and $P<0.05$ were considered significant.

RESULTS

In the present study, the total infection rate of samples by *H. pylori* was 24.13%. All obtained samples from Zayanderud River were positive while 12.5% of the samples from springs including Dehcheshmeh in Farsan city, Baram in Lordegan, and Badi in Shahrekord, and 50% in subterranean (Pez in Saman city) were positive (Table 1).

Table 1: Frequency of existence of *Helicobacter pylori* in springs of Chaharmahal and Bakhtiari Province

Springs	Positive N(%)	Negative N(%)	Total N(%)
Shahrekord	1(16)	8(83)	9(100)
Farsan	1(33.3)	2(66.6)	3(100)
Boroujen	0(0)	2(100)	2(100)
lordegan	1(100)	0(0)	1(100)
Kiar	0(0)	3(100)	3(100)
Kohrang	0(0)	3(100)	3(100)
Ardal	0(0)	3(100)	3(100)
Saman (river)	3(80)	0(0)	3(100)
Saman (subterranean)	1(50)	1(50)	2(100)
Total	7(24.13)	22(75.87)	29(100)

To achieve these results, at first 16S rRNA gene was amplified to determine the existence of *H. pylori* genus in samples using genus- specific 16S rRNA primers for *H. pylori* (Figure 1).

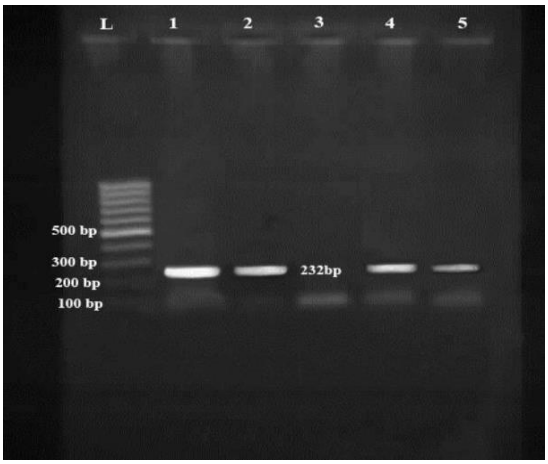

Figure 1: agarose gel electrophoresis of an amplified 446-bp fragment of 16S rRNA

Left to right: lane L: 100bp marker, lane1: negative control, lane2: positive control, lane 3-7: positive samples.

Samples harboring 16S rRNA genes revealed a 446bp band on agarose gel. In the

next step, samples were used in other rounds of PCR, amplifying cagA and glmM genes. PCR amplification of cagA gene was resulted in a 232bp band as it has been shown in Figure 2. Finally to determine the presence of *H. pylori* infection in spring water samples, glmM gene amplification was performed on DNA samples and produced a 294bp band (Figure 3).

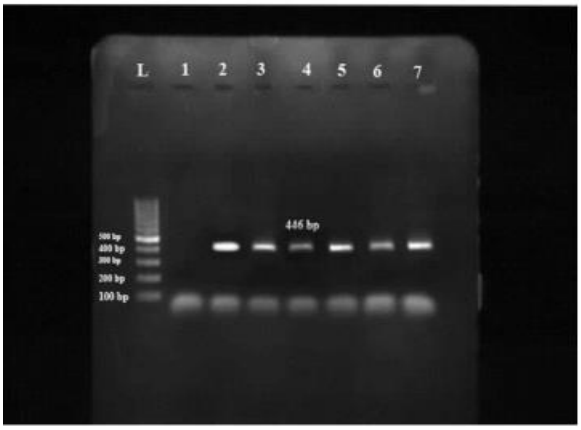

Figure 2: Gel electrophoresis of an amplified 232bp fragment of cagA gene on agarose gel (1%)

Left to right: lane L: 100bp marker, lane1: positive control, lane 2-4-5 cagA positive samples, lane 3: cagA negative sample.

Figure 3: electrophoresis of an amplified 294bp fragment of glmM gene on 1% agarose gel

Left to right: L: 100bp marker, lane1: positive control, lane2-3-5-6-7 glmM positive samples, lane4: glmM negative samples.

DISCUSSION

H. pylori could be transmitted via the fecal-oral as well as the oral-oral route.[12] Short-term survival period of *H. pylori* in water and inadequate sensitivity of some methods in isolation of *H. pylori* caused some problems for detecting *H. pylori* in water samples.[13,14]

Since the culture of *H. pylori* is difficult because of a limited period of time in a specific temperature, in the present study molecular methods were chosen.[15] The dead *H. pylori* cell's DNA in water sample could be detected by PCR, but the detection is usually impossible by culture.[16] In addition, the presence of *H. pylori* rRNA in a PCR assay strongly suggests that the samples contain intact cells.[17]

In the present study, *H. pylori* has been identified in several water sources using PCR and total infection rate of drinking water by *H. pylori* was 24%. The prevalence rates of *H. pylori* vary in different geographical regions and ethnic groups.[12] Similar findings were reported in other regions. In the study of Bahrami et al in Isfahan province, Iran by culture method only five cultures were positive while PCR method detected *H. pylori* ureC gene in 14 water samples.[7] However, in the study of Amirhooshang et al in Kermanshah, the overall prevalence of *H. pylori* DNA in the water samples was 56% with a frequency of 36% in tap water samples and 85% in wells.[8]

Twing et al studied about the existence of *H. pylori* that was determined by PCR assays from DNA and RNA for the 16S rRNA gene, as well as DNA for the ureA and cagA genes in fresh water, estuarine and beach sites in Delaware. In this study, 20% of the samples were positive for *H. pylori* 16S rRNA gene.[18]

Tanih et al in a study evaluated the prevalence of *H. pylori* vacA, cagA and iceA genotypes in South African patients with upper gastrointestinal diseases. This

study revealed a high prevalence of vacA, cagA and iceA2.[19]

Khan et al conducted a study with the aim of investigation the existence of *H. pylori* in drinking water samples of Karachi, Pakistan. Samples were concentrated and subjected to PCR for the detection of 16S rRNA gene of *H. pylori*. In this study, only two out of 4% of samples collected from two different densely populated town areas were found to be positive for *H. pylori*.[1]

In a particular condition the water contamination by *H. pylori* might be considered as a potential source of human infection.[18] Furthermore, Goodman et al study in Colombia reported that drinking water can be a source of *H. pylori* infection in children who drink, swim, or bathe in infected water.[20]

Presence of *H. pylori* in some of the major sources of drinking water in Chaharmahal and Bakhtiari province especially in low hygiene areas highlighted the necessity of more attention to public health, sanitary condition and other environmental related factors in this province. On the other hand, further studies will be conducted to determine the prevalence of *H. pylori* in water and potential risk of human infection with *H. pylori* via drinking of infected water.

CONCLUSION

These findings show the existence of *H. pylori* in springs and surface water in Chaharmahal and Bakhtiari province and additional evidence for waterborne transmission of *H. pylori* in some environments.

CONFLICT OF INTEREST

The authors declare that there was no conflict of interest.

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